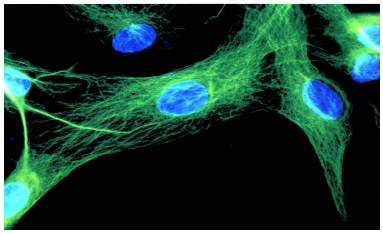
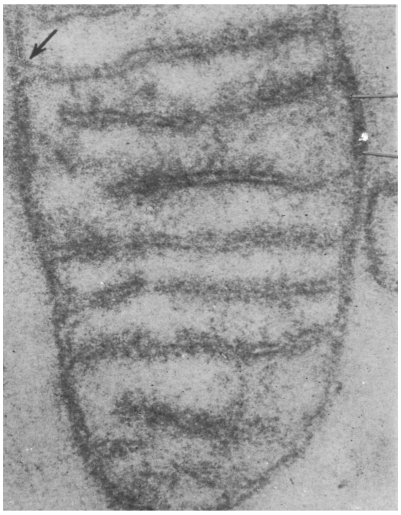
2.

Practical class: **Electron microscope and cell structure.**



astrocyte's microtubules

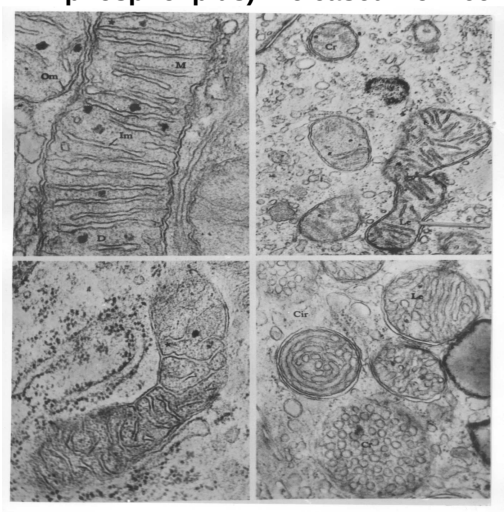
- **mitochondria (EM # 42, 51),**
- **endoplasmic reticulum (EM # 2),**
- **the Golgi complex & microtubules (EM # 12)**
- **endosomes & lysosomes (EM # 54),**
- **microtubules (EM # 33),**
- **proteasomes (text & EM # 98)**
- **peroxisomes (EM # 8),**
- **amino acids (text # 27),**
- **lipid rafts & caveolae (text #143)**
- **biologically active compounds (derived from fatty acids and phospholipids) - released from cell membranes (text & fig. # 13)**



EM - 42.

Mitochondrion.

Arrow indicates the site of crista formation by inner membrane.



EM - 51.

Various forms of mitochondria in various cell types.

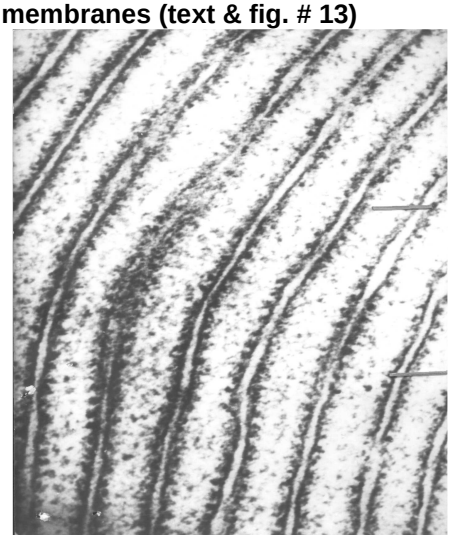
A - Epithelial cell from kidney tubules with lamellar cristae.
- Om - outer mitochondrial membrane;
- Im - inner mitochondrial membrane;
- M - Matrix; - D - Dense bodies;

B - Epithelial cell from epithelial lining of gallbladder.
- Le - mitochondrion with transversely arranged cristae (lamellar cristae).
- Cr - mitochondrion with longitudinally arran-ged, tubular cristae.

C - Liver parenchymal cell. Mitochondrion with lamellar cristae.

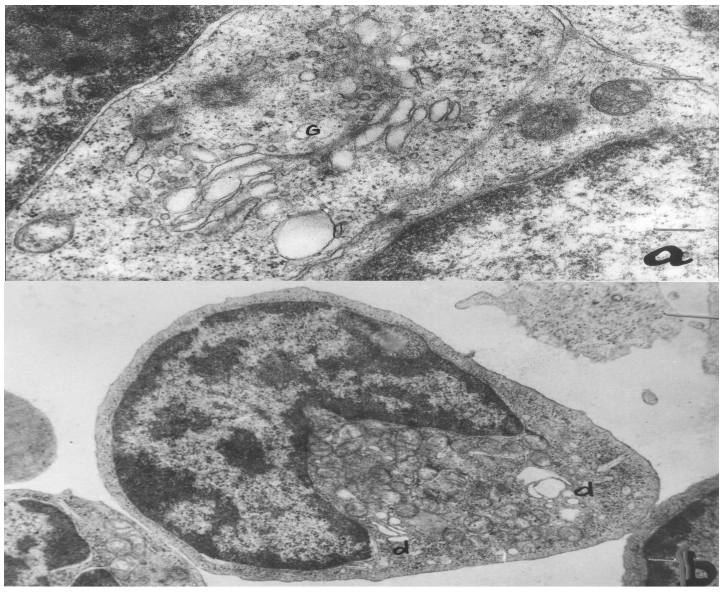
D - Theca lutein cell from corpus luteum (from ovary).
- Le- and -Cr - as in B;

- Cir - mitochondrion with circular cristae



EM - 2.

Rough endoplasmic reticulum in a cell from the pancreatic secretory acinus. It consists of flattened cisternae (lamellar forms) studded with electron dense particles, ribosomes. The ribosomes are bound to rough endoplasmic reticular membranes by ribosome-binding proteins called ribophorins.

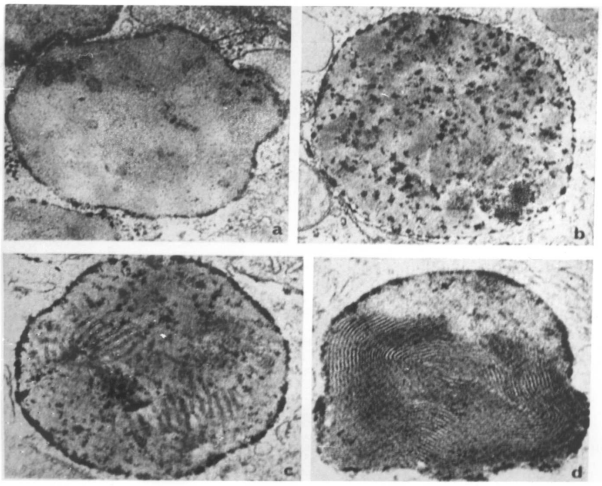


Electron micrograph 12a.

The Golgi complex (G) located close to the celi nucleus consists of
dictyosome composed by stack of flattened, narrow or moderately
distended cisternae accompanied by a few vacuoles.
In cells containing more than one dictyosome, these structures always lay in close vicinity to each other. The structural integrity and function of the Golgi complex as well as localisation of it's dictyosomes depend on the presence of intact microtubules.
Magnification 35, 000 x.

Electron micrograph 12b.

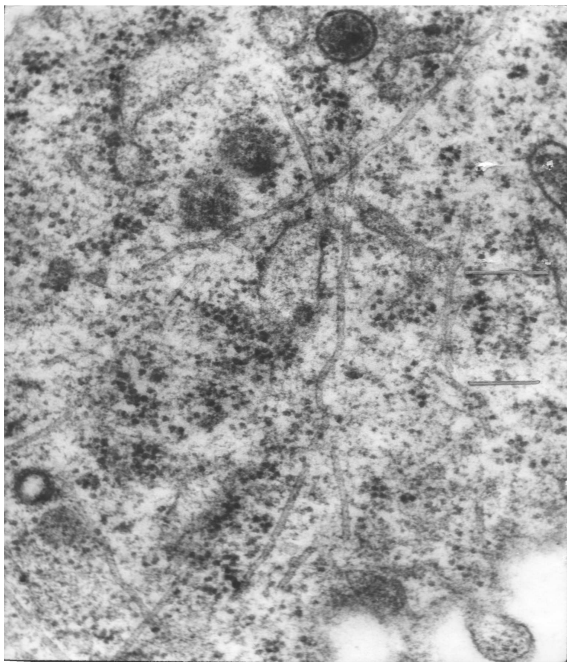
Leukaemic lymphoblast treated *in vitro* with vincristine. Two dictyosomes (d) lie at a considerable distance from each other. Incubation of cells with vincristine induced not only separation of dictyosomes but also alterations in their morphology. The cisternae are less numerous, shorter and more distended than in controls and some of them transformed into large vacuoles. The nucleus with deep indentation is also visible. Destruction of microtubules by inhibition of tubulin połymerisation using cytostatics such as colchicine, vinblastine or vincristine leads to changes in morphology and function of the Golgi complex.
Magnification 11, 000 x.



EM -54.
Old theory of lysosome origin

a - lysosome;
b - lysosome + phagosome = phagolysosome;
c - lysosome + autophagosome = autophagolysosome;
d - residual body

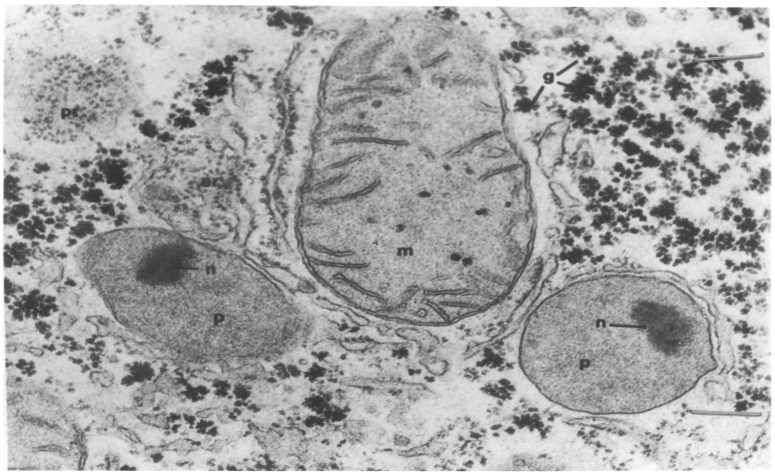
Dark, granular precipitate in a - d corresponds to sites of acid phosphatase activity.
New theory of lysosome origin is explained in connection with early and late endosomes.



EM - 33.
Microtubules in ovarian granulosa cell.

Each microtubule consists of 13 protofilaments composed of dimers of α- and ß-tubulin.

EM - 8. Peroxisomes (microbodies)



Electronmicrograph showing rat liver cell (hepatocyte).
m - mitochondrion; p - peroxisome; n - crystalloid containing enzyme urate oxidase; er - rough endoplasmic reticulum; pr - polysomes (polyribosomes); g - glycogen granules.
In mammals large peroxisomes (diameter about 0.5 μm) occur mainly in liver. In other tissues peroxisomes with a diameter 0.15-0.25 μm predomi-nate. They usually contain at least three oxidative enzymes: d-amino acid oxidase, urate oxidase and ca-talase. The crystalloid core is composed of urate oxidase. Peroxisomes protect the cells from harmful effects of hydrogen peroxide produced in various enzymatic reactions.

Peroxisomal enzymes use molecular enzymes to remove hydrogen atoms from specific organic substrates (designated as R) in an oxidative reaction that produces hydrogen peroxide (H_2O_2).

$$RH_2 + O_2 = R + H_2O_2$$

Catalase utilizes the H_2O_2 generated by other enzymes to oxidize (and detoxify) various substrates (phenols, formic acid, formaldehyde, alcohol, in the reaction:

$$R'H_2 + O_2 = R' + H_2O_2$$

Almost half of the ingested ethanol is oxidised to acetalaldehyde in this way. Furthermore, when excess H_2O_2 accumulates in the cell, catalase converts it into H_2O:

$$2H_2O_2 - 2H_2O + O_2$$

Text, schema and EM 98

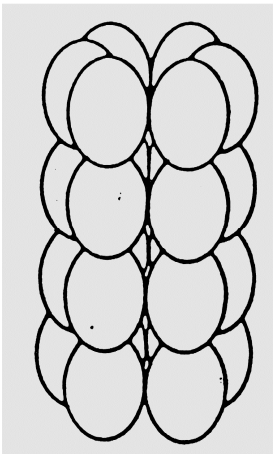
PROTEASOMES

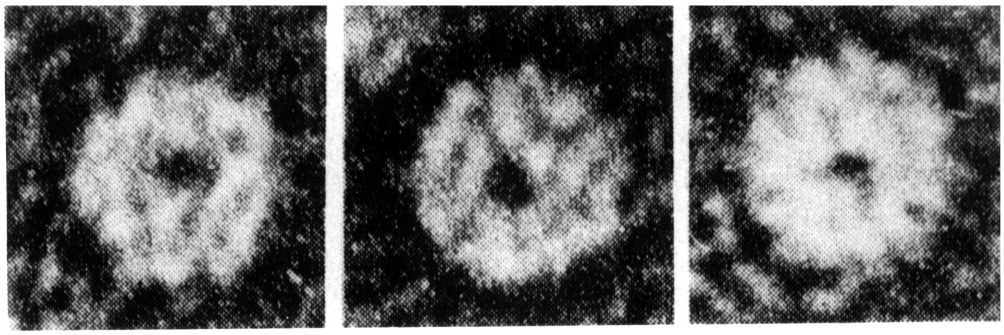
Proteins taken up by endocytosis are degraded in the endosomal and lysosomal compartments by proteases (cathepsins). Proteins produced by cells are continually degraded. This eliminates abnormal proteins or proteins which already ac-complished their function. The protein molecule to be degraded is first conjugated to the polypeptide ubiquitin. In this reaction (requiring ATP) the carboxy terminus of ubiquitin becomes attached to the amino group on lysine residues on the protein or on other ubiquitins. In this way protein to be digested acquires a long

chain of ubiquitin molecules which mark it for destruction.

Proteasomes are proteolytic complexes that degrade proteins connected with ubiquitin.

Proteins which are supposed to be destroyed soon after their synthesis contain at the amino-terminal residue one out of twelve destabilizing amino acids. The remaining 8 amino acids have stabilizing properties. Nevertheless, even proteins with stabilizing amino acids may be degraded by proteasomes after denaturation. Proteasomes (20S or 26S particles) are composed of 13-15 units of similar size. They contain various proteolytic activities and occur both in cytosol and nucleus. Proteasomes may be isolated from cells and observed in EM after deposition on a suitable surface. EM demonstrates 3 proteasomes isolated from cells by ultracentrifugation. The spacial arrangement of particular enzyme subunits is shown in the schematic drawing.





EM 98

Text & EM 143.

LIPID RAFTS AND CAVEOLAE

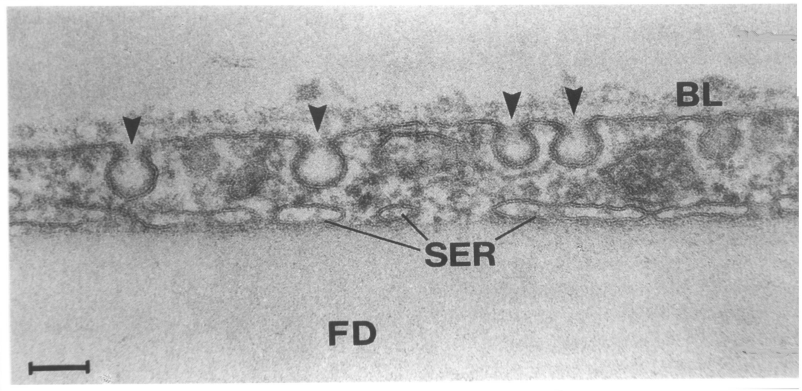
Lipid rafts represent a specific cell membrane microdomain. They are characterized by an increased cholesterol and sphingolipid content. Owing to an increased cholesterol and sphingolipid content these parts of cell membrane are less fluid and more stable than other parts of the cell membrane. This also enables raft isolation and purification as; at low temperature they are resistant to nonionic detergents.

Lipid rafts serve as sites of some specific cellular functions such as vesicular transportation and receptor-mediated signal transduction.

In addition to rafts that do not contain any structural proteins, there exist rafts enriched in some particular specific protein, which may influence raft morphology and function. This may be exemplified by caveolin-1, a protein that enables transformation of rafts into caveolae. In many rafts the proteins are anchored via glyco-phosphatidyl-inositol, one of cell membrane phospholipids.

Lipid rafts are also present in intracellular membranes, *e.g.* Golgi apparatus membranes. It is believed that Golgi apparatus is a site of lipid rafts formation.

Caveolae are regular cholesterol-enriched cell membrane invaginations existing owing to the presence of caveolin-1. They participate in cholesterol transportation, transcytosis, potocytosis as well as signal transduction from cell membrane receptors, especially growth factor receptors and antigen receptors of T and B lymphocytes. Caveolae may serve as an invasion site for numerous intracellular pathogens. This is possible because ceveolae do not fuse with lysosomes and pathogens are not destroyed by lysosomal enzymes.



EM 143

EM 143 CAVEOLAE

Fragment of rat adipocyte
BL - basal lamina
SER - smooth endoplasmic reticulum
FD - fat droplets
- 100 nm

Caveolae (caves) are omega-shaped pits of cell membrane having diameter of 50 – 100nm. They are covered with protein named caveolin. This protein can be visualized by immunocytochemical methods (for example with antibody conjugated with gold particles). Caveolae are engaged in the signal transmission, transcytosis (transportation of high–molecular weight molecules across cytoplasm, for example in the endothelial cells), as well as in the concentration of low-molecular weight particles (p
otocytosis).

see: Histology with elements of molecular biology, ed. S. Moskalewski & W. Sawicki, Warsaw.

TEXT & FIG. 13

BIOLOGICALLY ACTIVE COMPOUNDS (DERIVED FROM FATTY ACIDS AND PHOSPHOLIPIDS) RELEASED FROM CELL MEMBRANES

The components of the cellular membranes are lipids and proteins. The lipids, in the membranes of human cells, contain phospholipids and cholesterol (in the ratio 50:50), as well as glycolipids. The phospholipids of the membranes involve:

1) phosphatidylcholine,

2) phosphatidylserine,
3) phosphatidylinositol
4) phosphatidylethanolamine.

Phosphatidylcholine, cholesterol and glycolipids are the main components of the outer layer of lipid membrane, while in the inner layer of the membranes there is no glycolipid and instead there is phosphatidylinositol.

Transmembrane and membrane bound enzymes catalyse the matabolism of membrane phosphatidylcholine and phosphatidylinositol. As a result the primary messenger- hormons - and secondary messengers are being formed.

BIOLOGICALLY ACTIVE COMPOUNDS DERIVED FROM PHOSPHATIDYLCHOLINE

The hydrolase, phospholipase A cuts out from phosphatidylcholine 20 - carbon fatty acid known as **arachidonic acid (AA)**. The AA can also be derived from fatty acids of food and phospholipase occurs in the body fluids as well.

The products of metabolism of AA are known as **eicosanoids** and their formation is catalysed by:

a) cyclooxygenase
b) lipoxygenase.

The enzymes are membrane bound but may also occur in cytoplasm and as a result of cell activation they may be bound to the membrane.

Cyclooxygenase (and other enzymes - synthase and hydrolase) converse the AA to prostaglandins (PG) including prostacycline (PGI_2) and thromboxans (TX). **Lipoxygenase** (and other enzymes - synthase and hydrolase) converse AA to leukotriens (LT) and lipoxins (LX). The molecular mass of these compounds is approximately 350. Cyclooxygenase and lipoxygenase oxygenate the molecules of AA, i.e. they insert 0_2 into the specific sites of molecule. Cyclooxygenase, besides of oxygenation, catalyses the formation of cyclopentane fragment in AA molecule. Cyclooxygenase and lipooxygenase give rise to formation of the family of more than 20 eicosanoids which have various effects.

CYCLOOXYGENASE PRODUCTS DERIVED FROM AA

Cyclooxygenase (an intramembraneous enzyme) produces the cyclic endoperoxides (PGG_2) from AA. The latter is reduced to PGH_2. PGG_2 and PGH_2 are intermediate compounds in production of all PG and their synthesis is inhibited by some drugs, e.g. aspirin and indometacin.

Alltogether 16 kinds of PG are contemporarily known and they are marked with A-I letters and digits. The latter reffer to the number of insaturated bonds in fatty acids.

PROSTAGLANDINS

The most important PG:

1) PGA_2 dilates blood vessels and increases blood flow through the kidney and accelerates natriuresis,
2) PGB_2 has the poor physiological effect since it is an intermediate compound,
3) PGD_2 is released mainly from blood platelets and the cells of the lung. It aggregates the blood platelets and increases permeability of blood vessels,
4) PGE_1 inhibits lipolysis, blood platelets aggregation and dilates blood vessels,
5) PGE_2 dilates blood vessels and contracts uterine muscles, increases release of calcium ions from bones, inhibits natriuresis and causes blood platelets aggregation,
6) PGF_2 contracts blood vessels and bronchi as well as the muscles of uterine at the parturition (it is used to accelerate parturition and for the abortion), it causes luteolysis and decreases progesterone level in blood (it may initiate abortion),
7) PGI_2 known also as prostacycline is in physiological condition highly unstable, although its more stable analogs strongly dilate blood vessels (they decrease the blood tension) and prevent blood platelet aggregation as well as binding fibrynogen to the platelets. Prostacycline is mainly released from the endothelial cells,
8) Tromboxane A_2 (TXA_2) is a type of PG released preferentially from blood platelets and lung fibroblasts. It strongly contracts blood vessels and increases blood platelets aggregation.

PG RECEPTORS

Receptors for PG are localized in (or on) cellular membrane, in vicinity of G protein. The latter is an intermediate step in activation of adenylate cyclase and phosphodiesterase. Thus, the effect of PG on cells is mediated via activation/inactivation of cAMP synthesis as well as the synthesis of diacylglycerol and inositol triphosphate. For instance PGF_2 effect on a cell is synergistic with EGF (epidermal growth factor) in initiation of DNA synthesis and some classes of PG play the role in regulation of cell proliferation.

LIPOXYGENASE PRODUCTS DERIVED FROM AA

Lipoxygenase catalyses the conversion of AA into:

a) leukotrienes (LT)
b) lipoxins (LX).

LEUKOTRIENES

Lipoxygenase (and other enzymes - synthase and hydrolase) catalyses the reactions of formation of 5-HPETE (5-hydroperoxy, trans, cis-esatetrenic acid that is an intermediate compound), then LTA_4, LTB_4, LTC_4, LTD_4 and LTE_4.

LT are preferentially released from the cells originated in the bone marrow (leukocytes, mast cells and blood platelets) as well as from the endothelial cells.

LT effects:

1) they activate various types of leukocytes, increase permeability of venules (they do that by contraction of endothelial cells), they increase diapedesis and chemotactic effect of leukocytes. Therefore, they play the role of mediators of inflammation and are responsible for the symptoms of alergy.
2) they contract bronchi and increase the mucus secretion.
3) they control the terminal differentiation of granulocytopoiesis as well as of lymphocytopoiesis of T lymphocytes; they increase also the secretion of interleukine 2 and gamma-interferon by T lymphocyte.

LIPOXINS

Lipooxygenase catalyses reaction of formation of lipoxins (LX) from AA. Recently, two classes of lipoxins are known LXA_4 and LXB_4, that are form membranes of leukocytes. LXs dilate blood vessels of kidney and other organs, contract bronchi, activate kinase C and inhibits NK cells.

LT AND LX RECEPTORS

Receptors for LT and LX are situated in the membranes in vicinity to transmembrane protein G. The protein controls ligand (LT or LX) binding as well as the internalization of ligand-receptor complex. The signal transferred through the membrane affects the complex of actine-myosine intermediated by the system of protein kinases and causes either the contraction or

relaxation of myocytes or endothelial cells.

RELEASE OF EICOSANOIDS

Both eicosanoids and their precursor - AA are released from the membranes of most, if not all cells. Various cell types (including blood platelets) may cooperate in final production of various eicosanoids. AA or endoperoxides released from leukocytes or from blood platelets may penetrate the endothelial cells that in response, produce and release PI_2. In similar way, leukocytes, erythrocytes and endothelial cells may produce and release various compounds with production and liberation of the final eicosanoid.

Eicosanoids are the local hormons, i.e. their release from one cell affect the cells situated in their vicinity (paracrinic effect). The latter is in part an effect of unstability of eicosanoids. Their half life time ranges from seconds to minutes.

COMPETITIVE MODIFICATION OF EICOSANOID SYNTHESIS

Various unsaturated fatty acids, e.g. eicosapentainic acid (EPA, a constituent of fish oil) are introduced into human body with food. These kinds of fatty acids may competatively be bound with oxygenases and therefore, be converted by oxygenation. Their derivatives are being formed. Their biological activity is scarce (or they have not biological activity at all). The binding of fatty acids with oxygenases inhibits enzyme activity in competative way. Thus, the synthesis of TXA_2 as well as the formation of inactive analogues of LT is inhibited. As an effect the arteriosclerosis and inflammation is prevented.

BIOLOGICALLY ACTIVE COMPOUNDS DERIVED FROM PHOSPHATIDYLINOSITOL

In a membrane phosphatidylinositol may be pbosphorylated by protein kinases giving rise to phosphatidylinositol diphosphate. Intramembranous enzyme - phospholipase C may cut phosphatidylinositol diphosphate giving rise to two kinds of secondary messengers: diacylglycerol (DG) and inositol triphosphate (IP_3). Phospholipase C is an intramembranous enzyme activated by binding of hormons (e.g. endothelins) to receptors of cellular membrane. DG released from membrane to cytoplasm activates kinase C (that increases pH of cytoplasm). IP_3 released from membrane to cytoplasm mobilises calcium ions that flow from smooth endoplasmic reticulum to cytosol. Calcium ions are secondary messengers that trigger many cell reactions, e.g. contraction of smooth muscles, cell divisions etc.

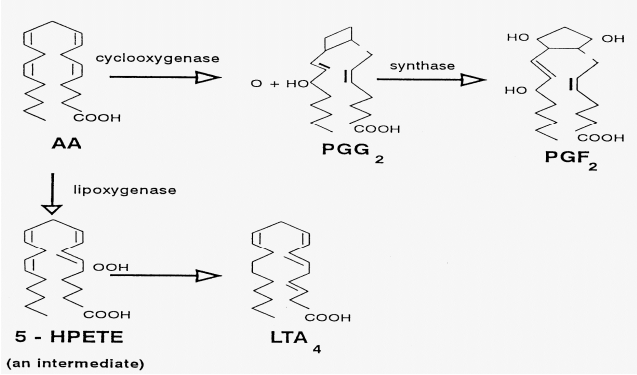


Fig. 13.1

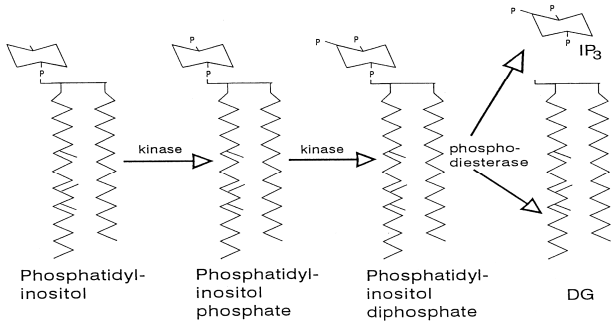


Fig. 13.2

No. 27 AMINO ACIDS

Amino Acid	Short	Abbrev.	Avg. Mass (Da)	pI
Alanine	A	Ala	89.1	6.01
Cysteine	C	Cys	121.2	5.05
Aspartic acid	D	Asp	133.1	2.85
Glutamic acid	E	Glu	147.1	3.15
Phenylalanine	F	Phe	165.2	5.49
Glycine	G	Gly	75.1	6.06
Histidine	H	His	155.2	7.60
Isoleucine	I	Ile	131.2	6.05
Lysine	K	Lys	146.2	9.60
Leucine	L	Leu	131.2	6.01
Methionine	M	Met	149.2	5.74
Asparagine	N	Asn	132.1	5.41
Proline	P	Pro	115.1	6.30
Glutamine	Q	Gln	146.1	5.65
Arginine	R	Arg	174.2	10.76
Serine	S	Ser	105.1	5.68
Threonine	T	Thr	119.1	5.60
Valine	V	Val	117.2	6.00
Tryptophan	W	Trp	204.2	5.89
Tyrosine	Y	Tyr	181.2	5.64