THE “ESSENTIAL” PHOSPHOLIPIDS AS A MEMBRANE THERAPEUTIC

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PREFACE

Research on "essential" phospholipids (EPL) has already attained but not yet accomplished its history. In the year 1989 it was 50 years since Hans Eikermann extracted the highly purified fraction of phosphatidylcholine molecules from soya beans. During these 50 years dozens of symposia and scientific meetings took place, and hundreds of publications appeared. Although a large number of texts on EPL have been published so far (mainly in the form of proceedings of scientific conferences), an up-dated and systematic scientific presentation reflecting all the achievements of EPL has still been missing. Therefore, this book describing all the results obtained from both experimental and clinical studies on EPL should be greatly appreciated. Moreover, some prospects of studies showing issues to be solved (strictly scientific questions like immunologic and receptor studies) and practical problems, such as new principles of dosage, new galenic forms or new indications, have also been pointed out. Another value of this book is the fact that some theoretical issues and practical indications have been consistently combined.

The title of the book as well as the titles of the chapters constitute a recapitulation of the existing knowledge on EPL, which with its special and main ingredient - dilinoleoylphosphatidylcholine - is of great importance in all diseases characterized by damaged membrane structures, reduced phospholipid contents and/or reduced membrane fluidity. The titles of the main chapters (phospholipids in the human membrane, the "essential" phospholipids, mode of action of EPL, pharmacological investigations, clinical studies) only give a general view, while careful reading presents a broad range of indications to the reader: liver and kidney diseases, dyslipidemia and atherosclerosis, gestosis, gastric and intestinal inflammation, neurologic disorders, lung and skin diseases.

For a clinical pharmacologist the results of pharmacokinetic investigations (absorption of the 14C-labelled substance after oral administration) are of special interest as well as the fact that 1,2-dilinoleoylphosphatidylcholine, which is physiologically present in the human body only in trace amounts, may substitute endogenous phospholipids and be incorporated into all membrane-containing fractions, thus improving the fluidity of membranes.

Summing up, the book is a broad source of modern knowledge on EPL and an interesting publication from both theoretical and practical points of view. It can also serve as an inspiration to start further studies on EPL.

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1. PHOSPHOLIPIDS IN THE HUMAN MEMBRANE

1.1 Phospholipids Composition
Phospholipids are amphipathic molecules. They contain a polar region and a hydrophobic part. The former is the so-called "head group" of the phospholipid molecule and can be one of several different possible groups, e.g. phosphocholine or phosphoethanolamine (fig. 1).

Fig 1: Structures of various types of glycerophospholipids (761) %>

Sphingomyelins (ceramide-1-phosphorylcholines) contain a choline polar head but are not linked to a glycerol backbone.

Furthermore, not all phospholipids have ester linkages as the covalent bond between the glycerol backbone and the tail regions, nor must they contain the linkages -P-O-R-3. In some phospholipids, e.g. the phosphonolipids, the polar group R3 is directly attached to P via P-C bond. Generally all mammalian tissues contain qualitatively the same phospholipids (742).

Phosphatidylcholine predominates in most parts of the human body (tab. 1).

Tab.1: Phosphatidylcholine content in different human organs in percent of the total phospholipid composition (742)

The hydrophobic region of a phospholipid consists of the long hydrocarbon chains esterified to the 1- and 2-positions of the glycerol backbone. These chains are at least 14 carbons long in humans (761).

Typically naturally occurring phospholipids contain an unsaturated fatty acid (such as oleic acid, linoleic acid or arachidonic acid) in position 2 and a saturated one (such as stearic acid or palmitic acid) in position 1 (718). Comprehensive analysis of the fatty acids of phosphatidylcholine of whole tissue and subcellular fractions (742) and their possible distribution to the 1- and 2-positions of the glycerol group elucidate the wide variety of different phosphatidylcholine molecules (and of the other types of glycerophospholipids) in the human organism.

1.2 Phospholipids for Structuring and Composition of Membranes

Beside glycolipids, cholesterol and proteins phospholipids belong to the main components of biological membranes (519). The total lipid content (40-80 % of dried weight) and the proportion of phospholipids are relatively high in mammalian cell membrane preparations but vary appreciably from one type of membrane to another (182).

Phosphatidylcholine and phosphatidylethanolamine predominate quantitatively in the lipid fraction of most biological membranes (742) and they mainly constitute the matrix of these membranes (fig. 2).

The majority of phospholipids are present in a bilayer structure (519). The bilayer may be discriminated in three parts:

The polar, hydrophilic groups are arranged at the outer sides of the cellular membranes, whereas the acyl residues face each other at the inside. The hydrophobic core of the bilayer can be subdivided, into the steroid stiff chain and the pliant chain. The pliant chain is a rather disordered region in the middle of the core with high flexibility, the steroid chain is the area where van der Waals forces are very strong (681). The different phospholipids need different spaces in the membrane:

1. The size and chemical structure of their polar region influence the tightness with which the acyl chains can pack. Ethanolamine, for example, is
smaller than choline due to N-substitution with methyl groups in the latter, and acyl chains in phospholipids containing ethanolamine can simply for this reason pack more tightly. Furthermore, phosphatidylethanolamine can form an extensive network of hydrogen bonds, which additionally enhances the tightness of packing (761).

2. The space is definitely influenced by the type of the acyl chains. With the help of the 'Langmuir balance technique' it was shown that sphingomyelin with its mostly saturated acyl chains occupies an area of 36 Å²/molecule. If there are two unsaturated acyl chains, as presented by dilinoleoyl-phosphatidylcholine, the space extends nearly twofold at a pressure of 25 dyn/cm which is assumed to be the intracellular pressure exerted on a bilayer (681).

Below the phase transition temperature (see 1.4.1 Membrane Fluidity) phospholipids containing only fully saturated acyl chains adopt a configuration in which the acyl chain carbons are in extended all-trans conformation. The introduction of a cis double bond precludes the adoption of the trans conformation in at least one position in the chain. A 'kink' is found in the chain, disrupting the close packing which is possible with the all-trans conformation (fig.3). Van der Waals energies are weakened. Correspondingly the presence of even one single double bond is sufficient to exert a profound influence on physical properties (684, 761).

Cornell and Separovic (123) found that an increase in the phosphatidylcholine hydrocarbon chain length was accompanied by an increase in bilayer thickness of only 40 % of the value expected for the fully extended chains. The major change was an increase in the area per phospholipid indicating that the ends of the fatty acid chains are highly dynamic and have an increased molecular area. To fulfill its vital functions the membrane must operate differentially on the two compartments it separates, and thus it must be asymmetric. The outer surface must differ chemically from the inner one: Some negatively charged phospholipids (phosphatidylinositol, phosphatidylserine, phosphatidic acid) and the zwitterionic phosphatidylethanolamine accumulate predominantly at the inner surface, while other species such as the strongly acidic phosphatidylglycerol and the zwitterionic sphingomyelin prefer the outer surface of the bilayer (53). Figure 4 shows the asymmetrical distribution of phospholipids in the membrane of human red blood cells (723).

The asymmetry is maintained by the lack of transmembrane diffusion (585). Apart from the asymmetric disposition of phospholipids (and of cholesterol), the fatty acids are also asymmetrically distributed (684). Asymmetry can be present also in the same layer, where lipids can form domains of different composition and diffusion characteristics (649). These lateral phase operations are characterized by a specific selection of several components which frequently occur in precisely localized positions and are obviously excluded from lateral movements by intramembraneous or exogenous (cytoplasmic and extracellular and intravesicular, respectively) constraints (197). Beside the fact that membrane parts may be laterally divided into distinct phases, the dynamic state of cell and organelle membranes has been demonstrated
by experimentally evidenced exchange processes of phospholipids within a membrane and between several membranes:

1.) Lateral diffusion, rotation of the lipid molecules around their longitudinal axis and rotation around the carbon-carbon bonds of the acyl chains are the main motions, the flip-flop motion is of less importance; the latter describes the movement of a phospholipid molecule from the extracytosolic to the cytosolic part of the bilayer (681, 761). The lack of flip-flop can be explained, at least in part, by the high activation energy that would be required to bring the polar group through the hydrocarbon core of the bilayer (585).

2.) Transfer proteins have been isolated for example from rat and cow livers which facilitate the phosphotipid transaction. Exchange studies on sarcoplasmic reticulum have shown that the phosphatidylcholine translocation had at 1/2 of either over 15 days (481) or less than 10 min (137), which brings up the possibility that these results reflect "closed" or "open" status of the phosphatidylcholine pore protein (719). This is important for the extracellular uptake of phospholipids. The 'phosphatidylcholine translocation' displays stereospecificity, which means that it facilitates the translocation in both directions (719). In this whole context it is relevant to note that phospholipids can undergo relatively fast transbilayer movements in several biological membranes which do not contain such a strictly organized cytoskeleton such as the microsomal membrane of rat liver (526).

3.) Also an exchange of phospholipid molecules between different membranes takes place. This has been demonstrated, for example, in-vitro between microsomes and mitochondria of rat liver (748). Such transfer processes have been described also to occur between natural and artificial membranes (e.g. between liposomes and rat liver mitochondria) (769). Membrane synthesis is initiated in the endoplasmic reticulum leading to the formation of precursor vesicles whose fusion with already existing plasma membrane results in formation of a new plasma membrane. During the fusion process the original transmembrane asymmetry of the vesicle is inverted (53) (fig. 5).

1.3 Protein Distribution in Membranes

In the fluid lipid bilayer various proteins are associated (fig. 2). Biological membranes contain two types of membrane proteins (680):

1. proteins which are only loosely associated with the membranes and are easily removed ('extrinsic' or peripheral proteins), and
2. those which are firmly bound to the membrane and can only be removed by detergents ('intrinsic' or integral proteins). The latter consist of ectoproteins which are arranged towards the extracytoplasmic side, and of endoproteins which are arranged towards the cytoplasmic side only. Some proteins penetrate the whole lipid bilayer. Most membrane proteins possess a hydrophilic region (protruding from the phospholipid bilayer) and a hydrophobic region embedded in the phospholipid bilayer.

1.4 Function of Phospholipids in Membrane Bilayer

In principle, phospholipids can affect a number of cellular functions, including carrier-mediated transport, the properties of certain membrane-bound enzymes, binding to receptors of biologically active substances (neurotransmitters, peptide-hormones and proteo-hormones, lectins, antigens and antibodies,
lipoproteins, complement factors, chemotactic substances and others), phagocytosis, endocytosis, exocytosis, prostaglandin and acetylcholine production, cell-cell interaction, cell cycle and cell differentiation. The effects of lipid modification on cellular function are very complex and vary often from one type of cell to another. Generalizations are problematic and the response of a membrane to a particular type of modification is difficult to predict.

However, more and more functions of a membrane system as an interplay with the membrane composition are understood and put together like elements in a puzzle. As membrane fluidity belongs to the main factors influencing membrane functions, it will be first described in detail.

1.4.1 Membrane Fluidity

At physiological temperature, x-ray diffraction studies have indicated that the fatty acids of membranes have a "liquid crystalline" structure (194). When the temperature of the membrane is decreased, phospholipids are converted from a "liquid crystalline" phase to a gel phase (194, 683).

The transition temperature and the membrane fluidity depend on (531, 718):

- a) chain length, degree of unsaturation and type of pairing of the hydrocarbon chains of the phospholipids,
- b) the nature and charge of the polar headgroups of the phospholipids,
- c) the phospholipid/sterol ratio and the chemical structure of the sterol, and
- d) the (phospho) lipid-protein interactions.

which will be described by a few examples as follows:

ad al (519, 649, 683, 761)

Acyl chain order increases with chain length but decreases with cis-unsaturation. A double bond has the greatest effect when it is introduced into a fully saturated chain, for example, when a stearoyl chain is converted to an oleoyl chain; the introduction of a second double bond, like from oleoyl to linoleoyl, has a lesser effect on reduction of viscosity. At higher degrees of unsaturation, the effect becomes progressively less pronounced.

The increase in specific volume, when a cis double bond is introduced, is parallel with a decrease of the thickness of the bilayer (phosphatidylcholine from 0.48 to 0.58 nm, resp. 0.46 to 0.41 nm).

A fluid-phase bilayer (= 45 Å thick) is 15% thinner than a gel-phase bilayer. In a more disordered membrane, where the acyl chains do not pack as tightly, even holes or gaps can form between the chains.

ad b) (401, 531, 649)

An increase in the phosphatidylcholine / sphingomyelin ratio is associated with increased erythrocyte membrane fluidity. In fact, natural sphingomyelin and lecithin are at the extreme edges of contribution to rigidity (sphingomyelin) or fluidization (phosphatidylcholine).

The sequential methylation of phosphatidylethanolamine to phosphatidylcholine increases lipid fluidity. Table 2 presents the phase transition temperatures for various species of phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

<%Tab. 2:%>

The remaining common phospholipids, i.e. phosphatidylycerine, phosphatidylglycerol and phosphatidylinositol, are all of a relatively high degree of unsaturation and may, therefore, be considered as fluidizers, similarly to phosphatidylcholine. The sphingoglycolipids (e.g. gangliosides), on the other, have a hydrophobic region which is similar to that of sphingomyelin, and as such act as lipid rigidifiers.

ad c) and d) (519, 585, 649, 683)
Cholesterol is known to restrict the mobility of neighbouring phospholipid fatty acyl chains, creating an ordered surface layer. The acyl chain order increases with cholesterol content above the phase transition temperature and decreases below this temperature. The effect of proteins on lipid dynamics is in essence similar to that of cholesterol. They also rigidify and increase the order in fluid lipid domains and act conversely below the lipid phase transition. Moreover, the fluidity can be influenced by such factors as temperature, pressure (osmosis), volume (lipid microviscosity is inversely correlated to the free volume of the lipid constituents), membrane potential, acidity and calcium (649). In the following sub-chapters the correlation of many membrane functions and membrane fluidity will be continually recognized.

1.4.2 Membrane Passage

Biological membranes delimit both the cell and its organelles 'from outside and from inside'. As a consequence, all membrane reactions have to pass through the membranes insofar as these reactions exceed the cellular or subcellular space. All the information, molecules and substances reach the cell through the membranes, so that these have to accomplish two contradictory tasks: on the one the membranes delimit the cells and the organelles and, on the other, the cell membranes are transmitting the signals and substances for the exchange processes between these unities.

For the molecular transport through the membrane both the lipid bilayer and a kind of pore system are at disposal. The passage of substances may happen
a) according to the rules of passive diffusion,
b) as facilitated carrier-mediated diffusion,
c) as active transport,
d) and as endocytosis or exocytosis (389).

The fluid mosaic model of a biological membrane offers three possibilities of direct participation of phospholipids in the membrane transport:
a) facilitated bilayer transport of polar molecules (e. g. of ions) by forming inverted micelles,
b) fusion, and
c) transport by changing the membrane morphology (e.g. by forming channels with nonbilayer structures) (128).

It is considered as sure that certain pairs of fatty acids in phospholipids require a certain kind of tissue and membrane specificity. As a consequence of their varying interactions with stearins, of the structural difference in their polar component (charges ranging from extremely negative to positive) and of different structures of hydrocarbon chains, phospholipids are able to regulate the multiple biological functions of the membrane. This applies in particular to permeability and to ion passage (quoted from 97, 117, 133, 183, 194, 365, 624, 680, 724).

The effects of increasing phospholipid unsaturation in increasing passive diffusion, for a series of defined phospholipid molecular species, has been shown to follow closely the increase in molecular area (143).

In carrier-mediated transport this pattern is not always followed (684) (tab. 3).

<Tab. 3: Effect of polyunsaturated fatty acid enrichment on carrier-mediated transport in cultured cells (658)>

When the membrane lipids are rich in unsaturated fatty acids, transport can proceed at rates up to 20 times faster than it does when the membrane lipids are poor in unsaturated fatty acids (194). Or, with other words, when the density of the phospholipid structures is loosened the exchange rate is accelerated (680).
1.4.3 Activity of Membrane-Bound Proteins and Receptors

Many enzymes, receptors and transport systems are linked to or embedded within membranes of cells and sensitive to the structure and physico-chemical properties of the lipids with which they interact. Because of this, the functions of these membrane-bound proteins and receptors can be regulated to some extent by changes within the lipid portions of biological membranes. The sensitivity may involve conformational changes that affect the binding sites of receptors of neurotransmitters and hormones, of transport systems (such as the calcium pump of the sarcoplasmic reticulum) or of the active site of enzymes. Lateral mobility and clustering, their vertical orientation in the lipid bilayer or their interaction with other membrane components may be affected. Thus, the membrane lipid structure mechanism may involve either bulk lipid fluidity, localized changes in specific lipid domains, or a combination of both (482, 658, 761).

The presence of lipids provides a medium for reaction; in general, they are needed for normal function, in single cases additionally for stimulation (117). Analogous to Zakim (761)

1) the many proteins which are embedded in the lipid bilayer and which are dependent from its physico-chemical properties
2) can be influenced in their functions by changes in the chemical composition of lipids and
3) since the composition of membrane phospholipids is not fixed but modifiable by diet and disease states, they can be manipulated by diet/drugs.

Listed in table 4 are some enzymes whose activities are showing lipid, especially phosphatidylcholine requirements (117). This list is not meant to be exhaustive but to illustrate that many vital processes are catalyzed by enzymes that are embedded within membranes.

<html>
<table border="1">
<thead>
<tr><th>Enzymatic activities showing lipid requirements</th></tr>
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<tbody>
<tr><td>Many enzymes such as ATPases, adenylate cyclase, 5'-nucleotidase, acetylcholinesterase, desaturases, succinate dehydrogenase, B-N-acetyl-D-glucosaminidase and drug metabolizing enzymes show modulation of activity by changes in fatty acid unsaturation (684).

Various regions of enzymes move during the catalytic cycle, and these movements may be critical for catalysis. These enzymes generally have higher catalytic activities in phospholipid environment of low versus high viscosity (761). Membrane enzymes could be modulated differently in the two bilayer leaflets which has been demonstrated in detail for adenylate cyclase, for example (684). Adenylate cyclase consists of a number of functional subunits, distributed in the different halves of the lipid bilayer, which are interrelated in a complex manner (for reviews see references 120, 402).
1.4.4 Cell Differentiation and Proliferation

The influence of phospholipids on cell differentiation and proliferation will only be highlighted by presenting a few characteristic points:
- Maturation of the hepatic endoplasmic reticulum is correlated with an increased production of phosphatidylcholine that contains greater amounts of unsaturated fatty acids. This microsomal phospholipid synthesis seems to be not only essential to the membrane structuring but also as to increase the enzyme activities during development (180).
- The available literature does not indicate any general profile for the change in membrane lipid fluidity or protein mobility during the cell cycle. While in a lot of different cell types such as lymphocytes, fibroblasts and hepatocytes (142) a lower microviscosity in mitosis than in the S-phase or interphase was seen neuroblastoma cells acquire in mitosis a higher membrane microviscosity (122).
Differentiation is affected by a progressive increase in microviscosity of the cell membrane (649).

1.5 Functions of Phospholipids in Monolayers

1.5.1 Phospholipids in Lipoproteins
Lipids occur in the blood as water-insoluble complexes which are transported only after binding to certain apoproteins. Phospholipids, which are lipophilic and hydrophilic, serve as solubilizers. Lipoproteins accordingly represent the transport forms of lipids in the blood. On the basis of their migration rates during electrophoresis, their density and their flotation in ultracentrifugation, lipoproteins are classified in chylomicrons, VLDL, LDL, IDL and HDL particles (29). Common to all lipoproteins is that the phospholipids as solubilizers surround these particles as monolayers (fig. 6).

Beside transporting lipids through blood lipoprotein-phospholipid exchange processes occur which probably contribute to the enrichment of phosphatidylcholine and sphingomyelin in the external phase of some plasma membranes (139).

1.5.2 Biliary Micelles
Another polar medium by which unpolar substances (mainly cholesterol) are transported is the biliary fluid. Bile is an aqueous solution with detergent-like molecules called 'bile salts', phosphatidylcholine and unesterified cholesterol; other components are conjugates of bilirubin and a mixture of proteins of diverse origin (96). Cholesterol is transported in small bile salt-cholesterol micelles and with, minimum-sized mixed bile salt - phosphatidylcholine-cholesterol micelles. All molecules are in rapid exchange from micelle to micelle via the intermicellar monomeric concentration of each lipid. The predominant subclasses of phosphatidylcholine are the 1-palmitoyl-2-oleoyl and 1-palmitoyl-2-linoleoyl species.

1.5.3 Emulsification
One function of bile is that of providing a reservoir of detergents and emulsifiers for efficient fat digestion in the gut (96).
The fine distribution of the fat droplets in the aqueous digestive secretion reduces the surface potential between oil and water and induces the formation of an emulsion, in which the lipids can easily be split by lipases.
For the intestinal resorption the emulsified fats are transferred into a micellar form (387). Again, these micelles consist mainly of lipids, bile acids and phosphatidylcholine.

1.6 Pool Function of Phospholipids In Membrane

1.6.1 Eicosanoid Precursor
Membrane phospholipids also play a major role in the prostaglandin synthesis: the principal prostaglandins, thromboxanes and leucotrienes are formed from precursors like eicosatrienoic and eicosatetraenoic acid. These precursors exist in the cell as components of the membrane lipids. They are both formed from essential linoleic acid by extending the chain and by desaturation (12, 583). In the case of the a.m. eicosanoid formation, the phospholipid fatty acyl modifications probably exert their effect primarily through a change in substrate availability. The chemical basis of the mechanism is the same, a change in membrane lipid composition (658).

1.6.2 Choline Donator
Cerebral choline is made by hydrolysis of acetylcholine, phosphorylcholine, membrane phosphatidylcholine and sphingomyelin as well as by the uptake of plasma choline (198). A small de novo synthesis is also possible (342). After the passage of the blood brain barrier choline may be used for the synthesis of acetylcholine in cholinergic neurons (58). It possibly also induces postsynaptic effects at nicotinic and muscarinic receptors (652).

1.7. Blood Corpuscles
In erythrocytes phosphatidylcholine comprises about 30 % of the total phospholipid complement and is distributed asymmetrically over the two layers: 76 % is found in the outer layer and 24 % in the inner one (526). A crucial part for structure and function of the erythrocytes plays the degree of unsaturation of phosphatidylcholine. The unsaturation index (i.e. the number of double bonds per fatty acyl residue in the phosphatidylcholine) should be 0.5-1.0, otherwise the packing of (phospho)lipid molecules within the erythrocyte membrane is no longer efficient and leads to abnormal structure and behaviour up to hemolysis of the cells (526). Experimentally it was shown that the cells turned echinocytic when the amount of di saturated phosphatidylcholine was increased, and stomatocytic on replacement by dilinoleoylphosphatidylcholine (fig. 7).

Even if the applied phosphatidylcholine could undergo a relatively fast trans-bilayer movement, 76 % phosphatidylcholine in the outer layer compared to the interior pool still result in predominant localization of the substituting molecules in the outer layer. The maximal substitution of phosphatidylcholine is about 53 %.

Beside the degree of unsaturation of phospholipid acyl chains the shape of red blood cells is influenced by the cholesterol/phospholipid ratio and the phosphatidylcholine/sphingomyelin ratio (121).

The normal molar ratio of cholesterol to phospholipid in the erythrocyte membrane is about 0.8-0.9. In diseases such as advanced cirrhosis of the liver spur cells are seen on dry smears with erythrocyte cholesterol/phospholipid molar ratio of about 1.22 (602). By exchange between the membranes of blood
cells and the serum lipoproteins the cholesterol/phospholipid ratio can be regulated/normalized (649).

The rigidifying effect of cholesterol, is similar on macrophages (104), monocytes (542) and lymphocytes (130, 542). In general, it appears that cholesterol acts as a natural modulator of immune responses (649). Unstimulated lymphocytes have an unusual fatty acid distribution of phosphatidylcholine. A high percentage of saturated fatty acids are formed in the 2-position of this phospholipid; stimulation, however, increases the content of polyunsaturated fatty acids in this position (519). It is apparent that the overt lymphocyte stimulation needs an optimum microviscosity for maximal response (170).

1.8 Surfactant
According to Hills (268) the surface of the gastric mucosa is covered with a hydrophobic coating due to the presence of surface-active phospholipids, the phosphatidylcholines in particular, both in the gastric mucosa and in the gastric juice. Probably a monolayer of the surfactant molecules is bound to the cell surface, thus protecting it against luminal acid (270). Hydrophobicity is most pronounced in the region of the acid forming mucosa; conversely, those regions of the gastointestinal tract absorbing aqueous luminal fluid do not possess a similar hydrophobic mucosal surface (268).

Surface active phospholipids identified in gastric juice and along the gastointestinal tract are also absorbed onto other mucosal surfaces to render them hydrophobic, these including alveolar (267), placental (124), eustachian (269), tracheal (266) and peritoneal (233) epithelium/mesothelium.

Protection against gastric luminal acid is one important function of a surfactant. Other main functions are:
- regulation of surface tension (622) (mainly down-regulation to prevent collapse of tissue such as of alveoli)
- anti-oedematous function (249) (to prevent shits of liquid e.g. from the lung interstitium to the alveoli)
- anti-glue function (21, 617) (e.g. to prevent adhesion in the peritoneum or to support mucociliar clearance)
- improvement of immunological effects (522) (such as broncho-pulmonal defence by alveolar macrophages)
- regulation of exchange of substances (157, 166) (e.g. increase of ultrafiltration in CAPD).
2. THE ESSENTIAL PHOSPHOLIPIDS (EPL)

2.1 Chemistry
The term of "essential" phospholipids indicates the highly purified extract of the semen of Glycine max (Linne Merril) with a standardized content of 76 % or 94 % (3-sn-phosphatidyl) choline in the oral preparations and of 94 % in the intravenous forms. The extract with 76 % (3-sn-phosphatidyl) choline contains besides phosphatidylcholine molecules further phospholipids, in detail 7 % phosphatidylethanolamine, 0.5 % phosphatidylinositol, 11.5 % other lipids such as glycolipids and 5 % oil. The (3-sn-phosphatidyl) choline purified to 94 % presents no more detectable quantities of other phospholipids, only 3 % of other lipids and 3 % oil. The word "essential" phospholipids results from the characteristic fatty acid composition of the phospholipids of the soya bean which is distinguished by its particular high content of polyunsaturated fatty acids, predominantly linoleic acid (approx. 70 %), linolenic acid and oleic acid. According to D. LeKim and H. Betzing (404) the fatty acid composition of these phosphatidylcholine molecules is in mol %:

About 52 % of these molecules consist of 1,2-dilinoleoylphosphatidylcholine, its main active component:

The atomic formula is: C₄₄H₈₀N₀₈P x H₂O, the molecular weight: 800. According to J.M. Fox (195) typical analytical data of the phosphatidylcholinmolecules are:

- Phosphorus content: 3.8 %
- Choline content: 14.9 %
- Fatty acid content: 69.0 %

with the following molecular proportions:

- Phosphorus:choline = 1.00
- Fatty acids:choline = 2.02
- Fatty acids: phosphorus = 2.00

EPL is a colourless or slightly yellow compound with waxylike consistency and nutlike taste.

2.2 Nomenclature
Thanks to the development of modern, patented techniques of separation it has become possible to constantly improve the chemical purity of the (3-sn-phosphatidyl) choline fractions from the soya bean, up to the phosphatidylcholine of 94% today. This historic development and country-specific peculiarities are the reasons why various names have been used in literature. The terms such as "EPL - Special" (= up to 82 % PC), "EPL - Purissimum" (= 94% PC), polyenylphosphatidylcholine (PPC) or polyunsaturated phosphatidylcholine (PUPC), or polyunsaturated lecithin (PUL) (=76 % PC or 94% PC) designate the same group of preparations with a very high content of phosphatidylcholine molecules with predominantly polyunsaturated fatty acids. According to the knowledge on the human membrane structures and functions (chapter 1 and 3) it is undisputed that phosphatidylcholine is a very important active ingredient. For this reason the soya-lecithins are standardised to the content of phosphatidylcholine. In this thesis, therefore, the generally known term of "EPL" is used to express the terminologically identical names of PPC, PUPC and PUL, phospholipon 100 (and with restriction LethiconR with a standardized content of 55 % (3-sn-phosphatidyl)choline).
2.3 EPL-Containing Preparations

The clinical trials described in chapter 5 were mainly performed with the following preparations:

1. Essentiale™ capsules:
   1 capsule contains:
   - 175 mg of EPL (76 % 3-sn-PC)
   - 3 mg of thiamine nitrate (vit. B1)
   - 3 mg of riboflavin (vit. B2)
   - 3 mg of pyridoxine-HCl (vit. B6)
   - 3 ug of cyanocobalamin (vit. B12)
   - 3.3 mg of -tocopherol acetate (vit. E)
   - 15 mg of nicotinamide

2. Essentiale™ forte capsules:
   1 capsule contains:
   - 300 mg of EPL (76 % 3-sn-PC)
   - 6 mg of thiamine nitrate (vit. B1)
   - 6 mg of riboflavin (vit. B2)
   - 6 mg of pyridoxine-HCl (vit. B6)
   - 6 ug of cyanocobalamin (vit. B12)
   - 6 mg of -tocopherol acetate (vit. E)
   - 30 mg of nicotinamide

3. EPL capsules:
   1 capsule contains:
   - 250 mg of EPL (76 % 3-sn-PC)
   [used only in Japan]

4. Essaheoan™ capsules:
   1 capsule contains:
   - 450 mg of EPL (94 % 3-sn-PC)

5. Essentiale™ for i.v. injection
   1 ampoule of 5 ml contains:
   - 250 mg of EPL (94 % 3-sn-PC)
   - 2.5 mg of pyridoxine-HCl (vit. B6)
   - 10 ug of cyanocobalamin (vit. B12)
   - 1.5 mg of sodium-D-pantothenate
   - 25 mg of nicotinamide

6. Essentiale™ for infusion
   1 ampoule of 10 ml contains:
   - 1000 mg of EPL (94 % 3-sn-PC)
   - 5 mg ot pyridoxine-HCl (vit. B6)
   - 15 ug of cyanocobalamin (vit. B12)
   - 3 mg of sodium-D-pantothenate
   - 100 mg of nicotinamide
   (The Essentiale™ preparations are also available without vitamins)

7. Lipostabil™ capsules:
   1 capsule contains:
   - 175 mg of EPL (76 % 3-sn-PC)
   - 1.5 mg of pyridoxine-HCl (vit. B6)
   - 1.65 mg of -tocopherol acetate (vit. E)
   (The preparation is in some countries also available with 30 mg of ethophylline.)

8. Lipostabil™ forte capsules:
   1 capsule contains:
   - 300 mg of EPL (76 % 3-sn-PC)
   (The preparation is in some countries also available with 50 mg of ethophylline.)

9. Lipostabil™ for i.v. injection
   1 ampoule of 5 ml contains:
   - 250 mg of EPL (94 % 3-sn-PC)
   1 ampoule of 10 ml contains:
2.4 Pharmacokinetics

The phospholipids reaching the organism by the way of EPL differ from endogenous phosphatidylcholines by their fatty acid pattern. 1,2-dilinoleoylphosphatidylcholine, the main active ingredient, is usually not present in the body. Therefore, in pharmacokinetic investigations radioactively labelled 1,2-dilinoleoylphosphatidylcholine was used, which can be obtained by synthetic or semisynthetic ways. In order to find an answer to the questions of distribution and excretion, different isotopes (32P, 3H, 14C) and sometimes multiple labels, at various molecular components were applied.

2.4.1 Absorption in Animal Experiments

The absorption rate determined by D. LeKim and E. Graf (405) following oral administration of radiolabelled EPL to rats was higher than 90 % (fig. 9). This confirms the results of J. M. Fox et al. (196) who found that EPL is almost completely taken up from the intestinal lumen within 24 hours. S. Parthasarathy et al. (537) also obtained concordant results. Almost 100 % of the administered dose of EPL is hydrolysed to 1-acyl- lysophosphatidylcholine during absorption (fig. 10). Of this, about 50 % is reacylated to intact EPL in the intestinal mucosa (405).

2.4.2 Distribution in Animal Experiments

EPL enters the liver via the lymph and the blood pathways. In the liver, it is partly taken up by cell membranes and subcellular membrane fractions. In the blood, phospholipid fractions of the lipoproteins exchange with EPL, the latter being taken up preferentially by the high-density lipoproteins (HDL) (403, 404, 584, 680, 764, 766-768). Studies in rats and mice given EPL i.v. showed that approximately 80 % of the injected dose was eliminated from plasma after 15 minutes, approximately 92 % after 75 minutes and approximately 100 % after 10 hours (282, 283).

After a single oral dose (350mg/kg b.w.) of 3H/14C-labelled EPL in rats, maximum concentration of radioactivity in the liver reached 24.5% after 24 hours of application and slowly declined to 4% within 8 days. Significant quantities of radioactivity were found in the striped muscles: 5.8% of the dose after the first 6 hours, an increase of up to 25% being observed within the first 8 days of dosing. Lower amounts of radioactivity were distributed in the kidneys, the lung and the myocard: 2.7%, 0.9% and 0.3% resp. of the administered dose within 24 hours. Following single oral administration of labelled EPL, radioactivity
was detected in the plasma only for 48 hours. After 96 hours approx. 22%, and after 8 days up to 54% were absorbed from the cells (105).

After a 5-day repeated oral administration of 3H/14C-labelled EPL to rats, significant concentrations of the 14C-radiolabel were detected in the liver (18.6 %), the depot fat (7.0 %), the striped muscles (31.4 %) and the bones (5.6 %). Lower concentrations of C-radioactivity were located in the lungs, kidneys, testes, gastrointestinal tract, blood and plasma. Six hours after the 5th administration of labelled EPL 22.9 % of the dose were accumulated in the liver. This value declined to 2.3 % after 16 hours. 14C-radioactivity in the striped muscles rose to 36.9 % within 4-day. Little radioactivity was measured in the bones and the depot fat: a maximum of 4.7 % and 5.6 % resp. of the dose 24 hours after the 5th application. Kidneys and adrenals presented 1.3 % and 1.7 % resp. of 14C after 6 hours. The 14C-radiolabel in the blood showed a fairly homogeneous distribution in blood cells and plasma throughout the 4-day dosing.

Eight days after the 5th dosing up to 65 % of the total radioactivity were concentrated in the blood. After another 8 days already 76 % of the 14C-radiolabel was detected in the cells (105).

Similar results were also obtained in rhesus monkeys after single and repeated dosing of 3H/14C-labelled EPL: 6 hours after the last repeated administration of the radiolabel 11 % of the EPL dose was detected in the liver, 7 % in the depot fat and 16-20 % in the striped muscles. After 17 days the values in the liver and depot fat declined to 1.78 and 4.5 % resp. to the applied dose, whereas the concentration in the striped muscles remained fairly constant. Six hours after the last dosing, the 14C-radiolabel in blood was mainly located in the plasma: 74-75 % of the dose. After 17 days the values declined to 46 and 28% resp. (282).

Whole-body autoradiographs taken after oral administration of 1,2- 3H-dilinoleoyl-3-sn-phosphatidyl- 14C-choline to Wistar rats, showed principally the following results (105, 406): 6 hours after a single dose of labelled EPL, radioactivity was mainly located in the liver, kidneys and the intestinal mucosa. Low concentrations were found in the secretory glands, the lymph nodes and the gut contents. 12 hours after application the distribution of radioactivity was somewhat more homogeneous, major concentrations, however, still being present in the liver, kidneys and the intestinal mucosa. A similar distribution was detected after 24 hours, low activity still being found in addition in the bone marrow, lungs, spleen, epidermis, testes and the secretory glands. 48 hours after application of the labelled EPL the substance was completely distributed, major concentrations being located in the liver and the gastrointestinal tract. In addition, considerable amounts were still found in the kidneys, epidermis, testes and the secretory glands (salivary, thymus and thyroid glands). There were signs of a general distribution in the striped muscles. After 96 hours the concentrations of radioactivity had sharply declined without specifically accumulating in one of the mentioned organs. Only low amounts of radioactivity, distributed homogeneously, could be detected after 8 days. Slight local accumulations were found in the intestinal mucosa, epidermis, testes and the seminal vesicles. A similar distribution pattern was described after repeated EPL application, low concentrations still being present additionally in the brain, spinal marrow and in the striped muscles.

To put the available results of investigations on the uptake of the intact molecule into the brain into more concrete terms it can be said that less than 1 % of an orally (196) or intravenously (407) applied dose is found in the brain. The autoradiographs show no special affinity for neurons (196, 406).

Analytic examinations of the incorporated EPL radiolabel by means of cell fractionation of the liver homogenate after 6 hours of oral application of 3H/14C-EPL clearly showed that the radioactivity was located to a quantitatively high degree in the membrane-containing fractions, the distribution being ubiquitous in all liver cell fractions (405). This result is in accordance with former investigations on i.v. application of EPL (403).
Hydrolysis by phospholipase A2 showed that 6 hours after application of the radiolabel, the phosphatidylcholine isolated from the liver still contained 96% of the labelled fatty acids in the 1-position, whereas there were only 4% in the 2-position. Moreover, the determination of the expired CO₂ after oral application of differently labelled 14C/3H-dilinoleoyl-phosphatidylcholines showed that a high concentration of the 14C-radioactivity was present only when the fatty acid in the 2-position of the phosphatidylcholine molecule was labelled with 14C (403).

Renal excretion after a single dose in the first 5 days is 17.4% of the administered dose in rats and 17.7% in rhesus monkeys (105, 106). After a single dose about 15% of the radioactivity is expired in rats and rhesus monkeys with the breathing air (105), a finding which may be attributed to degradation of fatty acids during absorption of phosphatidylcholine (516). As the excretion in the feces is low with 3-8% of the dose in the first 5-7 days in rats (405), a considerable part of the EPL must be subjected to a vast enterohepatic circulation.

2.4.3 Pharmacokinetics in Man

The results of pharmacokinetic studies in man using 3H/14C-labelled EPL agree largely with the data derived from animal experiments. Here again, the rate of absorption was found to be higher than 90%.

In man the maximum concentration in blood reached 6 hours after oral administration of the 14C-labelled substance was about 20% of the administered dose and thus approximately four times that measured in rats and monkeys (fig. 12).

Examination of lipoproteins revealed that the specific activity of polyenylphosphatidylcholine in HDL was 2 to 6 times higher than in apo-B-containing lipoproteins, and up to 20 times that of red blood cells or total blood. Thus, in man, EPL is also incorporated preferentially into the HDL fraction. According to Zierenberg et al. (766, 768) "essential" phospholipids are exchanged for phospholipids of membranes and lipoproteins.

After intravenous administration and a quick initial decrease in total plasma lipids (redistribution into other lipid fractions in plasma), the concentrations decreased slowly with an elimination half-life of 59 days (728) indicating largely catabolic reactions. The pharmacokinetic studies in animals and in man show that upon parenteral administration of EPL the radioactivity may be detected practically in the liver and there especially in the membranes. After oral application it amounts to about 50% of the applied compound. EPL is incorporated in toto.

2.5 Toxicology

The following gives a short survey of the most significant studies on the toxicology and teratology of EPL after mainly oral or intravenous application.

2.5.1 Acute Toxicity

Acute toxicity in single administration of EPL has been tested in the mouse, the rat and the rabbit in oral (p.o.), subcutaneous (s.c.), intravenous (i.v.) and intraperitoneal (i.p.) applications (199, 209). The following maximum doses were used:

For reasons of volume, obviously, no higher doses could be applied. As no animal died within the 7-day follow-up period, it can be affirmed that the highest tolerable dose, as used in the mentioned species and application forms, corresponds to the given doses or is even higher. From the clinical viewpoint,
only sporadically slight symptoms were observed, mostly none at all. Only in subcutaneous application, the section showed sporadically rests of substance at the site of application.

Under the afore-mentioned trial conditions, EPL has to be judged as practically non-toxic for the mouse, the rat and the rabbit.

In acute toxicity studies with LipostabilR or EssentialeR 5 ml ampoules for intravenous application (1 ml contains 50 mg of EPL and 23 mg of deoxycholic acid; (506)) the LD50 was found to be as follows:

2.5.2 Toxicity in Repeated Administration

Neither in subchronic nor in chronic toxicity tests were observed any reactions of intolerance when daily oral doses of up to 3,750 mg/kg b.w. were applied to rats, or up to 2,500 mg/kg b.w. to dogs:

In investigations with the Lipostabil and Essentiale ampoules on rabbits and dogs after 4-week application the "no-effect" dose was found to be 1.2 ml or 0.6 ml/kg b.w. for the dog and 0.6 ml/kg b.w. for the rabbit (667-669, 672).

13 weeks of application in dogs showed a "no-effect" dose between 0.3 and 0.9 ml/kg b.w. for local tolerance and more than 0.9 ml/kg b.w. for systemic compatibility (431, 433). The topic and systemic tolerance in rhesus monkeys ranged from 0.3 to 0.6 ml/kg b.w. (432).

2.5.3 Teratogenicity and Embryotoxicity of EPL

No toxic effects of EPL on pregnant rats or fetuses were observed either when doses of up to 1,000 mg/kg b.w. were administered to rats or up to 500 mg/kg b.w. to rabbits.

Similar results were found when using Essentiale or Lipostabil ampoules in doses of up to 1.2 ml/kg b.w. in rats or rabbits (411, 435, 671, 673).

2.5.4 Perinatal and Postnatal Toxicity, Fertility and Reproductive Performance

Perinatal and postnatal development of rats is not influenced by oral EPL doses of up to 3,750 mg/kg b.w./day. When the substance was applied until the end of the third lactation week all parameters were found to be within the normal histological range (210).

Male rats received EPL 10 weeks and female animals 2 weeks before the beginning of the test. Oral doses of 150, 750 and 3,750 mg/kg b.w. were applied a day (211). Even with the highest dose no effect of EPL on male and female fertility nor on the reproductive performance was observed. An increase in dominant lethals could not be found.

The Essentiale and Lipostabil ampoules neither influenced the fertility nor the reproductive performance of the rats (413, 434).

2.5.5 Mutagenic and Carcinogenic Potential

In-vitro investigations with Salmonella and yeast strains and with a human cell line, and in-vivo test systems such as the urinary assay (intraperitoneal) and the host-mediated assay (subcutaneous) did not yield any mutagenic potential (215).

According to a statement of the German Cancer Research Centre, Heidelberg, EPL does not belong to a class of substances suspected of carcinogenicity (626). Also in the analysis of the FDA Report on lecithins (176, 177) it is explicitly said that there is "no significant incidence of tumor formation" and "no evidence of carcinogenicity". The GRAS-Report (Generally Recognized As Safe) ordered by the FDA even refers to findings according to which carcinogen-induced tumours in mice are rather inhibited by simultaneous lecithin administration (234). As a consequence of these results, studies on carcinogenicity have not been performed.
2.6 Safety Pharmacology and Pharmacodynamic Effects of EPL

In 1976 an extensive publication appeared on the pharmacology, particularly on general safety pharmacology, of the hitherto obtained experimental results (729).

The pharmacological properties of EPL were tested in a series of trials into rodents. The animals were kept under standard conditions. They were fed an appropriate pelleted food according to the species. In most cases EPL was given in the form of a max. 10% suspension in aqua dest., freshly prepared every day, at doses of 10 to 5000 mg/kg by the oral route. The volume was usually 10 ml/kg b.w., in exceptional cases up to 50 ml/kg.

In-vitro and ex-vivo trials were carried out to study the influence of EPL on heart function of guinea-pigs, and on the haemolytic index in sheep and human erythrocytes.

In table 10 are summarized the results.

In these general screening tests was demonstrated that EPL, even at high doses, has no effects on the central or peripheral nervous system. Neither analgetic, spasmolytic or spasm-influencing effects, nor any effects inhibiting coordination or reflexes were found. Neither were observed antiphlogistic nor locally anaesthetising effects. No influences on renal function or on heart function or circulation were seen, no sympatholytic effects were reported. Only extremely high doses produced an inhibition of capillary permeability. With lower doses, in contrast, was observed a marked dose-related broncholytic effect of EPL in histamine asthma of the guinea-pig. In several species a slightly choleretic action was seen. Reduction of free and total cholesterol, and of the total lipid and triglyceride levels was found.

In the general pharmacological screening no toxic effects of the EPL substance were observed.

Tab. 10: Survey of results of safety pharmacology and pharmacodynamic effects in different standard models with single or short term administration of EPL
3 MODE OF ACTION OF EPL

As already mentioned in chapter 2.1 "essential" phospholipids supply the organism with (3-sn-phosphatidyl) choline molecules with a very high standardized content of polyunsaturated fatty acids, in particular linoleic acid. Approximately 15 kg of soya beans are required to obtain a daily dose of 1.8g EPL.

3.1 EPL as Structural Elements for Formation and Regeneration of Biological Membranes

First of all, like phospholipids in general (see chapter 1), the "essential" phospholipids are high-energy basic structural elements of all biological membranes such as of cells, lipoproteins and the surfactant. Labelled material has been found in all the mono- and bilayer-membranes of the organism, predominantly in the monolayer of the lipoproteins (217, 283, 680, 764, 767, 524, 584, 765), in the bilayer of erythrocytes and hepatocytes (217, 403, 404, 767), in the spleen, muscle, lungs (404, 405) and in the gastric mucosa (442). The distribution of radioactivity in the organs of normal rats and 0-galactosamine-treated rats, 6 hours following i.v. administration of labelled EPL, is shown in table 11 (404):

The application and incorporation of one phosphatidylcholine molecule of the "essential" phospholipids spares the body to supply the energy quantity of 5.600 cal/Mol required for the cellular biosynthesis of phosphatidylcholine (391).

A stimulation of membrane formation and regeneration can also be seen on the biochemical and histological level, for example in a reduction of cellular enzymes in blood and of membrane deformation, and in an increased protein and membrane synthesis (37, 131, 280, 304, 361, 597, 695, 696, 754).

3.2 Membrane Fluidity and EPL

The main active ingredient in EPL is 1,2-dilinoleoylphosphatidylcholine, which is present to about 52 % of the applied mixture of phosphatidylcholine molecules (128). 1,2-dilinoleoylphosphatidylcholine is not physiologically present in the human body. Endogenous phospholipids are substituted by "essential" phospholipids, especially by the 1,2-dilinoleoylphosphatidylcholine, which are incorporated in all membrane-containing fractions (404, 405). This means that the phosphatidylcholine molecules in the membranes with a saturated fatty acid at position 1 (718) are partly exchanged against those with a linoleic acid or a linolenic acid at this position; additionally, the amount of phosphatidylcholine molecules with a linoleic acid at position 2 is increased (524). In total the number of double-bonds in the group of phosphatidylcholine molecules in the membrane increases.

One of the important consequences of such a substitution of body-own phospholipids by these highly unsaturated phosphatidylcholines is a change of membrane fluidity.

The influence of EPL on membrane fluidity has been investigated and is continuously observed by measuring the
- cholesterol/phospholipid ratio (68, 311, 344, 466, 599),
- Arrhenius plots of enzymatic reactions (257, 353),
- regulation / stimulation of membrane-bound enzyme activities (49, 64, 92, 145, 285, 291-293, 295, 322, 384, 450, 539, 541, 584, 618, 644, 706, 730)
- and of receptor properties (515),
- influence on membrane permeability (46, 92, 705)
- decrease/normalization of aggregation of erythrocytes and platelets (46, 47, 68, 74, 110, 170, 345, 483, 520, 642, 705)
- and by Immunomodulation (36, 251, 513, 651, 750).
There is no doubt nowadays that EPL with its main active ingredient 1,2-dilinoleylphosphatidylcholine maintains or promotes many membrane functions of different organs and tissues by influencing membrane fluidity.

3.3 EPL and the Activity of Membrane-Bound Enzyme Systems

Two groups of enzymes which can be stimulated with EPL, will be mentioned as examples: detoxifying enzymes and enzymes relevant for the lipid metabolism.

3.3.1 EPL and Detoxifying Enzymes

Changes in lipid composition in membranes of aging rats lead to a decrease in fluidity (661, 684).

W. Klinger et al. (354) studied enzymes for the biotransformation of lipid-soluble xenobiotics in aging rats. The microsomal electron transport chain slowed down with the consequence of lower activities of cytochrome P 450-dependent monoxygenases and also lower conjugation activities. However, EPL reincreased the ethylmorphin N-demethylation and ethoxycoumarin O-deethylation activities. The Arrhenius plots for the monoxygenase reactions indicated a higher fluidity of the microsomal membranes. UDP-glucuronosyl-transferase activities were also enhanced. Glutathion concentration was increased as well as H2O2 production, and lipid peroxide concentration lowered.

G. Benzi et al. observed in two experimental studies with old rats that EPL (49, 50)
- increases the activity of the anti-oxidative enzymes: cytoplasmic Cu-Zn-superoxide dismutase and glutathion reductase in some brain areas and,
- increases the glutathion redox index of the forebrain.

Reoxygenation after experimental hypoxia induced a decrease of activity of the superoxide dismutase in the myocard of rats while the activity remained increased when EPL was applied in parallel (246).

3.3.2 EPL and Enzymes of the Lipid Metabolism

The influence of "essential" phospholipids on the essential enzymes of fat metabolism was studied after oral and intravenous applications as well as after in-vitro incubation. C. Desreumaux et al. (145) obtained lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) from adipose tissue, myocard, lungs or the liver of rats and assessed enzyme activity after in-vitro incubation with either dipalmitoylphosphatidylcholine, egg-lecithin or EPL (tab. 12). The highest increase in activity was achieved with EPL. Similarly V. Blaton et al. (64) observed a rise in the activity of milk lipoprotein lipase with EPL.

Several authors report an increase in the activity of cholesterol-esterase while the activity of acyl-CoA:cholesterol-acyltransferase (ACAT) (291, 292, 539, 541) was found to decrease after 2 to 6 months of oral or intravenous application of EPL. In addition, authors (155, 219, 285, 322, 344, 584, 644, 706) observed that EPL is a substrate for the lecithin:cholesterol- acyltransferase (LCAT) (supply of linoleic acid) and that it increases its activity.

The significance of these changes in the enzyme activities is that
- HTGL and LPL accelerate the breakdown of lipoproteins rich in triglycerides, and the reduction of serum lipoproteins;
- the decrease of the ACAT activity reduces the deposition of cholesterol esters in the cells;
- on the contrary, the cholesterol-esterase hydrolyzes cholesterol-esters in the cells, so that the free cholesterol can be transported to the blood and bound to the surface of HDL;
- LCAT transforms this mobilized free cholesterol to cholesterol esters, mainly cholesterol linoleate, for the uptake into the HDL particles.

3.4 Effects of EPL on Lipoproteins

O. Zierenberg and his group (764, 765) carried out comprehensive studies on the EPL pattern in lipoproteins after oral or intravenous administration to rats, rabbits and dogs. Depending on the individual species 50 to 80% of the activity administered was traced to HDL and about 20% to LDL and VLDL after intravenous administrations of radioactively labelled EPL. After oral administration 36 to 66% of the activity was traced to the HDL. EPL-enriched HDL showed an increased capacity for the uptake of cholesterol from LDL and peripheral tissue and its transport to the liver (68, 244, 344, 515, 650, 765-767) (fig. 13), the so-called reverse cholesterol transport.

3.5 Effects of EPL on Hemorrheology

Beside the more and more clear evidence that EPL influences the platelet and erythrocyte aggregation it improves the flexibility of the erythrocytes, especially by decreasing the cholesterol/phospholipid ratio in the membrane. This effect ameliorates the lifetime of these corpuscles, their functioning and the blood flow properties such as blood viscosity and microcirculation (63, 167, 311, 483, 597, 599, 641, 759).

3.6 EPL for Drug Transport and HDL Simulation

If solid phospholipids are treated with excess of water and detergents, for example with cholic acids we get the mixed micelles which are contained in the ampoules of Lipostabil and Essentiale. On the other hand, an EPL product with small (mainly) unilamellar liposomes is under experimental and clinical evaluation (390, 740).

Beside delivering polyunsaturated phosphatidylcholine molecules these particles can serve for intravenous and the latter one additionally for percutaneous drug transport (26, 222, 313, 740). E.g. C. Werner and A. Wendel (740) found in mice a liver protection of EPL liposomes against paracetamol intoxication only when these particles contained vitamin E. Intravenously applied EPL seems to be an excellent carrier for vitamin E to the liver.

Additionally, after injection (3-sn-phosphatidyl) choline in the micellar/liposomal form may simulate, to a certain extent, the transport function of HDL by absorption of apoprotein A-I and cholesterol (359, 662, 706, 744, 745).

3.7 EPL, Carrier of Polyunsaturated Fatty Acid and of Choline

Due to its high amount of polyunsaturated fatty acids (especially linoleic acid and linolenic acid in the phosphatidylcholine molecules (404)) EPL is a splendid supplier of eicosanoid precursors (318, 443, 484, 520, 755). Due to the substitution of body-own phospholipids in the membrane against EPL, the pool of these precursors is increased, which the organism uses on demand (486). A.o. cytoprotection can be favoured (443) and, as already described, platelet aggregation decreased.

Additionally, EPL influences certain neurological processes (175, 455, 486, 609). Beside being a membrane constituent, important for neuron structure, neurotransmission, transduction of biological signals and for metabolic processes in neurons/glia cells, its main function seems to be to act as a choline donator (455).

3.8. Influence of EPL on Lipid Peroxidation
Lipid peroxidation is one of the earliest measurable events of damage on the molecular level (389). Growing importance has recently been attributed to the part played by lipid peroxides in cell membranes and lipoproteins, in the development and progression of atherogenic lesions in the vascular wall, in liver diseases, in acute pneumonia etc. Therefore, it is very interesting to see, that EPL decreases parameters of peroxidation reaction such as of acyl hydroperoxides, of Schif’s bases, chemiluminescence, the diene/triene conjugates as well as malondialdehyde and the intensity of peroxidation-triggered hemolysis (46, 223, 242, 246, 333, 338, 363a, 394, 469, 571, 579, 609, 623, 639, 642, 653-655, 690, 717, 721, 722, 763).

As mode of actions are especially discussed a carrier-effect of EPL for vitamin E (740) or a direct influence on e.g. the peroxisomal membrane (471).

3.9 EPL as Fat Solubilizer

Beside some studies on the resorption of fat-soluble vitamins (363b), on lipid solubilization/resorption in the gastrointestinal tract (600) and on the stabilization of bile/influence on the lithogenic index (277, 598, 601, 655, 679, 704) the main focus on research in this field was and is on prophylaxis and therapy of tat embolism. As Lipostabil, intravenously applied, has, in this disease the character of a life-saving drug, its action has been intensively investigated. In particular its specific properties as a physiological surface-active emulsifier and stabilizer of blood lipids are essential criteria for the effect on lipid particles in human blood and the reduction of mortality in man (75, 87, 290, 298, 374, 375, 377, 379, 398, 400, 553, 627, 737):
- plasma fats are emulsified and stabilized,
- enzymatic degeneration of fats is increased,
- the number and size of fatty particles reduced in blood (and urine)
- fatty deposits in lungs and renal vessels decreased,
- blood flow properties improved and the risk of thrombus formation lowered, and
- microcirculation, especially in capillaries improved.

Figure 14a shows the possible influence of EPL on different indications in which these disorders/diseases are membrane-associated. Figure 14b summarizes the modes of action of EPL and their relation to each other. These actions are based on the assertion that EPL is a membrane therapeutic. The following chapters review the most relevant studies more in detail.
4. PHARMACOLOGICAL INVESTIGATIONS WITH EPL IN DIFFERENT DISEASE MODELS

4.1 Effect in Liver and Kidneys

4.1.1 Toxic Liver Damage

The suggested hepataprotective and hepatotherapeutic action of EPL could be corroborated in 7 in-vitro and in 55 in-vivo experiments. The results obtained from the in-vitro studies are summarized in table 13.

<%Tab. 13: In-vitro Studies with EPL%>

1) In mitochondria suspension, EPL prevents the suppression of cell respiration caused by snake venom (E.Petrushka et al., 1959) (556);
2) EPL prevents the loss of activity of microsomal glucose-6- phosphatase due to intoxication with CCI4 and allyl alcohol (S.Fujii et al. 1974) (214);
3) EPL protects liver cell membranes against the toxic influences of chenodeoxycholic acid (S.Sakisaka et al., 1984) (594);
4) EPL increases the survival rate of hepatocytes as compared with an endotoxin and simultaneously raises the rate of incorporation of L-leucine (C.Kodama et al., 1988) (358);
5,6) EPL pretreatment in rats protects the hepatocytes against lipid peroxidation by FeSO4 as pro-oxidant (A.Martelli et al. 1989) (469). Similar results had been found by L.Guiliano et al. (242) with even a positive influence on the survival rate.
7) EPL leads to a dose-related inhibition of collagen synthesis in human fibroblasts (A.Casini et al., 1992) (102).

In the presently available 55 in-vivo investigations, in which 20 different models in 5 different animal species were used, mostly such toxic substances were employed which are known to play a role in the origin of liver damage: CCI4, ethanol, galactosamine, acetaminophen, tetracycline, organic solvents, carbon disulphide, thioacetamide, indomethacin and others (tab. 14).

<%Tab. 14: Tabular survey on the liver models used%>

Effect in Intoxication Induced by CCI4.

Since the hepatotoxic effect of carbon tetrachloride was first described in 1923, this substance has often been applied in experiments and is considered as one of the best examined models. The damages are attributable to the formation of radicals and to lipid peroxidation. A single administration in mice and rats already produces membrane damage, centrolobular necrosis and fatty degeneration of the liver. Also the alterations in phospholipid exchange processes have been thoroughly examined, in particular with regard to microsomes and mitochondria (118, 593, 749)

The findings of the EPL effect from controlled studies demonstrate that the damages induced in the animals can be reduced by intravenous, intraperitoneal or oral administration of EPL at doses between 25 and 3,300mg/kg body weight. The assessment was made on the basis of reduced serum values of transaminases (ALT, AST), LDH and AP, the reduction of lipid values and peroxide formation, as well as on the basis of normalized protein synthesis in liver cells. The findings convey the impression as if it was first of all the mitochondrial (and microsomal) membrane which is protected against CCL4 destruction by the protective oral administration of EPL.

After chronic CCI4 intoxication for 9 months, rats showed severe cirrhotic alterations of the liver. The animal group which in addition to CCI4 was given i.m., i.p. or oral EPL at doses between 100 and 300 mg/kg b.w. during the last 3 months, in contrast, presented markedly less damages: histology and electron micrographs showed in all cases a reduction of hyperplastic connective tissue, fibrosis, formation of pseudo-acinus and biliary pseudocanaliculi; the content of liver hydroxyproline was reduced and the collagen/DNA ratio was normalized; on the biochemical level, the transaminase activities minished; mortality was markedly lower than in the controls.
The simultaneous EPL treatment produced a clear reduction of liver lipids and fatty degeneration of liver cells in rats exposed to chronic carbon tetrachloride intoxication and simultaneous feeding of alcohol.

Effect in Intoxication Induced by Ethanol:
The administration of toxic doses of ethanol in the acute trial produces increased lipid values in serum and liver cells of rats (as well as of most other laboratory animals). In chronic ethanol intoxication electron microscopic pictures show clear alterations in mitochondria, endoplasmic reticulum and microvilli. Disorders of the phospholipid metabolism in cellular and subcellular membranes are at the origin of all alcohol-induced alterations.

In acute ethanol intoxication, prophylactic administration of EPL (7 mg/kg b.w. i.p., 145/265 mg/kg b.w. s.c. and 10 mg/kg b.w. i.m.) could prevent the expected rise in serum and liver cell lipids. Normalization of membrane structures in the endoplasmic reticulum and mitochondria was evidenced by histology.

Similar results were obtained in models with subacute (several days) or chronic (up to a maximum of 150 days) ethanol feeding, in which doses of 7 to 450 mg EPL/kg b.w. (i.p., oral) were applied in addition to ethanol. Beside the aforementioned influences on lipid values, normalization of the reduced protein synthesis and of the alcohol dehydrogenase activity was observed and, by electron microscopy, normalization also of the ultrastructural damages.

Very promising are the results of Ch.S.Lieber et al. (449): polyunsaturated phospholipids significantly reduced ethanol-induced fibrogenesis in baboons (fig. 15). They did not progress beyond the stage of perivenular fibrosis and had a significantly lesser activation of lipocytes to transitional cells. One reason might be the observed increase of collagenase activity. The withdrawal of the phospholipids accelerated the process of fibrosis to finally cirrhosis.

Chronic ethanol feeding in combination with toxic allyl isothiocyanate leads to pronounced fatty liver in the animals. This effect could be prevented in the group receiving EPL (7 mg/kg b.w., i.p.) simultaneously. The problem of particularities according to the animal species becomes obvious in examinations with minipigs. In these animals, no sure reproducible alterations of serum lipids or increased transaminases were found after 10-day ethanol feeding. It appears that minipigs are not appropriate for such trials.

Effect in Intoxication Induced by Triton:
Due to the action of triton, laboratory animals exhibit dose-dependent fatty degeneration of liver cells. This triton-induced damage, which is well known from experiments and which can be aggravated by simultaneous alcohol feeding, was largely prevented by EPL administration. At the same time, reduction of serum lipids was found.

Effect in Intoxication Induced by Galactosamine:
D-galactosamine was first described as hepatotoxic by D.Keppler in 1968 (339). Until today it is considered as a most interesting substance for the production of liver damage because the resulting pathological picture resembles most to viral hepatitis in man (even if fatty infiltrations in liver cells are present as in hepatitis NANB). Acute exposure leads to vast liver cell necrosis; long-term application provokes cirrhosis of the liver.

In controlled trials with rats, EPL proved to have a measurable positive influence on histological and biochemical parameters as compared to controls, both in prophylactic and therapeutic treatment: reduced liver cell necrosis, reduction of vacuolar dystrophy, of fatty degeneration of liver cells, of periportal inflammation, angioneerosis and labilization of lysosomes; increase in RNA and glycogen storage was found; laboratory findings showed only a slight rise in transaminases, catalase and peroxidase; radioisotopic examination showed an improved protein synthesis.
According to A.S. Saratikov (618) EPL stimulates D-galactosamine-suppressed antitoxic liver functions: it increases the contents of RNA, cytochrome P-450 and b~
the activity of amidopyrine-D-demethylase; hydroxylases of hexobarbital and aniline, improves the activity of the respiratory chain of microsomes and counteracts inactivation of cytochrome P-450 into cytochrome P-420. EPL also activates conjugation of xenobiotics with reduced glutathione.

All trials showed that the most effective and reliable results were achieved when EPL had been given prophylactically, i.e. several days before intoxication.

Effect in Intoxication Induced by Allyl Alcohol:
In cytosol, allyl alcohol produces lipid peroxidation with ensuing liver cell necrosis and the corresponding transaminases activity, through its metabolite acrolein.

Prophylactic and curative administration of EPL (100-1,330 mg/kg b.w., i.v. or i.p.) inhibited in all cases an increase in transaminases, reduction in the glutathione content in the liver and, partly or completely, lipid peroxidation.

Effect in Intoxication Induced by Ethionine:
Ethionine has a cytotoxic effect and in laboratory animals it provokes fatty degeneration of liver cells, sometimes liver cell necrosis, and in long-term application cirrhosis of the liver. Also these experimental liver cell damages are attributable to the frequently evidenced disorders of the phospholipid metabolism in the biological membranes due to ethionine application.

With a 14-day EPL treatment in addition to the toxin, liver cell necrosis and fatty degeneration of liver cells was prevented in rats. As a sign of the activated metabolism enzyme activity of lipases, phosphatases and succinic dehydrogenase increased.

Effect in Intoxication Induced by Thioacetamide:
Under long-term exposure to thioacetamide laboratory animals develop centrolobular necrosis and fatty degeneration of liver cells, which finally may lead to cirrhosis.

In a long-term trial for 102 days, rats received i.p. EPL at a dose of 1000 mg/kg b.w., but only 3 times a week. Even this low dose could avoid fatty degeneration of liver cells and normalize the amino acid spectrum in serum. The development of liver necrosis and cirrhosis, however, was not influenced.

Effect in Intoxication Induced by Organic Solvents:
Rats exposed for 6 months to a mixture of cresol, benzol, xylene, toluene disocyanate and benzene in a toxicologic chamber developed fatty degeneration of the liver and displayed decreased liver cell glycogen. Simultaneous administration of EPL led to a clear reduction or disappearance of these liver cell alterations. At the same time, improved cholinesterase synthesis in the liver was observed.

Effect in Intoxication Induced by Paracetamol or by Indomethacin:
Paracetamol intoxication, known in man, can be reproduced in the mouse. Centrolobular necrosis develops. On the biochemical level, Increase of lipid peroxidation and transaminases activity as well as reduced liver glutathione are reported.

The prophylactic i.v. administration of EPL entailed a dosa4ependent correction of the affected parameters and prevented mortality.

In acute indomethacin-induced liver damage the simultaneous application of EPL (Essentiale) led to improvements such as normalized metabolic function of the liver, reduced membrane damage, reduced lipid peroxidation, and rise of the reduced glutathione content.

Effect in Intoxication Induced by Tetracyclines or by Rifampicin:
As compared to the control animals, tetracycline-induced rise of serum enzyme activity (ALT, AST) and suppression of cholepoiesis were inhibited with EPL; moreover, reduced lipid peroxidation and increased glycogen storage in the liver were found.
Similar as in man, animals present an increase in transaminases, bilirubin and the enzymes indicating cholestasis after repeated rifampicin administration. With the concurrent administration of EPL during the whole trial period transaminases activity, protein synthesis and DNA and RNA synthesis were largely normalized in the control group. The isolated hepatocytes were completely normalized and corresponded to those of untreated rat hepatocytes.

Effect in Intoxication Induced by Carbon Disulphide:

The inhalation of carbon disulphide leads to pronounced fatty degeneration of the liver both in laboratory animals and in workers of the artificial fibre industry. In a study for a period of 36 weeks, the parallel administration of EPL reduced histological alterations, transaminases and mortality. In another trial phase fatty degeneration of liver cells was observed only sporadically in the EPL-treated animals.

Effect in Intoxication Induced by Anaesthetics:

In a controlled study, mice were anaesthetized with solvent ether, trichloroethane or halothane: the control group pretreated with EPL showed clearly shorter times of anaesthesia, waking-up and recovery.

In a further study, secobarbital-induced reduction of the cytochrome P-450 activity was determined in 2 control groups: with the simultaneous administration of EPL there was only a slight reduction of the cytochrome P-450 activity.

Effect in Intoxication Induced by Cholic Acid:

Rats fed with a diet rich in cholic acid develop fatty degeneration of liver cells with periportal inflammation and liver cell necrosis. These symptoms usually lead to cirrhosis within 12 weeks. A control group received EPL in their drinking water. In the 26th week only fatty liver was found, whereas all untreated animals of the diet group presented cirrhosis.

Effect in Intoxication Induced by Cholestasis:

Bile salts remove phospholipids almost completely from hepatocyte membranes, a sufficient "repair-mechanism" with a high capacity of phospholipid synthesis guaranteeing intact membrane conditions. Here, a phosphatidyl transfer protein seems to play a decisive role (K.W.A. Wirtz; 748). It has been demonstrated that monohydroxy bile acids provoke cholestasis by a direct toxic effect on the canalicular membranes and the associated actin-containing microfilaments, which entails increased membrane permeability in the bile (A.L.Baker et al. 1976; 33). By means of bile duct ligature, considerable cholestasis with morphological damage to hepatocytes and bile capillaries can be obtained.

In an acute experiment with rats and in a subacute trial with dogs (2-weeks ligature) therapeutic EPL administration (400 mg/kg b.w. s.c. or 40 mg/kg b.w. resp., orally) proved to clearly reduce the damage of the measured parameters and to promote restoration. In another comparison with antibiotic and vitamin K treatment, normalization of liver values was twice as rapid in the EPL group. From the histological viewpoint, the increased rough endoplasmic reticulum indicated an activation of the protein synthesis as well as cell regeneration. The ultrastructural alterations of hepatocytes and bile capillaries were largely normal.

4.1.2 Immunological Hepatocyte Damage

Immunological pathomechanisms are of major importance in liver disease in man. To these pathomechanisms belong autoimmunological reactions and Insufficient Immunological reactions. The latter plays an important role in viral infection. An insufficient immunological reaction against the virus-infected cells leads to a chronic process of cellular infection and extinction of the affected cells. An activation of the primary immune response by EPL in mice sensitized with sheep erythrocytes has been reported by F.Barbarino et al. (37). Formation of haemolytic plaques was increased against controls as was the number of plaque-forming cells which were reduced as a result of galactosamine intoxication.
J. Neuberger et al. (513) studied the influence of EPL on the (autoimmunological) antibody-dependent cell-mediated cytotoxicity (ADCC: fig. 16 and mitogen-induced lymphocyte cytotoxicity.

Rabbits were pre-treated with EPL for 6 weeks. Hepatocytes were subsequently isolated and either incubated with antibody-containing sera from patients with HBsAg negative, anti HBs-negative and anti-HBc negative chronic active hepatitis or brought into direct contact with the mitogen-stimulated lymphocytes.

The following results were obtained:

In contrast to the studies using hepatocytes isolated from control rabbits not given EPL, the antibody-containing sera from patients and the lymphocytes from healthy individuals did not lead to increased ADCC in the hepatocytes obtained from EPL-treated rabbits (fig. 17a). Again in distinction to the findings in the controls, the mitogen-stimulated lymphocytes were not found to be directly cytotoxic to the EPL-treated hepatocytes (fig. 17b).

The results suggest that EPL, which is incorporated into the hepatocyte membrane in-vivo, obviously reduces the susceptibility of hepatocytes to lymphocyte cell damage.

Up to this date three groups have investigated the effect of EPL on endotoxin-induced liver damage (358, 447, 739, 740). Either Propionibacterium acnes, Corynebacterium parvum or Salmonella abortus equi endotoxin followed by lipopolysaccharide were added to isolated liver cells or intravenously to mice. The pretreatment with EPL protected against this immune-mediated inflammatory liver model.

The levels of cytolytic enzymes and lipid peroxidation in serum were reduced, while cholinesterase, protein synthesis and the viability of the hepatic cells were increased.

4.1.3 Irradiation

The liver is very sensitive to irradiation: the influence of free radicals, produced by x-rays, on ultrastructure and enzyme activity of different cellular membranes was demonstrated by morphological, histochemical and biochemical actions. Plasma and lysosomal membranes were found to be extremely sensitive, whereas the membranes of mitochondria and the endoplasmic reticulum were damaged to a lesser extent (W.A.R. Huijbers et al., 1976; 326). Ionising radiation first disturbs the phospholipid metabolism (634), then provokes severe inflammatory reactions similar to hepatitis, and finally leads to death.

In experimental whole-body irradiation of EPL-treated animals (mice, rats, rabbits) the survival rate served as an important parameter (51, 52, 498, 608) an in one exception liver circulation was measured (534). The survival of rats exposed to lethal doses of radiation was clearly prolonged with EPL. These results were confirmed in the mouse, and, the damaged energy-supplying systems of the liver cell mitochondria (oxidative phosphorylation) were found to be normalized under the influence of EPL (498, 608).

After partial hepatectomy and following whole-body irradiation, DNA synthesis increased in the liver of EPL-treated rats as a sign of stimulated regeneration (42). In another trial, rabbits received "essential" phospholipids (EPL) 24 hours and 30 minutes each before being exposed to partial body irradiation at different doses. On the basis of the observation that liver circulation, and thus also colloid clearance, was neither influenced by low radiation doses (producing an increase) nor by high doses (producing a decrease), but remained within normal, the authors concluded that the
incorporation of EPL into the hepatocyte membranes makes the liver cells less sensitive to ionising rays (534).

4.1.4 Stimulation of Regeneration

Histological examinations of the hepatic tissue to prove regeneration are completed by those concerning the synthetic performance of the hepatocytes. Important parameters were: the content of total protein, the rate of incorporation of radio-labelled amino acids, content of DNA and RNA and the concentration of albumin in serum, the activity of cholinesterase (CHE) as well as the albumin/globulin ratio (A/G).

The following pharmacological studies performed with EPL refer to these and corresponding parameters (tab. 16):

21 studies during the period of 1962 to 1991 were carried out in mice, rats or dogs on different models of induced liver damage: CCI4, galactosamine, ethanol, organic solvents, cholestasis, partial hepatectomy, etc. EPL was administered orally, intravenously, intraperitoneally, intramuscularly or subcutaneously, preventively as well as simultaneously or curatively. Nine of these 21 studies showed either an increase in the protein content of the liver with EPL therapy or an intensified protein biosynthesis in the hepatocytes due to the increased incorporation of 35S-methionine or 14C resp. 3H-L-leucine (3 studies). An increase in the content of RNA (6 studies), DNA (2 studies), the CHE activity (1 study) and the serum albumin (4 studies) could also be proven. Cytological and histological examinations give an additional hint at an increase in the metabolic and regenerative activity of the hepatocytes under the administration of EPL.

4.1.5 Renal Disorders

It is discussed that eicosanoids are particularly important in pathological pictures associated with limited glomerular filtration rates and urinary excretion. They are especially active in the kidneys as potential modulators for the regulation of renal haemodynamics and of glomerular filtration. In this process prostaglandins PGI2 and PGE2 exert vasodilating effects on the smooth muscle cells, whereas thromboxane A2 has a vasoconstricting action. It seems that eicosanoids provide for the maintenance of the glomerular filtration rate, and thus guarantee normal excretion of electrolytes and water, in situations when the intrarenal vessels are submitted to vasoconstricting influences. They further have tubular effects (441).

Taking into account this mechanism, in renal diseases with impaired glomerular filtration rate a positive influence can be expected from the enrichment of precursor fatty acids of arachidonic acid (165). This is possible with EPL. In addition, also direct effects of EPL can be triggered by their incorporation into renal membranes, e.g. the intracellular electrolyte transport due to improved membrane fluidity.

Only few experimental investigations into EPL and its effects on renal function are available which, in most cases, exclusively deal with the protection of the kidneys against noxious influences.

An exception is the publication by P. Bernardi et al. (54) who established a relationship between the effect of EPL and the influence on the prostaglandin metabolism. In New Zealand rabbits, EPL produced hypotonic polyuria as a result of a considerable increase in glomerular filtration rate and significant reduction of water reabsorption. The absorption of sodium and solutes remained unchanged, while potassium absorption increased considerably. On the basis that the pretreatment with sodium salycilate cancelled these effects the authors discussed that EPL stimulates the synthesis of renal prostaglandins, which can be blocked by the cyclooxygenase-inhibitor salicylate.
In chapter 5.2.3 will be shown that the authors confirmed their results clinically.

I. Zulic and co-workers (774) observed that the simultaneous application of EPL with sodium-heparin in rats for 1 week abolishes heparin increased diuresis and reduces heparin increased urinary excretion of labelled water. They concluded that EPL has protecting effects on the integrity of glomerulo-tubular structures and functions.

The other studies describe the protecting effects of EPL on renal disorders induced by fat embolism and by alcohol:

K. Hupe et al. (290) reported that experimental fat embolism by intravenous injection of homologous rat fat or olive oil is influenced favourably by EPL. The amount of fat deposition in the pulmonary and renal vessels was decreased significantly as compared to untreated controls.

The influence of "essential" phospholipids upon changes in rat kidneys caused by prolonged ethanol administration was investigated in the Pharmacological Institute of the Pomeranian Medical School in Szczecin in a series of studies (561, 605, 606). EPL had a prophylactic effect on the release of acid phosphatase from the lysosomes of the cell layer at the lumen of the renal tubules (605). Moreover, EPL protected against decreased activity of alcohol dehydrogenase, oxygen consumption (using the Warburg manometric method) and diuresis.

H.H. Wagener et al. (729) observed In normal rats no diuretic or saluretic effect of EPL.

A completely different effect of EPL was seen in chronic ambulatory peritoneal dialysis (CAPD), carried out in chronic renal insufficiency. In CAPD substances from the blood usually eliminated with the urine are continuously filtered through peritoneal vascular walls into a solution introduced into the abdomen.

In in-vitro studies, using isolated sections of the mesentery of rabbits, phosphatidylcholine increased the permeability of the mesothelium to water, urea and glucose from the vascular to the mesothelial side but not in the opposite direction (81). In their in-vivo studies the same group noticed an increased ultrafiltration (and urea clearance), mainly by decreasing the reabsorption phase, when 50 mg/I phosphatidylcholine were added to the dialysis fluid. The rate of glucose absorption from the peritoneal cavity was not affected.

The influence of EPL on the peritoneal mesothelium - although not applied in chronic renal insufficiency but to reduce post-operative adhesions - was recently investigated by the Swedish group of St. Bengmark (21). EPL appears to form a lubricant film on mesothelial defects and is kept in situ by attraction caused by negatively charged choline on the positively charged mesothelium.

4.2 Disturbances of Lipid Metabolism and Fat Embolism

On the basis of representative investigations the following tables show to what extent and variety research has been done on "essential" phospholipids and lipometabolism. The overview is divided into 6 tables, which reflect the main effects of EPL in animals:
1. Increase in linoleic fatty acid
2. Influence on enzyme activities
3. Reduction of serum lipids
4. Influence on lipoproteins
5. Antiatherogenic action
6. Cerebrovascular action

On the whole, 95 pharmacological studies were carried out in 11 different animal models using various forms of EPL application (i.v., p.o., s.c., i.c. and i.p.) prophylactically, simultaneously and curatively. The following table (tab. 17) summarizes the number of animal species and studies used for each model:

<%Tab. 17:%>
Table 18 describes the results of each study, differentiated according to model, animal, EPL dose, kind of application, results, details of reference (modified acc. to 767).

Table 19 comprehends an overview on the effects EPL have on human enzymes, lipoproteins, plasma, cells and tissues in-vitro (14 studies).

The results in tables 18 and 19 are mostly indicated by arrows:
= increase in concentration or activation of the measured variable = decrease of concentration or activation of the measured variable

Abbreviations:

p. = prophylactically
s. = simultaneously
c. = curatively
s./c. = simultaneous or curative application (etc.)
i.v. = intravenously
p.O. = per os (orally)
s.c. = subcutaneously
i.c. = intracardially
i.p. = intraperitoneally
b.w. = body weight

ACAT = Acyl CoA:cholesterol acyltransferase
ALT = alanine aminotransferase (GPT)
AST = aspartate aminotransferase (GOT)
C = cholesterol
CE = cholesterol ester
FA = fatty acids
FC = free cholesterol
FFA = free fatty acids
GE = glycerol ester
HTGL = hepatic triglyceride lipase
LCAT = lecithin:cholesterol acyltransferase
LDH = lactate dehydrogenase
LL = lysolecithin
LPL = lipoproteinlipase
m-RNA = messenger ribonucleic acid
MDA = malonedialdehyde
PL = phospholipids
RBC = red blood cells
SDH = succinic dehydrogenase
SOD = superoxide dismutase
TC = total cholesterol
TG = triglycerides
TL = total lipids

<%Tab. 18.1: Increase of polyunsaturated fatty acids in CE, PL and TG in the serum (aorta) following EPL therapy%>
<%Tab. 18.2.1: Influence of EPL on the enzyme activity in the aorta (in the serum)%>
<%Tab. 18.2.2: Influence of EPL on the enzyme activity in the aorta (in the serum)%>
<%Tab. 18.3.1: Lowering effect of EPL on serum lipid values%>
<%Tab. 18.3.2: Lowering effect of EPL on serum lipid values%>
<%Tab. 18.3.3: Lowering effect of EPL on serum lipid values%>
<%Tab. 18.3.4: Lowering effect of EPL on serum lipid values%>
<%Tab. 18.4.1: Influence of EPL on lipoproteins%>
<%Tab. 18.4.2: Influence of EPL on lipoproteins%>
<%Tab. 18.4.3: Influence of EPL on lipoproteins%>
<%Tab. 18.5.1: Antiatherogenic effect of EPL%>
<%Tab. 18.5.2: Antiatherogenic effect of EPL%>
<%Tab. 18.5.3: Antiatherogenic effect of EPL%>
4.2.1 Increase of Polyunsaturated Fatty Acids in Serum and Aorta

Seven publications, mainly from the seventies, describe the important fact that EPL, also when orally applied, increases the amount of polyunsaturated fatty acids, particularly of linoleic and arachidonic acids, in cholesterol esters. These investigations were performed in rats, minipigs and even chimpanzees receiving different cholesterol diets.

There are two reasons for this effect:
- the high amount of linoleic acid in the phosphatidylcholine molecules in EPL;
- the fact that EPL increases the activity of LCAT and that it is a preferred substrate (see chapters 3.3.2 and 5.4.6.1)

4.2.2 Lowering Effect on Serum Lipid Values

Using one of the noxae mentioned in table 20, an increase in serum lipids was triggered over a minimum of 2 weeks to a maximum of 18 weeks. (Tab. 20: Selected refs.: 159, 258, 350, 611, 612, 614, 692, 744, 751)

In the experiment of E.K. Wong et al. (751) rhesus monkeys were fed a high-cholesterol diet over a period of 10 years (fig. 18). (Fig. 18: Investigations on rhesus monkeys (n=7) after a 10-year period of high-cholesterol diet (120 mg/100 kcal) and a 7-week period of polyenylphosphatidylcholine application (1.7 g/100 g diet). Assessment of serum total cholesterol, LDL cholesterol and triglyceride values at baseline, 7 weeks after the start of medication and 16 weeks after completion of the medication (according to 751))

Irrespective of whether EPL was administered in parallel with or subsequent to atherogenic noxae, investigators reported a lowering of elevated serum levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), free cholesterol as well as a lowering of the TC/phospholipid ratio, and an increasing esterification of cholesterol with linoleic acid instead of saturated fatty acids.

4.2.3 Antiatherogenic Effect of EPL

This part highlights in particular the influence of EPL on existing atherosclerotic plaques.

Additionally are mentioned the factors playing a role in the development of atherosclerotic plaques; these factors may be influenced by EPL in the sense of protection and improvement.

Among these factors figure the following effects:
- a) Reduction of serum lipid values of total cholesterol, LDL cholesterol, triglycerides and increase in HDL cholesterol
- b) Inhibition of lipid peroxidation and particularly of the oxidative modification of LDL, which is considered to be a decisive factor aggravating the atherogenic effect of this lipoprotein class (665).
- c) Influence on the activity of important enzymes involved in the lipid metabolism (e.g. lecithin:cholesterolacyltransferase, acyl CoA:cholesterol acytransferase, lipoprotein lipase, hepatic triglyceride lipase)
- d) Diminution of increased platelet aggregability which, among others, is due to disorders of the lipid metabolism
e) Improved microcirculation of the blood by enhancing the fluidity of the plasma erythrocyte membranes
f) The stimulating influence of EPL on prostacycline formation
g) The particular property of intravenously applied EPL as to simulate HDL functions

Animal In-vitro Investigations into EPL:
The perfusion of normal rabbit aortas and of aortas damaged by an atherosclerosis-inducing diet was investigated in a medium containing radioactively labelled EPL. EPL was found to have been incorporated into the cells of the aortic tissue and especially at those sites where fatty streaks were present (79). These findings were confirmed ex-vivo in rabbit aortas by injection of radioactively labelled dilinoleoylphosphatidylcholine. Radioactivity appeared first in the lipoproteins and then in the aortic tissue (644).

Atherosclerotic changes in the tissues of arterial walls are induced by endocytosis, especially of the LDL, whereby the endocytosis can be receptor-dependent (88) or receptor-independent. Once the incorporation of EPL into the atherosclerotic damaged vessels had been demonstrated, the next step was to study the influence of EPL as a membrane substance on the endocytosis. In the model of smooth muscle cells from pig aortas was shown that the rate of endocytosis of radio-labelled tracers (125I-polyvinyl-pyrollidone, 14C-saccharose) was significantly reduced (p < 0.01) in the presence of EPL (80).

Trials with perfused rabbit aortas in the presence of EPL in the perfusate yielded a significant rise in fatty acids in the medium as well as a reduction of the biosynthesis of fatty acids in the tissue (p < 0.05; 78). First signs of atheroscleroticly changed vascular walls are the so-called "fatty streaks" which are characterized by an increased accumulation of cholesterol esters. Under the influence of EPL tissue cultures of rabbit aortas exhibited a significant reduction of cholesterol esters (284).

It is known that through the scavenger pathway macrophages are able to take up large quantities of cholesterol and cholesterol esters, and to transform themselves into foam cells. These are considered to be an early sign of atherosclerotic changes in the vascular wall. Rat macrophages, experimentally loaded with radioactively labelled cholesterol, were incubated for 6 hours in a medium rich in EPL micelles. The presence of the EPL micelles led to an approx. 15% release of cholesterol from the macrophages, whereas no cholesterol was released into the medium without EPL The effect of EPL could be increased to an over 20% release when apoproteins, such as Apo-A1, Apo-C1 or Apo-C3, which are contained in HDL, were added to the medium.

Preincubation of HDL with EPL micelles produced a modification of the HDL with respect to a changed density and an increased phospholipid content. It was surprising to find that these modified HDL were superior to native HDL as to the release of radioactively labelled cholesterol from rat macrophages (increase by 80%). This superiority of modified HDL was also seen when the rat macrophages were loaded with radioactively labelled cholesterol esters via acetylated LDL (650).

The existing in-vitro studies showed the preferential incorporation of EPL into atherosclerotically changed vascular regions. These studies further presumed that EPL is able to influence the lipid metabolism in the aortic wall and to provoke a release of atherosclerosis-inducing substances from atherogenic sources.

In-vivo Administration of EPL in Animals:
C.W.M. Adams (4) was one of the first investigators who found that with feeding an atherogenic diet the formation of atheromas can be induced in rabbits, which in their development is very similar with the human pathology. In the meantime, this model has been transferred to a series of other animal species. In a total of 22 studies in 7 different animal models the effect of EPL on atherosclerosis induced by an atherogenic diet has been investigated (tab. 21).

<%-Tab. 21: Pharmacological investigations into the anti-atherogenic effect of EPL (overview)%>
All studies demonstrated that the induced atherosclerotic changes in arteries, aortas and coronary vessels could be inhibited, prevented, reduced or eliminated in those animals which were fed EPL simultaneously or after the atherogenic diet had been discontinued. Two of the studies showed a clear reduction of total cholesterol, cholesterol esters and of free cholesterol in the aortic tissue after EPL treatment. The most important findings from the 22 studies can be seen in tables 18.5.1 to 18.5.3.

Below, some of the studies are described more in detail:

B.C. O'Brien et al. (521) investigated over 6 weeks the influence exerted by a 0.5% cholesterol diet on guinea pigs. No fatty deposits in the aortic tissue could be found. Guinea pigs which were fed soya lecithin in addition to the diet showed in their aortas a reduction of total cholesterol. In the serum, this effect was accompanied by reduced total cholesterol and free cholesterol and by a rise in HDL cholesterol.

W.W. Stafford et al. (662) found a significant (p < 0.05) reduction of total cholesterol in the aortic tissue of Japanese quails. As a consequence of a 2% cholesterol diet over 6 months the animals showed extensive plaques in the thoracic aorta as well as in the right and left brachio-cephalic arteries. The severity of the atherosclerotic changes was assessed by means of a score of 0-100 (score 100 = vascular intima covered by 100% with atherosclerotic plaques). Three investigators found independently of each other a significant reduction of the extension of plaques (p < 0.05) when the quails were given i.v. EPL over 3 months simultaneously with the atherogenic diet.

In a classic atherosclerosis experiment with New Zealand White rabbits aged 24 weeks, J. Patelski et al. (539) induced macroscopically detectable atherosclerotic lesions in the aortas of the animals by means of an 18-week diet with 20% beef tallow. In contrast to the control animals, the total surface of the aortic intima of the rabbits injected simultaneously with intravenous EPL presented not more than 5% of atherosclerotic regions in any case. With EPL also the content in cholesterol esters and in free cholesterol was reduced.

Investigations into male 1-2 year old baboons carried out by A.N. Howard et al. (291) showed that after a 6-month diet with 15% egg yolk the animals had developed macroscopically detectable atherosclerotic changes. When they were given simultaneously i.v. EPL, the percentage of affected surface decreased significantly from 46.3% to 9.5% (p < 0.05). Five of the 8 EPL- treated baboons showed no macroscopic changes in the aortic intima; on the microscope the thickened intima regions of these animals presented less fatty depositions; furthermore, cholesterol esterase activity in the aorta was found to be significantly increased by 50% (p < 0.05) in contrast to the controls.

In another study in baboons fed a 6-month 2% cholesterol diet with subsequent 4-month EPL therapy, A.N. Howard et al. (295) found a significant reduction of ACAT activity in the aorta (p < 0.01). These values were even below the level of normally fed baboons. ACAT (acyl-CoA:cholesterolacyltransferase) catalyzes the formation of fatty deposits rich in cholesterol esters in the peripheral tissue.

A 2-month curative treatment with EPL (dose: 28, 90 or 280 mg/ kg b.w.) of minipigs, which had been fed a 2% cholesterol diet during the preceding 2 months, provoked a dose-related reduction of the lipid values in the serum and in the aorta: total lipids, cholesterol esters, free cholesterol and triglycerides. With higher EPL doses (90 and 280 mg/kg b.w.) a clear reduction of atherosclerotic plaques was seen in the aortas and the valves. In the coronary vessels, however, no difference with respect to the normally fed control animals was found with these doses (L. Samochowiec et al., 613).

In an extensively documented study by L. Samochowiec et al. (614) a 20 % cholesterol diet over 2 months was given to rats to produce macroscopically detectable atherosclerotic changes in the form of nodular plaques and the formation of a rough intima surface. EPL-treated rats received the oral substance at doses of 280, 906 or 2800 mg/kg b.w. either during the atherogenic diet or over 2 months after discontinuation of this diet. With both simultaneous and curative EPL application the serum values of total lipids, total cholesterol, triglycerides and LDL decreased, whereas the phospholipid values
increased; in the aorta was found a reduction of the values of total lipids, cholesterol esters, free cholesterol and triglycerides, and an increase in phospholipid values. The afore-described macroscopically detectable atherosclerotic changes could be inhibited or even regressed in the animals receiving the highest EPL dose (2800 mg/kg b.w.). No lipid infiltrations were found in the tissue.

In a similar study design L. Rozewicka et al. (588) studied the influence of simultaneous and curative application of different EPL doses (280, 900 and 2800 mg/kg b.w.) on histological changes in the aorta and the coronary vessels induced by a 20% cholesterol diet. In the aortic tissue the atherogenic diet produced proliferations of connective tissue, degenerative lipid deposits, foam cells and cellular fatty infiltrations. In the coronary vessels were seen only minor changes in the form of some little fatty deposits. These atherosclerotic changes were found to be reduced after both simultaneous and curative administration of EPL over 80 days, the reduction being most pronounced in the highest dose group (2800 mg/kg b.w.) and in curative EPL application. In the myocard the 20% cholesterol diet caused fibrous changes and depositions of fat droplets. The fibrous changes in the myocard subsided, especially when 2800 mg/kg b.w. EPL were administered simultaneously. In the curative administration no differences in comparison with the rats on normal diet were seen. Fat droplets in the myocard were found neither in simultaneous nor in curative application of EPL.

In rabbits with extensive atherosclerotic lesions in the aortas after a 3-month high-cholesterol diet (32 + 4.3% of the total intima surface), E.A. Borodin et al. (68) showed that the i.v. administration of a total of 10 g EPL per animal over 5 weeks led to a reduction of the atherosclerotically damaged aortas (14 + 2.5 % of the intima surface).

J. Wojcicki et al. (750) obtained similar results in the same animal model upon simultaneous oral administration of a 1.35% cholesterol diet and 100 mg EPL/kg b.w. a day over 4 months: the aorta intima surface affected with plaques was reduced from 37.2% to 7.4%, which was not the case in the control animals; the intima was only slightly thickened. Considering that also the immune system is involved in the formation and continuance of atherogenic processes (A.N. Klimov, 352), in this study were examined also the influences of EPL on both unspecific and specific immunological mechanisms: especially unspecific immune reactions were found to be reactivated (e.g. the phagocytic and bactericidal activities of granulocytes).

In 22 studies on 7 different animals species could be demonstrated that diet-induced atherosclerotic changes in the aortas, arteries and coronary vessels of the animals were largely prevented or reduced with the help of simultaneous or curative administration of EPL (i.v., p.o., s.c. or i.p.). The analysis of the aortic tissue yielded a reduction of athero-genic lipids.

In-vitro Investigations with Human Cells and Tissues indicating an Antiatherogenic Effect of EPL in Man:
Tables 19.1 and 19.2 give a survey on the experiments carried out with EPL. The most important findings gave evidence of the following:
In detailed in-vitro studies, O. Zierenberg et al. (766) found upon incubation with EPL a modification also for human HDL. Up to 50% of the phosphatidylcholine in the modified HDL originated from the added EPL. The phosphatidylcholine/apoprotein ratio in the HDL increased and thus also increased the particle size and the fluidity of the monolayers. In contrast to native human HDL the modified HDL was able to take up 55% more 14C-cholesterol from the loaded human LDL (p < 0.001). The content in radioactive cholesterol in the LDL was also significantly reduced upon incubation with modified HDL (p < 0.01). These results could be corroborated with human serum by means of the 14C-cholesterol distribution test. The comparison of the effects of other more saturated phosphatidylcholines suggested that the more pronounced EPL effects are attributable to the high content in polyunsaturated fatty acids.

The already mentioned significance of oxidatively modified LDL for the development of atherosclerotic changes was studied by P. Avogaro et al. (31). They exposed human LDL over 48 hours to oxidative stress and thus provoked in
the oxidation medium a significant rise in the lipid peroxidation products: malonedialdehyde, diene conjugates, oxidized cholesterol esters and triglycerides. When under the same trial conditions 0.2 mg/ml EPL were added to the incubation medium, the mentioned lipid peroxidation products were significantly reduced again (p < 0.01).

J. Koizumi et al. (360) loaded macrophages of normolipidaemic volunteers with radioactively labelled cholesterol esters by preincubation of the cells with prepared LDL. The release of the radioactive cholesterol esters in human serum was significantly higher when 125 ug/ml EPL were added to the serum (p < 0.02).

A.N. Orekhov et al. (527) cultured smooth muscle cells obtained from atherosclerotic parts of aortas from patients, who had suddenly died of myocardial infarction. It was demonstrated that the typical atherosclerotic changes in the cultured cells (trend to proliferation, high lipid content) were maintained up to 12 days. When at least 250 ug/ml EPL were added to the serum, the content in total cholesterol and cholesterol esters was significantly reduced in the cultured cells in contrast to the control cultures (p < 0.05).

Using the same method, K.A. Khashimov et al. (345) showed with subendothelial cells from fatty streaks and from atherosclerotic plaques from intimas of the human aorta, that EPL was able to significantly reduce the content of total cholesterol in the cells (p < 0.05) by up to 40% (from 80.44 + 1.3 to 53.40 + 1.6 mg/105 cells). In-vitro tests with whole pieces of intima tissue also yielded a significant reduction of total cholesterol in the cell groups (p < 0.01).

After a 48-hour incubation with EPL, R. Niemann et al. (515) found an increase in LDL receptors in HepG2 cells. With the Scatchard's analysis could be excluded a change in the affinity of the receptors. In the control experiment with radiolabelled riboprobes was found a relative rise in LDL receptor specific m-RNA in the HepG2 cells incubated with EPL Human macrophages and fibroblast cell membranes loaded with radiolabelled cholesterol exhibited an increased cholesterol efflux depending on the EPL concentration and the duration of incubation. This effect was found to be more pronounced in the presence of HDL (particularly in the presence of HDL3), the cholesterol uptake capacity being improved when the HDL had been modified before in-vivo with EPL (for this model were used HDL from rabbits fed over 7 weeks with 250 mg EPL/kg b.w.).

The enzymes lecithin: cholesterolacyltransferase (LCAT), hepatic triglyceride lipase (HTGL) and lipoprotein lipase (LPL) are of outstanding importance for the human lipid metabolism. When incubated with different phosphatidylcholines the enzymes obtained from human plasma showed raised activity which was most pronounced with highly purified EPL (G. Assmann et al., 28; J.C. Fruchart et al., 213; C. Desreumaux et al., 145).

The afore-described 12 in-vitro studies with human plasma components, cells and tissues, show that
- by incorporation into HDL, EPL modify these HDL in the sense of an increased cholesterol uptake capacity;
- EPL are able to inhibit the formation of oxidized LDL which trigger atherosclerotic processes;
- the activity of the enzymes LCAT, HTGL and LPL, which are important for the lipid mobilization and metabolization, can be increased under the influence of EPL;
- the content in total cholesterol and cholesterol esters in atherosclerotic cells and tissues can be significantly reduced by the addition of EPL. In addition to the increased cholesterol efflux with EPL there are indications that the LDL receptor activity is improved with EPL on the level of gene expression;
- in platelets, the formation of lipid peroxidation products (malonedialdehyde, Schiff's bases) and aggregability are reduced with EPL.

4.2.4 Experiments on the Simulated Transport Function of HDL

During incubation of erythrocytes from IHD patients with human HDL it was noticed that the cholesterol/phospholipid ratio in the erythrocyte membranes was
decreased, the reduced activity of membrane-bound Na+, K+ -ATPase was normalized, and the microviscosity of the membrane had dropped. As it is difficult to supply sufficient quantities of HDL and as an administration of the substance would be complicated by its antigenicity, the therapeutic application of this principle is of purely theoretical interest (706).

Research teams from Chicago, London, Moscow and Chiba/Japan therefore tried to develop and investigate lipoprotein-like artificial phosphatidylcholine (PC) particies with the objective to find a possibility to simulate HDL function (68, 359, 560, 650, 706, 744, 745).

Several research teams have gained liposomes from phosphatidylcholine or EPL by different methods, which were investigated both in in-vitro trials and in-vivo after i.v. injection in animals with particular regard to their HDL-like cholesterol-accepting properties. These properties depend on the content of phosphatidylcholines with predominant binding to linoleic acid, as well as on the positive charge of the phospholipid monolayer at the HDL surface (706). This is the region where the incorporation of sterols takes place.

When they have the above-mentioned properties, artificial systems with a structure similar to HOL such as PC-liposomes are obviously most suitable to serve as model for the simulation of cholesterol-accepting and transport functions of HDL.

In general, the degree of effectiveness of the used PC-particles was found to be inversely correlated with their microviscosity. Due to its fatty acid bonds, in particular with linoleic acid, polyenylphosphatidylcholine (EPL; Lipostabil) exhibited the best capacity to form complexes with cholesterol and thus the best cholesterol-accepting activity as compared to other phosphatidylcholines (68, 706).

When erythrocyte ghosts were incubated with polyenylphosphatidylcholine liposomes the cholesterol content in these membranes was reduced by about 40%. This reduction was dependent of the quantity of liposomes (706).

J. Koizumi et al. (360) produced complexes of polyenylphosphatidylcholine (EPL; Lipostabil) and apoprotein A1, isolated from human HDL, and thus imitated HDL particles which, as it is known, consist primarily of phospholipids and apo A1.

The density of these apo A1/polyenylphosphatidylcholine complexes corresponded initially to the density of LDL. After incubation with plasma and after i.v. injection in the animal the density of these particles became gradually similar to HDL. Their flow rate in electrophoresis coincided with this characteristic (see also 697).

I.v. administration of polyenylphosphatidylcholine (Lipostabil) or apo A1/polyenylphosphatidylcholine-complexes to normolipidemic rabbits increased the phospholipid concentration in plasma. The enrichment of HDL with phospholipids, however, was higher upon injection of apoA1/polyenylphosphatidylcholine.

In in-vitro trials with perfused rabbit aortas in the presence of LCAT, the apo.HDL/polyenylphosphatidylcholine-complexes favoured the efflux of free cholesterol from the cells.

Experience has shown that circulating pure phosphatidylcholine (PC) liposomes are relatively quickly removed from plasma and degraded by reticulo-endothelial cells. The plasma clearance of apo A1/PC-complexes or of apoHDL/PC-complexes is much slower and can be compared with the clearance of native HDL. J. Koizumi et al. found in their investigation that after i.v. injection to rabbits polyenylphosphatidylcholine gradually reached the physico-chemical properties of the apo A1/polyenylphosphatidylcholine - complexes due to absorption of apo A1 from HDL (359).

After injection, both preparations provoked a slight temporary rise in the total plasma cholesterol level, which is due to the complex-binding of cholesterol to liposome-phosphatidylcholines. Afterwards, however, total cholesterol decreased markedly (359).
In earlier trials on dogs K.J. Williams and A.M. Scanu (744) confirmed the increasing density of phosphatidylcholine liposomes in plasma as well as the electrophoretic mobility similar to VLDL and HDL. They also observed that these liposomes acquired unesterified cholesterol from lipoproteins and tissue thus producing a temporary sharp rise in the cholesterol level in plasma. These observations are in accordance with early findings of other investigators (5, 539).

During incubation with plasma the PC-liposomes accumulated endogenous proteins, e.g. apolipoprotein A1 at the expense of HDL. The newly formed particles rich in phospholipids and apo A1 were very similar to native HDL. But there was a decisive difference between native HDL and apo A1-rich PC-liposomes: the HDL particles, the size and cholesterol-uptake capacity of which were increased (modified HDL) due to the uptake of phosphatidylcholines from the liposomes, transported predominantly esterified cholesterol in their nucleus. The PC-liposomes rich in apo A1 (modified liposomes), in contrast, transported only free cholesterol at their surface.

The Japanese research team of K. Shirai et al. (650) used dipalmitoylphosphatidylcholine (DPPC) and polyenylphosphatidylcholine (EPL; Lipostabil) vesicles to investigate the intensity of the release of 3H-cholesterol from macrophages isolated from the peritoneum of rats. Due to their lower microviscosity and their greater fluidity, the capacity of polyenylphosphatidylcholine vesicles to remove cholesterol from the macrophages after 2 and 6 hours of incubation was clearly superior to DPPC vesicles. In the medium without phosphatidylcholine no cholesterol removal occurred. By incubation of HDL3 with polyenylphosphatidylcholine vesicles the authors obtained complexes which they denominated modified HDL. The migration rate in electrophoresis resembled the rate of HDL. The capacity of these vesicles to release 3H-cholesterol from macrophages was about 80% higher than the cholesterol-releasing capacity of native HDL.

Recently K.J. Williams et al. (745) found out that hepatic uptake of soybean phosphatidylcholine liposomes is independent of LDL-receptors. According to the authors their findings in combination with earlier data support the hypothesis that the antiatherosclerotic effect of these liposomes/micelles - which is evident in animals even during continued feeding of a high cholesterol diet - results in part from scavenging of tissue cholesterol by these phosphatidylcholine liposomes, which transport this cholesterol to the liver. The antiatherogenic effect of infused phosphatidylcholine also occurs in LDL receptor deficient animals. These results suggest new approaches for the cholesterol extraction from tissues into plasma. On the basis of the described data, it is considered as plausible to also think of a compensation of Insufficient cholesterol release in ischemic heart disease in man (560, 706).

4.2.5 Fat Embolism

Fat embolism, now also termed fat embolism syndrome, does not represent a frequent but most certainly one of the most dangerous complications in surgery and orthopaedics. Although a more isolated event it also is observed in the field of internal medicine, where it may be a sequela of liver disease, intoxication, burns, infections and cold injury (86, 290, 504).
The question of etiology is not fully settled. It is safe to assume, though, that lipids from the site of injury (fracture, tissue lesions etc.) infiltrate venous circulation. Pulmonary fat emboli were identified, that definitely contained bone marrow cells (91, 237, 298).

Calculations showed, however, that in fat embolism more free lipids were measured in the blood and relevant organs (lungs, kidneys, heart, brain) than had been mobilized from the injured tissue (bones, muscles) (237, 400). Increased lipase activity, changes in the pattern of blood proteins, reactive reduction of phospholipids, release of free fatty acids, and shock all have to be considered contributing factors for the etiology of fat embolism (75, 278).

On the basis of these findings further theories have been advanced to explain the occurrence of fat embolism, such as segregation of fat, an enzyme theory and a supportive role of shock. Gresham summarized these divergent opinions on the pathogenesis of fat embolism in 1986 (237).

Whatever may be the objections to individual hypotheses and research findings, one fact is well established and is never disputed, viz.: segregated fat is found to appear in the blood in the form of major-sized droplets and to mostly deposit in the lung. If these fat droplets succeed to pass the lung filter, cerebral circulation will be compromised in the first instance. The resulting cerebral fat embolism is burdened with an extremely high mortality. Aside from this, the damage reported includes necrosis of the kidneys, heart, and eyes as an indication of invading fat droplets (374, 380, 398).

The ability of EPL to physiologically emulsify fats (properties as a surfactant), enhance the transport capacity of lipoproteins, improve membrane fluidity and to accelerate lipid catabolism (67, 167, 257, 650, 765) were the primary motives to employ this action profile in the control of fat embolism. Methods of identifying the segregation of fat as larger or smaller droplets in the blood of accident victims (89, 290, 298, 377) included the possibility of investigating pathological fat transport and its response to EPL in experimental and clinical studies (290, 375, 377, 378).

In the 18 pharmacological studies at hand the effectiveness of EPL in fat embolism has been assessed in five different species (rats, rabbits, cats, dogs, and monkeys). Fat embolism generally was induced by oil injections. In three investigations it was induced by bone surgery or bone fracture. In two trials a preliminary stage of fat embolism was provoked by anaesthetic agents (tab. 22).

Among the animals receiving EPL, a higher percentage survived otherwise lethal dosages of oil than among controls who had not been treated with EPL. This was particularly noticeable when EPL had been given prophylactically and subsequent curative doses had been administered at short intervals (at least every 12 hours). Shock symptoms associated with fat embolism, like e.g. respiratory depression, were found to diminish.

Fat deposits in the different organs (lungs, liver, kidney) were reduced just as were the size and agglomerations of fat droplets identified in plasma. Under the influence of EPL tributyrinase activity in serum, which has been found to decrease as fat embolism develops, proved to rise again. Tables 23.1 to 23.3 give a survey on the 18 studies already mentioned. Some of these will now be discussed in greater detail:

In an exhaustive investigation on a total of 480 rats K. Hupe et al. (290, 300) studied the influence of 122 and 244 mg of EPL/kg b.w. on the deposition of fat into the lungs, liver, and kidneys after the injection of olive oil. Blind histological evaluation established markedly lower amounts of fat in the pulmonary and renal vessels of animals who had received prophylactic or curative dosages of EPL than in the controls.

In several trials on a total of 290 animals, J. Kroupa et al. (375) were able to demonstrate a significant increase in the survival rate of animals receiving prophylactic or curative dosages of EPL (7.5 to 150 mg/kg body weight) after exposure to olive oil (0.5 or 0.75 ml/kg body weight). Preventive
applications of EPL given 30 minutes prior to the olive oil injection led to a further increase of the survival rate. As a rule tributyrinase activity was found to drop in the beginning of fat embolism. A single application of EPL (37.5 and 75 mg/kg body weight) restored normal enzyme activity.

H. Koch et al. (357) performed femoral surgery on cats. Histological evidence of fat embolism associated with surgical interventions of this type was obtained in the lungs of the animals. Without pretreatment massive postoperative fat embolism was demonstrable in the lungs of 6 out of 8 cats, while the preventive application of EPL (114 mg/kg body weight) reduced the incidence to 2 out of 10 animals as well as the degree of severity of fat embolism. Prognosis was further improved by introducing a tube drain at the site of surgery.

W. Wehner (736, 738) performed a whole series of trials investigating EPL in cats, dogs, and monkeys. His studies into dogs are impressive evidence of the fact that the animals survived lethal dosages of oil, if they had received preventive treatment with EPL (50 mg/kg body weight) and adminstration of the same dosages was repeated at 8-hour intervals after the oil injection.

In 18 studies including various species of animals, the prognosis of fat embolism, induced by surgery or fracture, oil injections or anaesthesia, was improved by the prophylactic and/or curative application of EPL. EPL increased survival rates, lessened the signs accompanying fat embolism, reduced fat deposits in organs especially in the lungs, and diminished the incidence of fat droplets as well as their agglomeration in plasma.

4.3 Effect In Mucosa Damage (by NSAIDs)

As has already been described in chapter 1.8 surface active phospholipids play an important role in quite a lot of different tissues. The following chapter focuses on the gastrointestinal tract and its disturbances by non-steroidal anti-inflammatory drugs (NSAIDs). The reason for this focus resides in the incidence of such side-effects: NSAIDs do not only directly irritate the gastrointestinal mucosa but also diminish cytoprotective prostaglandins.

The "essential" phospholipids, on the other hand, can be used as repair elements of the hydrophobic layers and provide with their high content in linoleic acid precursors for a reincrease of these prostaglandins.

4.3.1 Protection against Ulcerogenesis

Intragastral co-administration of EPL and acetylsalicylic acid (ASA), diclofenac, indomethacin, phenylbutazone, piroxicam or sudoxicam in an acute gastrototoxicity test in the rat showed a pronounced reduction of ulcer formation (tab. 24). EPL was more effective at the higher doses in the phospholipid-NSAID combinations (444).

| Tab. 24: Acute effects on the rat gastric mucosa of various anti-inflammatory drugs administered orally with or without EPL (1:2 molar ratio). Animals were fed a carbohydrate-rich diet (bread rolls) for 3 days, fasted for 24 h (to sensitize the gastric mucosa) and killed 3.5 h after drug administration. Data are expressed as mean ulcer indices with the range of individual values in brackets (444) |
|-------------------|-------------------|
| 1 Suspensions not sonicated; 1:1 molar ratio of diclofenac to EPL |
| 2 Animals received normal diet before 24 h fasting; suspensions were not sonicated *p < 0.05; **p < 0.01 (Mann-Whitney U-test) |
| 0 = no macroscopically visible lesions |
| 1 = 1-3 small (< 4 mm) haemorrhages |
| 2 = more than 3 small (< 4 mm) or 1 large (> 4 mm) haemorrhages |
3 = 1 large (> 4 mm) and further small haemorrhages
4 = several large (> 4 mm) haemorrhages
5 = perforation

Sensitization of the mucosa by feeding rats a carbohydrate-rich diet for three days did not appear to be an essential factor for the action of EPL, since a reduction of the gastrotoxicity of piroxicam was also obtained in rats which were fed a normal diet before fasting.

In addition to acute gastric mucosal tolerance the effect of EPL on the subacute gastrotoxicity of piroxicam and diclofenac was studied (444). Oral administration of piroxicam and EPL (1:2 molar ratio) to normally fed rats produced a significant reduction in the gastrotoxicity of piroxicam at the highest dose (tab. 25). The changes were dose-relatea. Administration for 3 days of diclofenac and EPL (1:1 molar ratio) produced a significant reduction in the gastrotoxicity of diclofenac at 100 mg/kg per day.

Tab. 25: Effects on the rat gastric mucosa of oral NSAIDs administration for 3 days with or without EPL. Animals received a normal diet throughout the experiment and were killed 3.5 h after the last drug administration. Data are expressed as mean ulcer indices with the range of individual values in brackets (n = 10) (444).

Oral administration of saturated phosphatidylcholine also reduced the acute gastrotoxicity of all NSAIDs tested, though the effect was not statistically significant with phenylbutazone (tab. 26) (444). However, in most cases the extent of the reduction was less noticeable than with equivalent doses of EPL (tab. 24). In the case of diclofenac, a molar ratio of drug to saturated phospholipid of 1:2 was even less effective than a drug to EPL ratio of 1:1.

Tab. 26: Acute effects on the rat gastric mucosa of various anti-inflammatory drugs administered orally after sonication with or without saturated phosphatidylcholine (1:2 molar ratio). Animals were fed a carbohydrate-rich diet (bread rolls) for 3 days, fasted for 24 h and killed 3.5 h after drug administration. Data are expressed as mean ulcer indices with the range of individual values in brackets (n = 10) (444).

The effect of EPL on the gastrotoxicity of ethanol was studied in rat experiments with or without simultaneous administration of diclofenac (281). Co-administration of EPL dose-dependently reduced gastric damage caused by high doses of these gastrotoxic agents. Considering the biphasic course of the ulcer index following administration of 100% ethanol (fig. 19) EPL (100 mg/kg b.w.) inhibited the second of these phases. In addition to gastrotoxicity, alcohol obviously induced adaptive cytoprotection at 2-3 h, as diclofenac at non-gastrotoxic doses converted this biphasic effect into a monophasic one and increased the severity of damage at all time points. Under these conditions EPL showed an earlier and increased inhibitory effect.

Fig. 19: Time course of gastric damage in rats (n = 10-20) following administration of 0 100% ethanol, o 100% ethanol + 1000 mg EPL/kg b.w.. Broken lines indicate extrapolation to time zero, *p < 0.05 and **p < 0.01 in comparison with controls; +p < 0.05 for intraindividual differences (281).

Mechanisms of action:

To evaluate the local effect, whole-body autoradiography was performed in rats given an oral dose of 3H-1,2-dilinoleoylphosphatidylcholine (fig. 20). The test revealed a high concentration of radioactivity in the gastric and intestinal mucosa. This was demonstrable 15 minutes after administration in the gastric mucosa and was still evident in the gastrointestinal region after 24 hours (406).

Fig 20a: Whole-body autoradiography of rats 6 h (a) and 24 h (b) after an oral dose of 70 mg/kg EPL labelled with 1,2-(9,10,12,13- 3H4)dilinoleoyl-PC (406).

In addition, we examined the absorption of EPL into the stomach wall (fig. 20b) (442).

Fig. 20b: Following oral or intravenous administration of EPL (400 u.Ci 3H/kg i.v., 400 u.Ci 3H/kg p.o., 250 u.Ci 14C/kg p.o.) rat stomachs were dissolved and radioactively measured by LSC (442).
For the labelled choline and fatty acid moieties of (3-sn-phosphatidyl) choline comparable incorporation rates were recorded in the gastric wall.

To assess a possible systemic protective activity of EPL we investigated the effect of i.v. injected EPL on diclofenac-induced gastric damage and that of orally administered EPL on mucosal PGE2 formation after indomethacin administration (443).

Three individual studies have shown that tolerance to diclofenac was clearly improved after i.v. EPL administration (fig. 21).

<Fig. 21a: Reduction of diclofenac-induced gastric lesions by i.v. EPL administration. After a 3-day bread diet EPL was administered to the rats by i.v. route. Oral diclofenac was given 1 or 2 hours later (443)> Mucosal PGE2 synthesis, inhibited by indomethacin administration alone, was significantly increased 60 and 120 minutes after simultaneous treatment with EPL (fig. 21b).

<Fig. 21b: PGE synthesis in the gastric mucosa after indomethacin administration (10mg/kg p.o.) with and without EPL (96 mg/kg p.o.); n = 5(443) I = indomethacin, C = control + and *: p < 0.05; + + and *: p < 0.01 vs. I or C, resp.

Using indomethacin, it was shown that simultaneous administration of EPL (200 mg/kg) reduced the inhibition of PGE2 generation; a similar effect on 6-keto-PGF1 formation (prostacyclin metabolite) was also detectable. The increase in mucosal leukotriene synthesis after indomethacin and diclofenac administration could be reversed with EPL (445).

4.3.2 Anti-Inflammatory and Anti-Arthritic Effects

In order to investigate possible changes in the anti-inflammatory properties of NSAIDs by co-administration of EPL, the efficacy of indomethacin, phenylbutazone, acetylsalicylic acid and diclofenac (each drug combined with EPL) was examined by means of the rat paw oedema test and then compared with the action of the anti-inflammatory drugs alone (tab. 27) (243,446).

<Tab. 27: Inhibition of rat carrageenin paw oedema 3.5 h after administration of NSAIDs with and without EPL. Mean values of t0-20 observations (243, 446)> The combination of diclofenac and EPL was also tested in rats using the adjuvant arthritis model (fig. 22a and b) (444).

<Fig. 22a and 22b: Inhibition of Adjuvant Arthritis in the Rat by a) 0.1 mg/kg (A), 1 mg/kg (B) and 10 mg/kg (C) of diclofenac and b) 0.316 mg/kg (A), 1 mg/kg (B) and 3.16 mg/kg (C) of piroxicam with (closed circles) and without (open circles) EPL; triangles represent untreated arthritic control animals. Values are means of 10 measurements made on uninjected paws (444)%>

No significant differences were noted between the groups. EPL did not affect the anti-inflammatory potency of the NSAIDs studied.

4.4 Cerebral Effects

The potential pharmacological and clinical properties of EPL in certain neurological diseases are principally based on the following mechanisms of action (a.o. 180):
- donor of choline = precursor of the neurotransmitter acetylcholine;
- increased fluidity of glialytic and neuronal membranes; this has an effect on all the activities related with neuronal membranes, such as neurotransmission, transduction of biological commands, metabolism between neurons and gliaocytes, regeneration of neurons, influence of the response of receptors as well as increased activity of membrane- bound enzymes;
- carrier of unsaturated fatty acids;
- antilatherosclerotic and hemorrheologic action.

As already mentioned in chapter 2.4.2, the absorption of EPL into the brain is very limited after single oral or intravenous application (< 1%). It should be underlined, however, that the phospholipid metabolism is quite complex: besides "pure" synthesis there are reactions of interconversion, and the lipids resulting from "pure" synthesis can be changed by such interconversions (570).
On the other hand, the application of phospholipids can also provoke endogenous synthesis, as has been demonstrated in the case of EPL. I.Montanini et al. were able to show this process in rats in the case of brain lipid synthesis during aging (494). This team showed also in-vitro the stimulation of the key enzyme CTP: phosphocholine cytidyltransferase by EPL; this effect could not be obtained with saturated phosphatidylcholines (495). It should be pointed out, in addition, that not only substrate concentration is decisive alone: phosphatidylserine, for example, is incorporated only by 0.5% into the parenchyma of the brain, although effects like stimulation of the catecholamine metabolism, increase of glucose content and of acetylcholine release, and increased cAMP have been observed (60, 100, 410, 699).

Further, no kinetic data are available on EPL levels in the brain after chronic application. In such a case, larger quantities of EPL might be incorporated.

On this background, the investigation into the influence of EPL in neurological diseases is of certain interest.

Although the number is quite reduced, the existing experimental trials are quite interesting:

Table 18.6 in chapter 4.2 reflects the dilation of retinal arteries, the normalization of ERG's, the increase of cerebral blood flow, of physical activity, and of the dopamin and noradrenaline levels in the brain, demonstrating the cerebrovascular effect of EPL (536, 563, 609).

In chapter 3.3.1 were mentioned already the favourable influences of EPL on the cerebral enzyme antioxidant system in old rats (49, 50). Fig. 23 shows the changes in superoxide dismutase and glutathione reductase in various regions of the brains of 25-month old rats after application of daily 100 mg EPL/kg b.w. for 2 months.

Fig. 23: Cerebral enzyme antioxidant system. Influence of aging and EPL (49)

In the following will be described more in detail an experimental study each on
- allergic encephalomyelitis (651)
- influence on the brain choline and acetylcholine levels in total brain (158)
- changes in the growth and branching of the dendritic trees (486)
- retinal oxygen supply (525)
- improved energy supply during anaerobic metabolism and tor accelerated restitution of the energy balance in the recovery phase (726), and
- damage to the cerebral tissue in fat embolism (492)

Experimental allergic encephalomyelitis (EAE) constitutes the most important animal model in multiple sclerosis research (582). It can be considered as a prototype of autoimmune disease, mainly mediated by a deviated cellular immune response (651). The application of polyunsaturated fatty acids is discussed as therapeutic measure (44) since the concentration of these fatty acids in the food appears to be decisive for the resistance of adolescent animals against EAE (512). These facts and deliberations were on the basis of an EPL study:

A dose of 100 mg/kg b.w. Lipostabil solution (containing about 50 mg unsaturated fatty acids) was inoculated i.v. in guinea-pigs starting on the 3rd day after sensitization with 100 µg of basic protein (BP) in complete Freund's adjuvant. A series of T-14 daily injections either completely inhibited EAE or reduced its severity. The production of anti-BP antibodies, detected by indirect immunofluorescence and radioimmunoassay, was not affected, whereas cellular reaction as measured by a skin test was markedly reduced.

The effects of orally applied EPL on mouse brain choline and acetylcholine levels were investigated by Domino et al. in 1983 (100). EPL in a dose of 250 mg/kg choline equivalent was given 1, 2, 4, 8, 12 and 24 h prior to brain assay to groups of fasted mice. Mouse brain choline levels increased significantly at 4 and 8 h after EPL administration. However, there was no change in the concentration of mouse brain acetylcholine. The same results were found when scopolamine was used in order to decrease the choline level in the brain. The authors discussed that perhaps regional brain differences would be more apparent, which are masked by total brain measurements.
Very interesting are the results by R.F.Mervis et al. (486). After a 13-month application of EPL to 11-month old mice the animals presented a significant rise in dendritic material, its growth and branching in the brain (fig. 24).

In another study was investigated the influence of 1 capsule Lipostabil a day, containing 175 mg of EPL and administered for 1, 2 and 4 weeks each, on tissue respiration of the retina of healthy albino rabbits (525). However, no effect was observed in this study model in comparison with the control group.

After 34 preliminary experiments on rabbits, pharmacological premedication with anorganic iodide, EPL and Persantin was administered to 50 dogs in order to improve the energy supply during anaerobic metabolism and to accelerate the restitution of the energy balance in the recovery phase of the organism after circulatory standstill by inflow occlusion (726). This premedication allowed the prolongation of the permissible time of circulatory standstill.

B. Montalto et al. presented an electron microscopy study on the cerebral tissue of rabbits, by means of an experimental model reproducing the injuries of post-traumatic fat embolism (anatomo-pathological pattern) in man (492). The damages concern some modifications in the fine structure of capillary vessels, in the pericapillary space (presence of glial cells with many multivacuolar osmiophilic formations in the cytoplasm) and in the surrounding parenchyma (increase of nervous cell axes and vacuolization of ganglial cells). The simultaneous application of EPL and methylprednisolone prevented these changes.
5. CLINICAL STUDIES WITH "ESSENTIAL" PHOSPHOUPIDS IN DIFFERENT DISEASES

5.1 Liver Diseases

5.1.1 Toxic Liver Damage

In conformity with experiments in animals, clinical trials were carried out to study the possible effects of EPL on liver damage caused by alcohol abuse, halogenated hydrocarbons, treatment with anti-tuberculous agents, or malnutrition. Subjective and objective signs of improvement were seen in the clinical picture, demonstrated on comparison of final results with pre-treatment findings by an improvement which was more pronounced in the groups receiving the active preparation; in these patients, a return to normal of test variables was also more frequent. EPL increased resistance of the hepatocytes to hepatotoxic agents, particularly when administered concomitantly with known noxious agents such as isoniazid. Clinical assessment of efficacy in intoxication and fatty liver resets on more than 70 clinical trials involving over 4,200 patients.

A representative selection of this work is described in the following chapters.

5.1.1.1 Alcoholic Fatty Liver

The success of treatment of alcoholic liver damage depends essentially on whether the patient is able to curb his alcohol intake or abstain from drinking entirely. Controlled studies (98, 255, 307, 308, 657, 735, 770) have shown that administration of EPL accelerated elimination of fatty deposits from hepatic tissue. Twenty patients with alcoholic fatty liver or alcoholic hepatitis, most of whom had had diagnostic biopsies, were studied on a double-blind basis against a placebo group of the same size (355). Eight weeks after treatment with EPL was begun, all variables showed highly significant improvement against control.

The EPL-treated patients registered a return to normal of the pathologically raised serum levels of AST, ALT, GLDH, AP, LAP, LDH and bilirubin. Gamma-GT, cholesterol and triglyceride reached nearly normal levels. There was a highly significant reduction in the relative proportion of saturated fatty acids in favour of polyunsaturated fatty acids with a particularly marked increase in linoleic and arachidonic acids following administration of EPL. While determination of immunoglobulins showed a decrease mainly in IgA following EPL administration, a complete return to normal levels was not attained during the period under review.

Subjective and systemic tolerance of the active preparation was good throughout. None of the placebo patients showed a return to normal of biochemical values. A controlled study in which EPL was tested against placebo in 40 patients corroborated these findings (628). In the actively treated group, the erythrocyte sedimentation rate and serum activities of AST, ALT and of gamma-GT were reduced. Though the group receiving the active preparation had higher baseline values than the placebo-group, their final values at the end of the 12-week treatment period were lower; an initial significant decrease in these values became apparent after 4 weeks already.

Determination of the area under the curve showed significant differences in gamma-GT and bilirubin values between the actively treated group and the patients given placebo: i.e. both levels were reduced as a result of treatment with EPL (p < 0.05).

After an 8-week combination treatment, consisting of diet, physical exercise and EPL (1.5 g/day), of 6 patients with fatty liver, compiled tomography revealed a significant reduction of the liver fat accumulation (735). There was no essential difference in effectiveness when comparing these results with another group who received diet and a derivative of nicotinic acid.

5.1.1.2 Drug-Induced Hepatic Injury
Concordant data from available studies indicate that concomitant administration of EPL can prevent, or at least substantially diminish, hepatic injury due to anti-tuberculous agents. The results suggest that co-administration of EPL with other known hepatotoxic agents may also be of value (276).

O. Djuric-Milosavljevic (154) compared 240 patients given EPL in addition to a combination of the antituberculous agents isoniazid, streptomycin and rifampicin, with a control group involving 140 patients. Liver damage had been ruled out in all patients before treatment.

In the EPL group, serum transaminase activities rose to levels of up to 50 U/I in 28 patients (11.7%). In contrast, 20 patients in the control group (14.2%) showed an increase to values reaching 50 U/I (p < 0.001). In 9 patients (6.4%) levels even exceeded 50 U/I (tab. 28).

In the EPL-treated group, the damage was apparent after 60 days, in the control group after only 30 days. Clinical symptoms in the EPL-treated patients were mild, 5 patients had no symptoms at all. In the control group, 20 patients complained of mild symptoms, 9 had jaundice, raised bilirubin and/or increased alkaline phosphatase levels.

In a study carried out by S. Hirose et al. (274), 42 patients with tuberculosis were treated with rifampicin and isoniazid. In addition to this treatment they were given EPL orally for 3 months. Only 5 of these patients showed mild liver function disorders; in none of the cases did these lead to the discontinuance of treatment with antituberculous agents.

Under these experimental conditions, EPL not only diminished the hepatotoxic effect of the antituberculous agents, but even ameliorated hepatic function in patients with pre-existing liver damage. In patients whose liver function had almost returned to normal following a 2-week treatment course with EPL, A. B. Insanov et al. (310) were able to resume administration of antituberculous agents without deterioration of hepatic function.

H.D. Kuntz et al. (392) treated 17 patients with tuberculosis with 1 ampoule of Essentiale (= 1000 mg EPL) solution for i.v. drip infusion daily for 3 months in addition to current therapy with rifampicin/isoniazid/ethambutol. 150 patients who were given the antituberculous combination only served as controls. Unlike the patients treated with EPL, who showed mean values of serum AST, ALT, AP, GLDH and bilirubin within the normal range, 95 out of 150 patients in the control group had increased ALT values of 42.3 U/I + 24.4 U/I and in 85 out of 150 patients AST values rose to 39.2 U/I + 17.5 U/I; in 13 of these 85 patients serum AST activity reached values exceeding 50 U/I.

The overall increase in serum transaminase activities was 2.3 to 2.5 times more frequent in the placebo patients than in those given EPL. Four and 8 weeks after the start of treatment with EPL gamma-GT values were reduced. In contrast, levels rose in the placebo group.

<%Fig. 25: Percentage of patients with an increase in serum AST and ALT activities during anti-tuberculous treatment (468)%>
5.1.1.3 Toadstool Poisoning
At least 47 cases relating to the use of EPL in Amanita phalloides poisoning have been published (169, 491a, 591, 693a). The open controlled studies performed by A. Monow and A. Hubenova (491a) demonstrated the value of EPL in addition to basic treatment as compared with basic therapy alone. Basic treatment included sugar solution, other hepatoprotective drugs as well as detoxification and laxative procedures. When treatment was begun between the first and second day of the onset of illness only 3 out of 24 patients died, while the death rate was 7 of 10 patients when treatment was started between the third and fourth day of onset. In the survivors the average duration of the acute intoxication and convalescent phases was shortened by additional treatment with EPL. This was associated with an earlier return to normal of liver function tests (p < 0.05).

5.1.1.4 Hepatic Injury Caused by Chemicals
Isolated reports have been made of very favourable results in cases of poisoning from halogenated hydrocarbons (22 cases of carbon tetrachloride poisoning, 5 cases of dichloroethane poisoning and 1 case of trichloroethylene poisoning), poisoning with antiepileptic drugs (n=38), organophosphorus drugs (n=5) and intoxication of varied origin (n=50) (276, 386, 491b, 497, 693b). As a rule, EPL was administered to these pre-comatose and comatose patients in conjunction with other therapeutic measures. In chronic intoxication with aromatic hydrocarbons (cable industry) EPL treatment improved uptake capacity and secretory function of the liver (577).

5.1.1.5 Diabetic Fatty Liver
In a randomized double-blind study involving a total of 30 patients with fatty liver associated with age-related diabetes, a significant decrease in liver size was observed in the EPL group, but in none of the patients of the control group, 6 months from the start of the therapy (229). As early as 4 weeks after the beginning of treatment, gamma-GT activity was significantly reduced against control. Histological assessment also revealed marked improvement, particularly in the active group; there were 4 successes with EPL compared to only one with placebo.

5.1.1.6 Fatty Liver Due to Malnutrition
Ninety-four children with fatty degeneration of the liver and membrane damage due to kwashiorkor were treated in a controlled study (392). The children were treated with EPL alone or in combination with a high-protein diet. Electronmicroscopic examination showed almost complete disappearance of fat deposits and marked restitution of the membrane structures in the cell nuclei, the mitochondria, the endoplasmic reticulum and the Golgi apparatus after 18 days of treatment. Moderate hypertrophy of the granular endoplasmic reticulum and numerous ribosomes were suggestive of increased protein synthesis. Signs of structural restoration of liver cells and organelles appeared earlier in the group treated with EPL than in the children given a high-protein standard diet only.

In another study by M.Cairella et al. (95) 14 out of 19 patients presented after a 90-days treatment of Essentiale forte (1.8 g EPL/day) + hypocaloric diet clearly improved findings, e.g. in the ultrasonographic picture. 6 out of these had even a normalization of the hepatic picture. In the control group, in contrast, only 3 out of the 20 cases showed slight improvements.

5.1.2 Acute Viral Hepatitis
Based on EPL Incorporation into the damaged membrane structures of the liver cell, the aim of the clinical trials of EPL in acute viral hepatitis (a.o. 307-309, 366, 373, 502, 685, 725, 731) was to facilitate the rapid normalization of
the metabolic processes in the liver and to reduce the susceptibility of the hepatocytes to cytotoxic agents. At present it cannot be determined with certainty whether the earlier disappearance of the HBs antigen from serum, repeatedly seen following treatment with EPL, can be equated with a clinically relevant reduction in the development of chronic hepatitis.

1,313 patients in total were treated with EPL, the duration of therapy being related to the severity of the disease. In contrast to patients receiving basic treatment only, the patients treated with EPL usually reported earlier improvement of symptoms such as dyspepsia, feeling of pressure, feeling of tension, sensation of fullness in the epigastrium, nausea and epigastric pain. Improvement of the clinical parameters (hepatomegaly, ascites) and biochemical tests (AST, ALT, total protein, bilirubin, AP) occurred more rapidly and histological assessment indicated earlier regeneration of the liver in patients treated with EPL (373, 731).

Hospitalization was shortened in the EPL-treated patients who were able to return to work sooner compared with the controls (373, 502, 731). Late sequelae such as progression to chronicity were less frequent. A randomized double-blind study performed by G.Visco (725) corroborated earlier findings from open trials.

After 30 days of treatment, HBsAg was no longer detectable in the sera of 15 out of 30 patients in the EPL group compared with 7 out of 30 patients in the placebo group (fig.26).

Six months after the beginning of treatment, 1 patient in the placebo group was still HBsAg positive, but none in the active group.

56 patients with moderately severe acute or protracted viral hepatitis were treated with EPL for 12-60 days depending on the severity of their condition (373). The investigators assessed the efficacy of the treatment by means of clinical symptoms, laboratory parameters and liver biopsies. A group receiving conventional treatment was used as a control. Histological assessment revealed clearer better results in the patients treated with EPL. Dystrophic and necrotic changes in the liver parenchyma were less pronounced, infiltration by lymphocytes in the portal stroma was reduced, cholestasis was less marked and regeneration of hepatocytes more evident.

H.Wallnofer and M.Hanusch (725) also found histological evidence of marked regression of infiltrations and less frequent individual and group necroses in the patients treated with EPL; a significant decrease in the abnormal content of diffusely distributed iron in the liver was also recorded.

V.Mudric (502) reported an earlier return to normal of total and direct bilirubin levels in their patients with acute viral hepatitis A (n=25) and B (n=25) treated with EPL compared with a control group (n=100). The duration of the illness was shortened in the EPL patients by 13 days on average in those with hepatitis A and 33 days on average in those with hepatitis B.

5.1.3 Chronic Hepatitis

In 67 studies, 2,245 patients with chronic hepatitis were treated with EPL for periods ranging from 3 to 14 months. The following are a representative selection of these studies.

In a controlled trial involving two groups each consisting of 17 patients with chronic hepatitis, M.Yano et al. (754) performed liver biopsies before and 6 months after the beginning of treatment with EPL (group A) or placebo (group B). Assessment of liver biopsy specimens was on a "blind" basis. A statistically significant improvement or a trend towards improvement with regard to ballooning of liver cells, appearance of liver cell membranes, focal necrosis and mobilisation of Kupffer cells was observed in the actively treated patients as compared with the control (tab.29).

Tab. 29: Degree of improvement of liver biopsy findings in CAH; n=25 (754). The figures in brackets refer to patients with normal values.
In patients with chronic hepatitis (219) a 2-week treatment with EPL increased LCAT activity up to normal. At the end of the trial the LCAT activity assessed in this group of patients was higher than in the control group without liver disease.

The number of patients with decreasing serum transaminase values and increasing albumin levels was significantly greater in the group given the active preparation than in the controls.

In a double-blind study (272) clinical symptoms of chronic hepatitis (hepatomegaly, pain on pressure, cirrhosis) showed significant improvement in the patients given EPL for 3 months (n=58) compared with the placebo group (n=66) (p < 0.10). Estimation of biochemical variables 4, 8 and 12 weeks after the start of treatment also showed a greater tendency towards normal levels in the group receiving the active preparation.

Chronic hepatitis varies greatly in severity from patient to patient resulting in large standard deviations in the variables of efficacy. For this reason, the authors stratified their cases. All patients whose pathological levels had returned to normal following treatment were analysed. Furthermore, results of pre- and post-treatment liver function tests in each individual patient were combined and evaluated. The results were published in a second report (273). It was shown that the worse the initial values, the greater was the improvement in liver function with EPL treatment. In patients with high serum transaminase activities placebo was almost ineffective while EPL was able to reduce transaminase levels by more than 50 units. (AST: p < 0.10 after 4 and 12 weeks; p < 0.05 after 8 weeks; ALT: p < 0.05 after 8 weeks; p < 0.10 after 12 weeks).

In a pilot study performed on a double-blind basis, R. Williams treated 10 patients with HBsAg negative chronic active hepatitis (HBsAg CAH) for a period of 6 months (746). In addition to immunosuppressive therapy in accordance with this clinic's standard practice, 6 patients received EPL, the other 4 were given placebo. Histological assessment showed a reduction in portal tract infiltration and piecemeal necrosis in 4 out of 6 patients in the EPL-treated group while histological deterioration was demonstrated in 3 patients in the placebo group. Because of these encouraging findings, a further prospective double-blind controlled trial was performed. This trial lasted 1 year and included 30 patients with HBsAg CAH confirmed by biopsy (321). In accordance with the results of the pilot study (746) only the EPL-treated group showed a statistically significant reduction in disease activity (p<0.05), particularly in portal tract infiltration and piecemeal necrosis. In 3 of the EPL-treated patients, but none of the control group, the disease was judged to have become inactive. According to the authors the results indicate that EPL is of value in cases of HBsAg CAH inadequately controlled by conventional therapy.

In a randomized double-blind trial V. Kordac et al. (364) treated 20 patients with chronic active hepatitis confirmed by 2 biopsies for a period of 1 year. The authors also observed a reduction in portal tract infiltration associated with a decrease in inflammatory activity in the EPL group. Following treatment with the active preparation there was a significant reduction in hepatomegaly (p<0.01), gamma-GT and BSP concentrations (p<0.05) compared with the controls. Serum albumin levels rose and gamma-globulin levels decreased in comparison with pretreatment values during the 9 and 12 months of EPL treatment. These changes were statistically significant at the 5% level.

In a further study, 14 patients with HBsAg+ CAH and signs of cirrhosis of the liver were given EPL for an average period of 11.2 months. They were compared with 11 controls receiving vitamins only (303). During the treatment period EPL led to statistically significant reductions in AST and ALT concentrations (p <
0.01) and prothrombin time (p < 0.05). Similar effects were not seen in the control group.

Inflammation in the liver lobules and portal fields was reduced and parenchyma and piecemeal necrosis diminished in several patients given the active preparation but in none of the control group. In contrast to the controls, no increase in fibrosis or cirrhosis was seen in any of the EPL patients.

These data have been confirmed by the results obtained in a recently performed double-blind trial (305; fig. 27 and 28).

5.1.4 Cirrhosis of the Liver

Assessment of the efficacy of EPL in clinical studies was based mainly on biochemical tests.
Marked improvement of the liver function following EPL administration was interpreted as a sign of a favourable effect on the course of the disease in terms of an increase in the metabolic and detoxifying capacity of the liver. A.P. Pogromov et al. (565) reported improved well-being in their 25 patients with cirrhosis of the liver. After 90 days of oral treatment with EPL nearly all biochemical parameters were found to be within the normal range. M. Kalab and J. Cervinka (332) who treated 30 patients with EPL for a period of 6 months also observed marked improvement in clinical and biochemical findings. Raised IgA decreased to normal levels. At the Liver Symposium held in Sofia/Bulgaria (1982), A.S. Loginov and M.N. Markova (452) suggested a direct correlation between decreased prostaglandin levels in the blood of cirrhotics and the severity of the disease. At the end of a treatment course with EPL an increase in plasma prostaglandin levels was found, which was in good agreement with a trend towards normal in bilirubin, transaminase activities and gamma-globulin levels.

G. Salvioli et al. (595) compared 8 patients with cirrhosis with 10 untreated controls in a study to evaluate the effect of infusions with EPL on morphological erythrocyte changes (thorn-apple forms, target cells) associated with haemolytic anaemia, and on the lipid composition of the erythrocyte membranes. Following treatment with EPL the morphological erythrocyte changes regressed; the molar- ratio of the cholesterol/phospholipids in the membranes returned to normal. Regression of the haemolytic anaemia led to a reduction in unconjugated bilirubin, so avoiding overburdening of the liver with bilirubin. The molar ratio of free cholesterol to phospholipid in the HDL fraction decreased significantly from 0.37 to 0.27. The proportion of unsaturated fatty acids in the membrane phospholipids rose significantly following EPL administration.

P. Fassati et al. (174) studied 61 patients with moderately severe to severe cirrhosis following type B hepatitis. They compared 34 patients given EPL with
27 patients receiving a vitamin preparation. While in the control group there was almost no change against pre-treatment values, the patients given EPL registered an improvement in liver function. In 5 out of 8 patients given EPL, but in only 1 out of 7 patients in the control group, HBsAg was no longer detectable at the end of the 3-month treatment period.

In 24 patients with cirrhosis, confirmed by laparoscopy and biopsy, blood levels of free phenols and ammonia were found to be reduced 1 and 2 hours after i.v. injection of EPL, while the urea level was only slightly raised. D. Müting et al. (505) suggested that EPL probably favours coupling of ammonia with glutamic acid and the conjugation of free phenols. When EPL was given orally to 27 patients for a period of 8 months, D. Müting et al. again observed a significant decrease in blood ammonia. Together with a decrease in BSP retention and AST, ALT, GLDH and SDH activities in serum, this was interpreted as a sign of improvement in the oxidative processes in the liver and the detoxification capacity of the organ.

V. Petera and V. Prokop (552), who administered EPL to 81 patients with compensated, moderately active cirrhosis for 6 months, achieved significant amelioration of the liver function parameters (tab. 30). According to Sh. Sherlock (643), the serum albumin levels reflect the severity of the disease, being of prognostic value and useful in determining the effectiveness of treatment. In the group treated by V. Petera and V. Prokop the highly significant increase in the serum albumin level and the albumin/globulin ratio against baseline (p < 0.001) were the most important changes.

The authors suggested that the improvement in protein metabolism may be an indication of a more favourable prognosis. The decrease of circulating immunological complexes (measured in serum by means of the thymol turbidity test) signals the diminishing intensity of immunological processes. The reduction in ALT activity and in ß2 and ß-globulin levels was significant against baseline at the p<0.01 level. Since their cases were patients with compensated cirrhosis and mildly pathological liver function, the authors attached particular importance to these findings.

Other hepatoprotective drugs (Silymarin, Cianidanol and others) had previously failed to produce a similar effect after such a short period of treatment (551).

5.1.5 Hepatic Coma

Assessment of the efficacy of a particular treatment is made difficult by the severity of the clinical picture, the heterogeneity of the underlying liver diseases and the large number of life-saving measures employed simultaneously.

First experiences with EPL in hepatic coma and pre-coma came from studies by E. Rottini et al. (586) and Y. Sakai et al. (592) who obtained good results. P. Davcev and V. Serafimovski (132) administered a basic treatment consisting of ammonia-lowering agents, glucocorticoids and a low-protein diet to 55 comatose patients by intubation.

35 of these patients received, in addition, EPL in form of a continuous drip infusion. In this group, the reduction in the highly pathological ammonia values as well as in the AST and AP activities in serum was more pronounced than in the controls. 18 out of 35 patients in the EPL group and 7 out of 20 patients in the control group awoke from their coma. However, the extent to which EPL contributes to a better prognosis in hepatic coma remains to be clarified.

In 1989 E. Kuntz (390) published his results with a new galenic EPL application (see chapter 6.2.2.). The patients with severe liver insufficiency received in this pilot study 3g of EPL/day i.v. for 8-16 days. Seven of the 10 patients showed a clear improvement. After termination of the 4-week period of observation 9 of the 10 patients were still alive and had a recompensated and stabilized condition.

In a recent randomized open clinical trial 28 patients with acute icteric fulminant hepatitis and 22 patients with fulminant hepatitis on chronic active hepatitis or on decompensated cirrhosis of the liver were divided into 2 groups.
One group received a standard treatment, the other one was treated additionally with 2-4 ampoules Essentiale i.v. (500-1000 mg EPL/day over 14 days (751). Subdividing the 2 groups according to the above-mentioned types of fulminant hepatitis showed a significant difference in survival rate and survival time for the Essentiale group in comparison with the standard treatment group (753).

5.1.6 Effect on the Composition of Bile
Open clinical studies have given first hints of an improvement in the composition of bile following EPL administration, which may point to an inhibition of cholesterol gall stone formation. The postulated mechanisms underlying this action are: decrease in the cholesterol/phospholipid ratio associated with increased cholesterol solubility due to the emulsifying properties of EPL, and replacement of biliary phospholipids by "essential" phospholipids containing a large proportion of polyunsaturated fatty acids.

In a study carried out by G.Salvioli et al. (598) the linoleic acid deficiency in biliary phosphatidylcholine seen in patients with gallstones was corrected by administration of EPL. K.R.Holan et al. (277) also observed an increase in the concentration of unsaturated fatty acids in biliary phosphatidylcholine following administration of EPL. J.Tououli et al. (704) found an increase in the biliary deoxycholic acid concentration in their patients. A trial performed by L.Stiehl et al. (679) showed that a combination of EPL and chenodeoxycholic acid was more effective in reducing the lithogenic index of bile than the individual compounds given alone. Combination with EPL substantially reduced the incidence of side-effects of chenodeoxycholic acid. In a double-blind trial (83) performed in 8 patients after gallbladder surgery, bile secretion in the common bile duct was examined for 10 days after the operation. The cholesterol content of bile decreased in relation to the EPL dose and the lithogenic index was reduced. The clinical relevance of currently available results is matter of further investigations.

5.1.7 Stimulation of Regeneration
18 clinical studies (5 open, 8 single-blind, 5 double-blind ones) with 714 patients carried out during the period of 1968 to 1990 prove the increase of albumin (12 studies 6 of which with significant results), the CHE activity (3 studies, 2 of which with significant results), and the A/G (4 studies, 3 of which with significant results) (tab. 31). From the histological and cytological points of view, especially an increase in the regenerative activity of the hepatocytes and the liver tissue (8 studies) is indicated. In a study on children suffering from kwashiorkor (11, 695), electronmicroscopic findings reveal modifications at the smooth and rough ER, the Golgi's apparatus and the mitochondria which hint at regenerative processes within the hepatocytes under the treatment of EPL. The remaining EPL studies were carried out during periods of 2 weeks to 12 months with patients suffering from chronic hepatitis, cirrhosis of the liver, fatty liver or alcohol intoxication. EPL was administered orally, and additionally intravenously by A.P.Pogromov et al. (565) and S.Grunevska et al. (240).

5.1.8 Summary of Clinical Findings
- In clinical studies EPL was given to patients with toxic liver damage, particularly fatty liver of varied origin, intoxication, acute viral hepatitis, chronic hepatitis, cirrhosis of the liver, hepatic coma and in conditions associated with changes in the composition of bile.
- Depending on the severity of their condition the patients were treated for periods of up to one year or even longer.
- The hepatoprotective effect of EPL previously demonstrated in models of experimental liver disease, was shown both in patients receiving an otherwise
therapeutic regimen known to be hepatotoxic and in those with manifest toxic liver damage. Comparison with controls indicated an earlier return to normal of hepatic enzyme activity following administration of EPL; fatty degeneration of the liver was less pronounced and structural restoration of damaged liver cells occurred more rapidly. In life-threatening intoxication associated with severe liver injury, EPL reduced the mortality rate and accelerated recovery of the patients.

- In patients with acute viral hepatitis EPL was also effective in speeding up recovery. Both the length of stay in hospital and the recovery phase were significantly shorter in the EPL-treated patients than in the controls.
- Patients with chronic hepatitis of varied origin experienced rapid improvement in well-being. Objective signs in support of this finding were changes towards normal laboratory parameters and in particular, substantial improvement in the histological picture with reduction in portal tract infiltration and piecemeal necrosis, reflecting a marked decrease in disease activity. All patients whose disease had even become inactive had received EPL treatment; in other words, in the controls disease activity was not arrested. EPL was also shown to be effective in cases of HBsAg CAH inadequately controlled with standard immunosuppressive therapy.
- In cirrhosis of the liver the success of treatment was determined by the stage of the disease. The effect of EPL in this condition was manifested as a marked improvement of the patient's sense of well-being and a reduction in free phenol and ammonia concentrations, reflecting amelioration of the oxidative processes and the detoxification capacity of the liver.
- The severity of the clinical picture of hepatic coma calls for a variety of therapeutic measures. By exerting a favourable effect on liver function, EPL, used in clinical trials in conjunction with other measures, helped to save the patients' lives. The use of EPL in hepatic coma requires further studies.
- The clinical studies in which a possible improvement of the composition of bile by EPL is under investigation, also await completion, but available results indicating a reduction in the lithogenic index of bile provide promise of efficacy.

5.2 Kidney Disorders

23 clinical studies on this subject are available and, in addition, 3 publications on CAPD. Nine papers date back to the sixties. From the seventies 3 reports are available, whereas since the eighties up to now the interest in the application of EPL in renal disorders has increased again.

The following subdivision into glomerulonephritis, renal insufficiency and changed electrolyte excretion serves rather to get a clear presentation of the obtained results, although the patient groups cannot be divided so clearly into these pathological pictures.

5.2.1 Glomerulonephritis

K.Jacyszyn and R.Szymanski from Wroclaw reported already in 1961 about favourable effects of EPL in the therapy of chronic glomerulonephritis associated with nephrotic syndrome in 11 patients (317). After a 6-day treatment with 1000 mg EPL i.v. daily, 6 of 9 patients showed already a reduction of edema. Serum albumin increased by 35% (0.4 g/dl), total cholesterol and total lipids fell by 13.7% and 17.2%, resp. The increase in albumin was statistically significant. No information is provided about the therapeutic action on proteinuria. The authors attributed the observed effects to the activation of albumin synthesis in the liver cells.

Afterwards, a large-scale controlled study was carried out by A.D.Petrushina et al. in 3-15 year old children suffering from acute and chronic nephritis (555). 24 patients received basic treatment, 25 additionally Essentiale i.v. for 10 days and subsequently Essentiale forte for 20 days at a daily dose of 2 to 2.5 mg EPL/kg body weight.
Despite the astonishingly low dose, renal and extrarenal manifestations of the disease disappeared significantly earlier, symptoms of intoxication were reduced (palor and dystrophic changes of skin and mucosa, asthenia, acidosis, dystonia), blood pressure was normalized, hepatomegaly and haematuria, proteinuria, hypoalbuminemia and leucocytosis disappeared. The effect of Essentiale became particularly clear in the presence of nephrotic forms.

Stabilization of the renal excretion of phospholipids - e.g. reduction of lysophosphatidylcholine excretion - was observed. Moreover, the ratio of easily oxidized phosphatidylserine and phosphatidylethanolamine to not easily oxidized sphingomyelin and phosphatidylcholine in the urine was stabilized.

Methodological uncertainties in this paper (e.g. kind of basic therapy, unusually low EPL doses), however, limit the value of these results. Therefore, in connection with the study by K.Jacyszyn and R.Szymanski it just serves as a hint that EPL can be helpful as adjuvant therapy in glomerulonephritis.

Further studies with patients suffering from glomerulonephritis (M.Dobiasova et al. (155), V.G.Kukes et al. (383)) will be described in the next chapter in connection with disorders of the lipid metabolism.

5.2.2 Renal Insufficiency

A series of studies into renal insufficiency confirm the presumption that EPL constitutes a valuable additional therapy in kidney diseases.

In another study K.Jacyszyn et al. (318) divided the patients into 2 groups: one group consisted of 10 patients with an urea level lower than 100 mg/dl (moderate renal insufficiency), the other consisted of 9 patients with an urea level exceeding 100 mg/dl. A first 5-day phase of EPL medication (1000 mg i.v. and 750 mg orally) was followed by oral administration of 1500 mg EPL over 10 days.

In group 1 were achieved significant rises of creatinine, urea and sodium clearance. In 5 patients was observed a complete clinical remission including normalized blood pressure. 3 further patients exhibited clear improvements, whereas no changes were observed in another 2 cases. The 9 patients with advanced renal insufficiency showed a significant reduction of creatinine and cholesterol concentrations in the serum. The increases of creatinine clearance, urine volume and of the clearance values of sodium and potassium were also significant. The authors consider the stabilization of renal cell membranes to be one of the main effects of EPL.

Similar results were obtained by V.Martinez Llinares in his investigations into raised non-protein nitrogen and blood pressure in 2 patients with nephrosclerosis (476).

The favourable action of intravenously injected "essential" phospholipids on renal function was corroborated by L.Mainieri and A.de Lutterotti even in single i.v. administration of 250 mg EPL (464). They examined the renal function of 12 patients without renal disease by means of a clearance test. With the exception of 1 patient with exudative pleuritis, in all of them were found increases of the glomerular filtration rate, of renal plasma flow and of renal blood flow. This effect was observed already 30 minutes upon administration, from which the authors deduced a direct effect of EPL on the kidneys.

K.Deibert and R.Juchems (141) also described improved glomerular filtration after a single EPL dose of 2 g, in this case orally administered. 22 patients were included into the study, among them 7 with hypertension, 4 with fatty liver, 1 with glomerulonephritis, 2 healthy persons, and other patients not suffering from renal disorders. A significant fall of serum creatinine from 1.54 to 1.37 mg/dl was found. Creatinine clearance also increased significantly from 74 to 90 ml/min. The duration of observation was 24 hours and started directly after EPL administration. Also these authors postulated a direct action of EPL on the cell membranes of glomeruli; it appears that the permeability coefficient is increased by the phospholipid administration.

Another study with 9 boys and girls, aged 9 months to 13.5 years, suffering from nephrotic syndrome, is of interest (572). Children younger than 3 years...
were given 330 mg and 5 school children 700 mg EPL daily. In 1 case EPL was administered in combination with deltacortisone.

7 children with nephrotic syndrome presented edema at the beginning of treatment. In 2 cases the edema disappeared and were reduced in the remaining 5 patients. Reduced diuresis, which had been 300–750 ml/24 h and 160 ml/24 h in 1 case, was increased by 64%. Albumin in urine was 0.5 to 10 g% before treatment, and was raised by 4% in 6 children. Serum cholesterol was reduced from 421 mg/100 ml to 321 mg/100 ml. In 6 of 8 children the serum lipids of 1392 mg/100 ml were reduced by an average of 33%, the extent of improvement depending on the initial values. The 9-month old baby with idiopathic nephrotic syndrome showed no reduced serum lipids; this was also true for the child receiving EPL and prednisone.

A positive result on lipid values was described in further studies (F.Pupita and C.Gagna 1969 (576), K.Moriya et al. 1979 (500), V.G.Kukes et al. 1985 (383), M.Dobiasova et al. 1988 (155), R.Kirsten et al. 1989 (350)). EPL improved significantly the secondary hyperlipidemia provoked by renal disorders.

M.Dobiasova et al. (155), for example, investigated into the effect of EPL on serum lipid levels and on LCAT activity in 18 patients with chronic glomerulonephritis accompanied by hyperlipemia and reduced rate of cholesterol esterification in the plasma.

The effects of therapy were evaluated immediately after the 2-month treatment period, and again after the subsequent 3-month interval without medication. The immediate effect of the therapy was reflected in a significant increase in the fractional esterification rate and in a marked reduction of triglyceride concentration. The discontinuation of the medication resulted in the return of the values of triglycerides and fractional esterification to the initial levels and in a rise of total and unesterified cholesterol, of HDL cholesterol and of the molar esterification rate. The activity of LCAT determined by radioassay in common and endogenous substrates varied at the same time. The increase in HDL cholesterol - which persisted even 3 months after the end of the therapeutic treatment - paralleled by raised LCAT activity and, surprisingly, by a rise in unesterified cholesterol, made the authors suggest that by influencing LCAT activity EPL can control the rate of cholesterol efflux from the tissue and its transfer into the plasma pool.

A.P.Peleshchuk et al. (548) obtained different findings in 11 patients with chronic renal failure. They found that EPL produced a positive effect on the phospholipid metabolism (reduction of the relative percentage of lysolecithin and sphingomyelin fractions, increase in lecithin), but that it did not improve the metabolism of neutral lipids (except free cholesterol) and of beta-lipoproteins.

In the last 2 trials of this series P.Dewailly et al. (147) demonstrated in-vitro under hypotonic conditions that EPL increases the osmotic resistance of erythrocytes of patients with renal insufficiency; and K.Hupe et al. (299) found less particulate fat in the urine of patients with large bone fractures and surgery of fractures when high doses of i.v. EPL were applied.

5.2.3 Electrolyte Metabolism

In 1960 D.P.Mertz et al. (485) reported for the first time about effects of a highly purified choline phospholipid fraction on the human electrolyte metabolism, after intravenous application. 20 probands received 10 ml of a 10% hospholipid solution. It is interesting that there were no acute effects on the glomerular filtration rate and on renal water excretion. Instead, the extracellular space increased, and potassium and phosphate excretion were significantly diminished with simultaneously raised serum values. Additionally, an isolated fall of the magnesium level was measured without concomitant changes in the magnesium excretion. The authors attributed the effect to an influence on the ion transport through the cell membranes and suggest correlation correlations with the tubular urine concentration.

In 15 patients with histologically confirmed fatty liver R.Juchems and W.Gross (327) investigated the effect of 3 x 2 capsules Essentiale daily (1050
mg) on urine electrolytes; 5 healthy volunteers served as control. Within the control period of 5 days both groups showed a clearly enhanced excretion of sodium and chlorine whereas potassium uresis was not significantly changed. The findings were discussed under consideration of an aldosterone effect and an effect on the glomerular filtration.

In a complementary study the authors demonstrated that the simultaneous administration of phospholipids combined with hydrochlorothiazide or furosemide leads to a further increase of sodium uresis, whereas potassium retention was highly significant (328).

It is only since the end of the seventies that the effect of the applied phospholipids has been related to an influence on the prostaglandin metabolism. P. Bernardi et al. had demonstrated already experimentally in rabbits that EPL increased the PGE2 synthesis in the kidney and provoked hypotensive polyuria as a consequence of enhanced glomerular filtration and reduced water permeability in the distal nephron (see chapter 4.1.5). These authors performed then a series of clinical investigations which were published in several medical journals between the years 1982 and 1986 (55-57). These studies focused on the acute effect and on that of a 3-day application of i.v. EPL on renal function in healthy volunteers, in patients with chronic renal insufficiency, cirrhosis and cardiac insufficiency. The acute effect of the preparation was assessed with a dose of 3.5 mg/kg b.w. over a period of 90 minutes. 30 minutes later, part of the patients were given additionally acetylsalicylic acid at a dose of 10.5 mg/kg b.w. In order to examine the continued effect of EPL the afore-mentioned dose was administered for 3 days. In a second study series acetylsalicylic acid was given the last day at the afore-described dosage.

Most of these persons showed an increase in renal plasma flow, of glomerular filtration as well as of renal sodium and water excretion after EPL application. These changes were reversed by acetylsalicylic acid, and additional EPL administration showed no further effects. Basically the same results were obtained in the investigation into the continued EPL effect. The changes were also reversed by acetylsalicylic acid. The hemodynamic changes due to EPL were explained by the stimulation of the renal prostaglandin synthesis. Part of the examined persons presented increased PGE2 excretion with the urine. Also this effect could be inhibited by acetylsalicylic acid.

In 1985, G. C. Agnoli and co-workers (6) reported about the effects of indomethacin and/or EPL on renal function during diuresis and antidiuresis. They carried out acute trials following indomethacin pretreatment (2.5 mg/kg b.w. p.o. daily for 2 days, and then 100 mg 60 min. before renal function test) with or without EPL (13 mg/kg b.w. daily for 2 days, and then 300 mg before the tests). In contrast to EPL, the indomethacin-pretreated group showed a reduction of water clearance and of renal prostaglandin excretion (PGE), and a significantly increased urine plasma/osmotic ratio and osmolar clearance. In the indomethacin group, additional vasopressin produced a further diminution of the urine flow, of free water clearance, of the creatinine clearance and of the reduced urinary excretion of PGE. Urine osmolarity and the blood pressure increased. Combined treatment with EPL reduced the effectiveness of indomethacin in potentiating vasopressin effects. No significant difference in the prostaglandin excretion was found between the pretreatment with indomethacin alone and with indomethacin and phospholipids.

5.2.4 Chronic Ambulatory Peritoneal Dialysis (CAPD)

N. Di Paolo and co-workers (149) were the first to perform interesting investigations into the EPL administration in CAPD, which is an interesting alternative of chronic hemodialysis allowing the patient much more independence and freedom to move at considerably lower costs. On the basis of their considerations was the fact that the peritoneal effluent of patients on CAPD not only contains substances to be eliminated with the urine, such as electrolytes, creatinine and urea, but also a surface-active material which is probably secreted by mesothelial cells. This surface-active material, mainly consisting
of phospholipids including phosphatidylcholine, was presumed to play a role in
the ultrafiltration during peritoneal dialysis since it lowers surface tension,
helps to repel water and acts as a lubricant. The phospholipid level in the
dialysis effluent of patients who had been on CAPD for a long time are lower in
comparison with patients undergoing their first days of peritoneal dialysis. A
more drastic and significant decrease in phospholipids is observed even in
patients with low ultrafiltration and in those with peritonitis.

The idea of N. di Paolo and co-workers was to check if the addition of EPL
into the dialysis fluid was able to modify the water transport in patients with
low ultrafiltration and peritonitis. The authors found that during dialysis
exchanges containing phosphatidylcholine (50 mg/I) the mean ultrafiltration
increased significantly in the 10 patients with low ultrafiltration or
peritonitis, indicating that the substance was able to restore normal
physiological conditions (fig. 29). The significant increase observed 72 h
following EPL addition was maintained throughout the investigation period of 15
days. Moreover, creatinine and urea clearance increased significantly. EPL
seemed to act also when administered intravenously (250 mg daily) and orally
(400 mg daily). No improvement was seen in patients with normal ultrafiltration.

A. De Vecci et al. (140) did not corroborate the positive results obtained by N.
di Paolo et al. in their study with 800 mg oral EPL daily given to patients with
reduced peritoneal ultrafiltration.

The fact that exact methodology seems to be of decisive importance for the
successful application of EPL in CAPD has been underlined by a recent
publication by N.V. Dombros et al. (157), who observed increased ultrafiltration
after EPL administration even in the normal peritoneum. This difference with
respect to the results obtained by N. di Paolo et al. may be explained by the use
of a higher dose of phosphatidylcholine (125 mg/I). It appears that the effect
of EPL in CAPD is dose-related.

The following and last study from the field of EPL and renal disorders will
be described apart because it serves at the same time as introduction for the
next chapter, 5.3 gestosis (R.I. Shalina et al., 642):

145 pregnant women with late gestosis were allotted to different groups
according to the severity degree of nephropathy, and treated with conventional
routine therapy (consisting of psychotropic substances, diuretics, spasmylics,
antihypertensives etc.) and additionally with vitamin E and C and/or Lipostabil.
The aim of the treatment was to maintain the
pregnancy to term, as far as possible, and to improve the chances of the
delivery of a viable child.

The more severe the gestosis was, the higher were the levels of lipid
peroxidation (LPO) products in both the serum and the erythrocyte membranes,
whereas antioxidative activity in serum decreased due to the reduction in the
ceruloplasmin levels and - the ceruloplasmin/transferrin coefficient; 
microviscosity of membranes increased.

In some patients, particularly in mild cases, already routine treatment led
to the disappearance of symptoms. When reducing the dose, however, lipid
peroxidation increased again. When additionally to routine treatment vitamins E
and C were given, lipid peroxidation activity could be normalized within 7–14
days. Especially in severe nephropathy, however, this treatment was not
sufficient to restore the structural and functional integrity of cell membranes.

In comparison with other therapeutic measures, Lipostabil (in combination
with vitamin E) favoured the antioxidative activity in serum to a larger extent
(raised ceruloplasmin/transferrin coefficient): malonedialdehyde level reduced
by 30% in contrast to 20% with Lipostabil alone. The inhibition of LPO activity within 7-14 days correlated with the restoration of the barrier function of the lipid bilayer of cell membranes, also in patients suffering from severe nephropathy.

The authors interpret the LPO activation and the structural modifications of cell membranes as important pathogenic mechanisms of late gestosis.

5.3 Gestosis

Gestosis, toxicosis, and preeciampsia are synonyms for a multifactorial manifestation during pregnancy, which may present different degrees of severity and occur in early pregnancy (e.g. hyperemesis gravidarum) or during the last months, or even short time before delivery (late gestosis).

Risk factors appear to be first pregnancy of the mother and, especially, when she is of an advanced age already, preexisting vascular alterations, chronic nephropathies, disorders of liver function and diabetes mellitus. A large number of hypotheses on the origin of the often dangerous syndrome have been established; none of them, however, proved to be convincing.

On the pathophysiological level can be seen, among others, uteroplacental-disorders of circulation with morphological changes of the placenta, partly abruptio of the placenta, modified vessel function (angiospasm) and coagulation disorders, hepatic and renal insufficiency with associated metabolic disturbances.

The fetus runs the risk of retarded growth, hypoxia and premature birth; at the same time, the syndrome threatens the mother's life. Considering the severity of the condition, particularly predisposed pregnant women need to be carefully controlled to impair the disease, or at least to early diagnose and treat it adequately.

Due to the multifactorial character of the condition, a large number of measures are required to retard the birth of a vital child to the calculated term, to reduce the rate of perinatal mortality, and to prevent the death risk for the mother.

First of all have to be taken measures to reduce hypertension, to dissolve vascular spasms, to improve renal circulation and liver function, and to support with high-protein nutrition.

On the basis of his wide-spread experience In this field H. Graf (232) recommended the administration of "essential" phospholipids (Essentiale) in addition to basic therapy, since EPL support liver function and favour the regression of edema.

Since 1963 have been carried out 13 investigations in a total of 684 pregnant women presenting the syndrome with various degrees of severity; the patients were treated additionally with Essentiale ampoules and/or Essentiale forte capsules (tab. 32).

a) Early Gestoses

H.G. Mücke (501) reported already in 1963 about the successful Essentiale treatment of 47 patients suffering from severe hyperemesis gravidarum. In 39 out of them symptoms disappeared already after 1-2 injections; in the remaining 8 patients 3-4 injections were necessary. The Essentiale ampoules (250 mg) were administered intravenously every other day.

Good results were obtained also in patients with severe premenstrual vomiting.

At a later date, J. Hartel (252) confirmed these positive results. In a patient with intact intrauterine pregnancy, who consulted the doctor in gestation week 18, nausea and vomiting subsided already after the first Essentiale injection (250 mg EPL/day). At the end of the 8-day treatment the patient was completely free from complaints.

In another 6 patients with less pronounced symptoms were also obtained positive results with Essentiale treatment.
F. Jaisle (319), in contrast, could not achieve relief from symptoms in his patients suffering from hyperemesis gravidarum, even when increasing the dose to 1 g EPL per infusion/day over 4 days.

b) Late Gestoses

In a much larger number of patients (n = 568) Essentiale (in combination with basic measures) was given in the last trimester of pregnancy. In a group of 52 patients in gestation months 6–9 (n = 22) or short time before delivery (n = 30) it was striking to observe how rapidly clinical symptoms disappeared with daily injections of 2 Essentiale ampoules (500 mg EPL/day). On an average 7 days of treatment were necessary. Particularly edema subsided, liver and kidney function values as well as diuresis were normalized. F. Bottiglioni and R. Tirelli (76) pointed out that with conventional treatment alone they rarely saw such rapid and complete improvements.

D. Arandelovic et al. (22) extended the daily application of 2 Essentiale ampoules or 6 Essentiale forte capsules to their patients (n = 42) to 10–15 days, and administered simultaneously progesteron substitution. In their study they concentrated on liver function parameters, because the symptoms in gestosis are largely related with impaired liver function. In comparison with previously treated patients perinatal mortality was reduced in these cases. Nuclear icterus was not observed in any of the patients.

The aim of the study by M. Kovacevic and S. Gavric (368) in 37 patients with preeclampsia was to eliminate gestosis-induced liver damage by means of 3–6 Essentiale forte capsules for 7 days. After the 7-day treatment total protein and albumin values increased, and improvement of the albumin/gamma-globulin ratio was obtained. Serum transaminases continued to fall within the normal range. The subjective well-being of the patients improved quickly.

D. de Aloysio (136) and E.K. Ailamazyan (7) concentrated on the clinical picture of disordered lipid metabolism. With EPL both authors observed clear improvements and even normalization of the pathological lipid values. According to E.K. Ailamazyan the penetration of total lipids and triglycerides through the placenta and their utilization by the fetus were increased, thus promoting prevention and treatment of intrauterine fetal hypotrophy.

G. M. Rendina et al. (581) assessed the duration until disappearance of symptoms. They administered infusions of 1 g EPL/day for 10 days to 50 patients with gestosis of different degrees of severity and compared the results with those obtained from another group of 50 patients who were treated in addition to basic therapy with infusions of glutathione, vitamin B12 and uridine-5-triphosphate glucose. In the EPL-treated group 46 patients presented no symptoms any more after 10 days already, and the remaining 4 patients after 15 days; in the reference group, 40 patients were free from symptoms after 10 days, and the remaining 10 after 22 days.

R. I. Shalina et al. (642) divided the patients with late gestosis according to the severity of nephropathy. The authors focused on the influence of EPL on the production of lipid peroxides (see also chapter 5.2.4, page 134).

Also F. R. Kurbanova (394) concentrated on the LPO products in the serum of pregnant women. In addition to basic treatment 52 patients received for 20 days 1.8 g oral EPL/day; 70 pregnant women served as control group. Lipid peroxidation was clearly inhibited with EPL, and the values almost reached the level of the control patients not suffering from impaired liver function. The effect on liver function was more pronounced in most cases than with basic therapy alone. E.K. Ailamazyan (8) confirmed the significance of the antioxidative effect of EPL for the treatment of late gestosis and the related fetal hypoxia in his investigation into 549 pregnant women. He treated 153 of them with Essentiale. The administration of the preparation in this indication is justified in his eyes to inhibit membrane damaging lipid peroxidation and to favourably influence liver and kidney function.

5.4 Dyslipidemia and Atherosclerosis

The first described trial links chapter 5.1 with the following one. The central position of the liver in lipid metabolism leads to the possibility that
EPL as a potential membrane therapeutic may be effective in liver diseases and in lipid metabolic disturbances at the same time.

L.Zvenigorodskaya and I.E.Speranskaya (776) reported that the additional treatment with EPL or silymarin in long-term cardiovascular insufficiency entailed improvement of liver function, increased subjective well-being and improved cardiovascular status. Essentiale i.v. was more efficient than silymarin.

5.4.1 Effects on Serum Cholesterol

In the study group of the European Atherosclerosis Society (EAS) there now is general agreement on the significance of serum cholesterol as a risk factor for early cardiac death, coronary/ischaemic heart disease and other manifestations of atherosclerosis. On the occasion of the European Consensus Conference on the Prevention of Coronary Heart Disease (119) the study group recommended concentrations of up to 200 mg of cholesterol/dl serum as the upper normal limit.

Although it is true that cholesterol is an essential constituent of all cell membranes and serves as a support for the membrane-forming phospholipid bilayers, an excess of it in serum (and in the membranes) renders them rigid. As a result of an unfavourable alteration of the activity of membrane-bound enzymes, membrane lipid metabolism is then reduced.

The aim of any treatment, therefore, is to lower cholesterol levels in serum.

The response of total serum cholesterol to EPL treatment has been assessed clinically in 3836 patients. Especially in the early studies (up to 1960) this parameter served as the main basis for evaluation of the influence of EPL; this was due to the fact that more sophisticated diagnostic methods had not yet gained ground, for one thing, and that authors were of the opinion that the reaction pattern of total cholesterol decided that of blood lipids, for another.

Reduction of Serum Cholesterol:

In the majority of trials an average reduction of total serum cholesterol by 12 to 19 % was observed under treatment with EPL; in some of the trial groups mean values were reduced by more than 20 % as against initial values, yet others were lowered by 7 to 10 % only.

In a documentation of 15 clinical trials with a duration of EPL treatment ranging between 1 and 12 months, total serum cholesterol was lowered by 8.8 to 28.2 % (172). The level of initial values, the route of administration, EPL dosage and duration of treatment seem to be the main determinants for the slope of the reduction. Nine to 20 days of intravenous EPL treatment, for instance, already caused a reduction of total serum cholesterol of approx. 13 % (65, 66, 99, 543-546, 557).

An initially simultaneous administration of EPL capsules and solution for injection led to a pronounced decrease in cholesterol (15); the author had introduced treatment on a dosage scheme of 250 mg i.v. + 875 mg orally and observed a further, though markedly slower decrease in cholesterol concentrations when continuing treatment on oral EPL alone.

After an initial 2-week intravenous administration of 1 g of EPL/d. Peeters et al. (543-546) even registered a slight rise in serum cholesterol values when therapy was continued orally on 1.8 g EPL/d, though they did not return to initial levels. Under oral treatment (1.05 to 2.7 g of EPL/d) successful lowering of total cholesterol obviously depended on basal values at the onset of therapy: starting from moderately elevated total cholesterol levels (up to approx. 400 mg/dl) the reduction became all the more noticeable, the higher initial levels had been (264, 312, 336, 543-546, 569, 635, 646, 714).

Duration of Treatment:

The duration of treatment is of varying importance for a reduction of total cholesterol. In trials using intravenous EPL, the preparation was only administered for periods of 9 to 20 days in most cases. In spite of these short
periods of time the reduction of total cholesterol levels in serum proved satisfactory (mean values between 12.8 and 13.8%).

Oral administration of EPL mostly covered periods of 4 weeks to 6 months.

12 trials involved long-term studies which included at least a small number of patients who had been subjected to treatment periods of 0 to 24 months (14, 301, 316, 480, 488, 523, 560, 589, 646, 647, 698, 720, 760).

Some investigators (99, 543-548, 560) reported slight transient elevations of serum cholesterol at the beginning of treatment. According to the authors mobilization of cholesterol from vascular walls can be offered as an explanation for this phenomenon in atherosclerotic patients.

Moreover, it is assumed that with increasing age there is a slowing down of cholesterol clearance, which also may be responsible for the effects observed.

In contrast to these results a double-blind trial by A.K. Horsch et al. (288) using 1.8g of oral EPL/d led to mean reductions of total cholesterol by 12.7% already within 14 days of treatment. After another 4 weeks reduction of initial values totalled 18.9% (2p<0.001) (tab. 33).

After oral EPL treatment over 3-4 (to 16) weeks, mean rates of cholesterol reduction in another 4 studies (101, 656, 700, 755), that were either controlled against a diet, double-blind, controlled against placebo or were open, ranged from 12 to 25% as compared with initial values.

Diet plus EPL:

While diet alone did not produce satisfactory reductions in most cases, it clearly enhanced the effect on serum lipids when applied together with EPL (356, 700, 720). G. Varkonyi (720), for instance, observed that in spite of continuing the diet, serum cholesterol rose once more when EPL treatment was withdrawn and decreased only when therapy was taken up again.

On the whole, the authors considered EPL to be effective in the sense of reducing raised total cholesterol in serum, especially when there was only a moderate elevation of initial values (up to 400 mg/100 ml). Sufficiently high EPL dosages and an adequate duration of treatment were of decisive importance.

5.4.2. Effects on LDL Cholesterol in Serum

Being the main carriers of cholesterol, low-density lipoproteins (LDL) are particularly atherogenic. High levels in serum damage the vascular endothelium and in this way facilitate a receptor-independent cholesterol diffusion through vascular walls. In other words, apart from the receptor-mediated physiological uptake, there is another, uncontrolled uptake of cholesterol leading to an enhanced accumulation of cholesterol in the cells. Preventive therapeutic measures against the manifestations of atherosclerosis, therefore, aim predominantly at lowering serum levels of LDL cholesterol.

According to our present state of knowledge, LDL cholesterol levels permit a relatively reliable rating of the risk of coronary sclerosis to be made. This function of LDL is backed by sound and acknowledged pathophysiological mechanisms (a.o.127).

The response of LDL cholesterol to EPL treatment has been observed in clinical studies in approx. 1160 patients with the reduction of LDL cholesterol ranging from 10 A to 31% of mean initial values. The extent of reduction was determined by the type of hyperlipoproteinaemia involved, the homogeneity of the case material, the EPL dosages as well as the duration of treatment.

The study of H. Peeters et al (543-546) demonstrated the need for an adequately long duration of treatment. A 14-day treatment with 250 mg/d of intravenous EPL did not lead to distinct changes in the serum profile of lipoproteins. According to U.Svanberg et al. (687), this initial failure to reduce LDL cholesterol may reflect an intensified catabolism of VLDL to LDL.

Reduction of LDL Cholesterol in Serum:
P. Dewailly et al. (148) and A.K.Horsch et al. (288) carried out double-blind trials against placebo with oral doses of 2.7 g EPL/d or of 1.8 g EPL/d resp.; already on the 14th or 21st day of treatment they registered a drop in LDL cholesterol of 12% and 20% respectively (tab. 34). In a controlled cross-over study (24) mean reductions of 25.8% in the initial LDL cholesterol levels were obtained within a 2-month therapy period. After a treatment period of up to 218 days M.Murakami and H. Sekimoto (503) achieved average reductions of 25.5%; P.Saba et al. (590) observed a mean reduction of patholgical initial values of 27.9% within 120 days of treatment.

Highest average reductions of LDL cholesterol, viz. 34.1% after 42 days of treatment, were recorded by A.K.Horsch et al. (288) in the double-blind trial described earlier.

In another double-blind trial (635) in which patients had received clofibrate and clofibrate plus EPL, J.Schneider et al. observed that EPL slowed down the rise in LDL cholesterol induced by clofibrate.

Tab. 34: Reduction of LDL cholesterol in 7 double-blind studies [lla = type lla according to Fredrickson; n.s. = not significant]

Cholesterol Esters:
H.Ditschuneit et al. (152) obtained evidence for an increase in cholesterol linoleic acid d esters in LDL, for instance, in a pilot study including healthy volunteers with diet-induced hyperlipoproteinaemia; this phenomenon had already been described by H.Peeters et al. (545, 546) in 1974 and later was confirmed by V.Blaton (66). Blaton attributed the increase in LDL cholesterol esterified with linoleic acid, to an activation of LCAT and to an increase in the enzymatic activity of cholesterol esterase under EPL treatment.

This result is of importance, because the rate of hydrolysis of cholesterol esterified with highly unsaturated fatty acids (e.g. linoleic acid) is higher than with saturated esters, so that while cholesterol linoleic acid ester is hydrolysed more rapidly, serum clearance of LDL cholesterol is accelerated, too. This effect triggered by the administration of EPL is a step towards the prevention or inhibition of vessel wall lesions induced by atherogenic LDL cholesterol (66).

In summary it is safe to say that a distinct lowering of LDL cholesterol in serum has been achieved in almost all investigations with EPL. As the results of the LRC-CPP Trial (Lipid Research Clinics Coronary Primary Prevention Trial (459)) have demonstrated, a decrease in cholesterol of 1% lowers the coronary risk to a patient by about 2%. Hence even a less pronounced reduction in total cholesterol and serum LDL cholesterol will be of decisive importance in the long run.

5.4.3 Effects on HDL Cholesterol in Serum
In association with the LDL cholesterol levels HDL cholesterol concentrations (as well as those of HDL subfractions and apoproteins) and the LDL-/HDL-cholesterol ratio may serve as an indicator of the atherosclerosis risk of a patient, and in this capacity represent a criterion for the requirement for drug therapy of raised serum levels of cholesterol and triglycerides.

HDL suppresses LDL-binding to smooth muscle cells and inhibits the proliferation of smooth muscle cells into the media of arterial vessels thus weakening the damaging effect of LDL cholesterol on the endothelium (260). Consequently, any drug therapy is aimed at enlarging the HDL capacity for cholesterol uptake from LBL and the vascular wall, so that serum LDL cholesterol as well as total cholesterol are reduced and an accumulation of cholesterol in the vascular wall is prevented.

The following gives a survey of studies assessing the response of HDL cholesterol, of the HDL subfractions HDL2/HDL3, the apoprotein A-I and/or the LDL/HDL cholesterol ratio to EPL treatment.

Increase of HDL Cholesterol in Serum:
Various authors (47, 316, 510, 663, 698) have given values after lipoprotein electrophoresis as percent of the total lipoprotein content. Within 1 to 3
months of treatment with EPL, the HDL cholesterol of the patient groups investigated improved by 1.5 to 2-fold.

H.Izumi et al (316) observed an increase in HDL cholesterol from 13.4 ± 1.3% to 20 ± 2.3% of total lipoproteins (normal range) when subjecting diabetic patients to a 12-month oral treatment with 1.5g of EPL daily.

The authors of other studies (24, 63, 112, 172, 288, 314, 336, 349, 462, 640, 686, 687, 691, 700) expressed mean HDL cholesterol in mg/dl or mmol/I. The increase rates obtained for HDL cholesterol as compared with initial levels ranged between 10 and 45% with values above 20% being the most frequently described. It was obvious that low initial levels of HDL cholesterol were raised, while high initial values were hardly influenced or remained normal throughout treatment.

In a controlled study V.K.Serkova (640) measured a mean increase in HDL concentrations from 1.1 ± 0.06 mmol/I to 1.42 ± 0.07 mmol/I (+29%) giving a significance of p<0.01 (n=42).

A. Maeda et al. (462) conducted a controlled study (n =32) and reported mean evaluations of 25% (2p < 0.001). The increase in HDL cholesterol was most pronounced when initial values were lowest. A.Fasoli (172) observed comparable reactions (mean increase in HDL cholesterol of about 26 /o). He demonstrated a significant rise in HDL3 as well as HDL2 (p<0.01).

Mean HDL cholesterol increases of 10 /o were observed in the controlled study of T.Suo et al.(686). However, when patients were stratified (for low or high initial levels), the group with baseline values of 30 mg/dl (n=17) showed mean increases of 26% (p<0.05), while there was only a slight increase when initial values were higher than 50 mg/dl (n = 23).

In his controlled study covering a 4-week treatment with 1.8g EPL/d M.Tomasevic (700) observed a mean elevation of HDL cholesterol of 22 %, while a mean increase of 30% (n=5) was reached in a patient group reported by U.Svanberg et al.(687).

In their double-blind test against placebo, A.K.Horsch et al. (288) observed a mean rise in HDL cholesterol of 30% after 2 weeks of treatment with 1.8g of EPL/d, while the corresponding value was 45% after 6 weeks (2p < 0.001)(fig. 30).

In a double-blind trial by R.Kirsten et al.(350) dialysis patients (n=10) were subjected to EPL treatment: 4 weeks after the onset of therapy HDL had increased by 23%,while at the end of the 6-week course of treatment the corresponding value was approx. 15%.

EPL and Hemabsorption:
In some studies EPL was given by intravenous and oral administration during and in between several hemabsorption sittings (63,456-458). The authors reported a substantial reduction among others of total cholesterol and LDL cholesterol. The authors are of the opinion that the rise in HDL is to be attributed without doubt to the action of EPL.

LDL-/HDL-Cholesterol Ratio:
This ratio was found to decrease from 4.3 to 2.8 in an open study including 14 patients who received a 4-week course of treatment with intravenous EPL injections of 0.5 to 1g/d (231).

The results of a controlled study by S.Uchida (713) showed that the decrease in the LDL-/HDL-ratio was clearly dose-related, i.e. it was most noticeable at doses of 1.5 to 2.25 g of EPL/d.

In a controlled trial, M.Tomasevic (700) observed a 24% reduction of the LDL-/HDL-ratio (n=30).

A reduction of the LDL-/HDL- cholesterol ratio from 5.6 to 3.7 was observed under double-blind conditions of the trial by R. Kirsten et al. (350).
In comparative studies by E.J. Diamantopoulos and L. Varsou (150) a group of CHD patients showed drop in the LDL-/HDL-cholesterol ratio to normal values (2p<0.01), while the ratio of patients suffering from hypercholesterolaemia but without CHD clearly approached normal values (ratio<2).

A substantial lowering of the LDL-/HDL-ratio indicating a lessened coronary risk has also been described in other controlled (660, controlled cross-over (24) or double-blind trials (512, 688). A similar interpretation was placed on the increasing levels of HDL cholesterol observed when patients with acute myocardial infarction (25) were given a 2-month treatment with 1.8g EPL/d. This rise was more strongly pronounced in non-smokers than in smokers and was significant as compared with controls.

A. Turnherr (698) claimed in his studies an increase in HDL cholesterol coincided with improved elasticity of the vascular wall. He interpreted this as showing cholesterol mobilization from vascular walls and elimination of cholesterol by HDL.

Fatty Acid Profile in the HDL Cholesterol Esters:

A number of authors (63, 66, 543-546, 595) have pointed out that with EPL the fatty acid profile in HDL among other molecules had improved: among the cholesterol esters transported in HDL, the proportion of cholesterol esterified with linoleic acid was found to be relatively higher under EPL treatment. According to G. Salvioli (595) EPL stimulates the enzymatic activity of LCAT, which, in turn, influences the rate of cholesterol transesterification (HDL as the preferred substrate for LCAT).

Although the differences in case material, dosages, duration of treatment and measuring methods are limiting factors for a comparison between the investigational results obtained, these may yet serve as distinct indications that - given a sufficiently high EPL dosage and adequate duration of treatment - EPL-enriched HDL take up more cholesterol. Hence not only is the HDL cholesterol in serum raised, but there is also a distinct improvement or normalization of the LDL/HDL ratio and of the relation between HDL cholesterol and total cholesterol.

This observation may well be understood as an EPL related lessening of the risk of atherosclerosis.

5.4.4 Effects on Serum Triglycerides

According to the recommendations issued by the Consensus Conference of the National Health Institute in 1984, fasting triglyceride levels below 250 mg/dl do not necessarily indicate an increased cardiovascular risk if total cholesterol in serum is normal. Fasting concentrations of neutral fats in serum exceeding 250 m/dl do, however, constitute a determinant factor for the progression of atherosclerosis, especially, when further risk indicators are present (148 350). This assumption is backed by the observation that the atherosclerosis risk is distinctly higher in patients suffering from endogenous hypertriglyceridaemia and in diabetics with high triglyceride levels.

As seen with the investigations on EPL, triglycerides in serum may vary considerably with the cold and warm seasons and high or low calorie intake connected with them (663, 773). Hence any drug therapy which lowers raised levels of high-triglyceride lipoproteins should always be accompanied by a long term reduction of carbohydrate supply in the daily diet.

The response of serum triglycerides and/or the triglycerides of individual lipoprotein fractions has been assessed in the clinical studies summarized below. Triglyceride data on a total of 2734 patients treated clinically with EPL have been compiled.

Reduction of Serum Triglycerides:

The following reports give a wide range of reduction rates for neutral fats in serum, with values of around 25% being the most common.

The extent of the reduction does not only depend on the duration of treatment and on EPL dosing. Several authors of controlled/double-blind trials have pointed out that relatively slight reductions of triglyceride levels in
serum were achieved when initial values had already been low or within the normal range (148, 755, 759).

Moderately elevated initial levels of neutral fats in serum however fell from 195 ± 56 mg/dl to mean levels of 146 ± 38 mg/dl (approx. 25%) (635), and from 198 ± 26.4 mg/dl to 134 ± 15.8 mg/dl (p < 0.001) in diabetics (316) after 2 months oral treatment with 1.5 g EPL/d.

Several studies revealed a particularly rapid drop in serum triglycerides: in a trial conducted by A.L.Grebenev et al. (236) 4 weeks treatment with FPL led to a marked reduction both in the patient group with high initial values (lowered from 274 ± 21 mg/dl to 116 ± 8 mg/dl) as well as in the group with only moderately increased baseline values (decrease from 201 ± 11 mg/dl to 94 ± 32 mg/dl).

In a double-blind trial (288) with patients on a standardized diet mean values had dropped from 353.7 mg/dl to 276.2 mg/dl (-21.9%) after only 14 days. In a further study including patients with coronary heart disease and angina pectoris, the investigators (12) reported a mean decrease in serum triglycerides of 23% after 14 days of intravenous EPL injections of 1 g daily. In a controlled study over 3 months T.Luther et al. (461) administered 1.4 g of oral EPL/d to patients after myocardial infarction. The mean decrease in serum triglycerides reached 24% after 4 weeks and 46% after 12 weeks.

Furthermore, the following research groups reported particularly marked reductions in serum triglycerides (tab. 35).

Influence of Nutrition or Occupation on EPL Efficacy:

In a controlled study against placebo I.Zulic et al. (773) investigated the influence of the daily food intake on the lipid-lowering effect of EPL. No dietary recommendations were issued.

A 6-week treatment with EPL (1.05 g/d orally) produced mean triglyceride reductions of 22.7% (p < 0.01) in winter when a greater supply of calories may be assumed, while levels dropped by 58.6% (p < 0.001) under comparable conditions in early summer.

In another study (772) (controlled against placebo, 3 weeks oral administration of 1.8 g of EPL/d), when EPL was administered to subjects performing strenuous physical tasks with a correspondingly high calorie intake, serum triglycerides only decreased by 13.3%, viz.: from 158.7 ± 50 mg/dl to 137.7 ± 48.2 mg/dl (significant as against controls). The levels of participants with office jobs, on the other hand, (and a correspondingly low calorie intake) could be reduced significantly from 171.9 ± 50 mg/dl to 97.3 ± 38.5 mg/dl (-43.4%).

The Use of EPL in Diabetes:

Various investigators screened the possible influence of EPL on the disturbed fat metabolism of diabetic patients (14, 153, 316, 499, 557, 635, 646, 659, 715).

Treatment of the well-adjusted, insulin-dependent patients with maturity onset diabetes and diabetics on oral antidiabetic agents comprised a combination of EPL injections and capsules in the first 2 weeks, to be continued on capsules alone for the following 10 weeks. Triglyceride levels in the insulin-dependent patients were shown to drop from 302 ± 58 mg/dl to 133.8 mg/dl on average; the respective values were 340 ± 67 mg/dl to 239.6 mg/dl for the controls (on oral antidiabetics). The authors explained this discrepancy in results on the basis of an insulin-related promotion of lipolysis (153).

The decrease in triglycerides was considered particularly favourable with regard to limiting the long-term complications of diabetes. In a trial against placebo (14) when 1.05g of oral EPL/d had been administered to patients with maturity onset diabetes over a period of 12 months, triglyceride levels were 37.7% lower than baseline values (from 210.6 mg/dl to 132.5 mg/dl).

Under comparable test conditions (316) mean serum triglycerides of non insulin-dependent maturity onset diabetics were found to drop from 198 ± 26.4 mg/dl to 134 ± 15.8 mg/dl (p < 0.001).
In general, no EPL-related influence on fasting glucose levels was observed. However, G.Martines et al. who treated 24 patients with diabetes mellitus type II for 60 days with diet + 1.2 g EPL daily in comparison with diet alone observed a significantly higher decrease of the blood sugar level (474). Only in the EPL group the blood sugar level was significantly reduced after a glucose tolerance test. The authors discussed a possible influence on the insulin receptor.

As a whole, trial results showed a moderate to pronounced reduction of serum triglycerides to be attributable to EPL, with the patient's diet and the test conditions (duration of treatment, dosage, level of initial values) contributing further important determinants for the intensity of the reduction. Hence EPL influences both triglyceride levels and cholesterol levels in serum.

5.4.5 Influence on Lipid Peroxidation
Growing importance has recently been attributed to the part played by peroxides, particularly by lipid peroxides in cell membranes and lipoproteins, in the development and progression of atherogenic lesions in the vascular wall.

In the studies discussed below, the authors tested the possibility that EPL inhibits lipid peroxidation in coronary heart disease and diabetes mellitus.

The levels of acyl-hydroperoxides, of Schiff's bases, the diene/triene conjugates as well as malondialdehyde and the intensity of haemolysis induced by peroxidation served as parameters when assessing the levels of the primary and secondary products of lipid peroxidation before and after EPL treatment.

In a controlled study by V.K.Serkova (640) a group of patients with angina pectoris was subjected to 3-week oral therapy with 1.8 g EPL/d. At the end of treatment the reduction in atherogenic serum lipids and the rise in HDL cholesterol correlated well with the favourable effect on the indicators of lipid peroxidation, i.e. EPL inhibited peroxidation and the signs of haemolysis due to peroxidation were reversed.

These results square with the observations V.G.Spesivtseva et al. (659) had already reported in 1984 on a controlled study with EPL.

V.I.Kalmykova and E.B.Zakharova (333) were able to confirm these results in 1989 when carrying out a trial with patients suffering from stable angina pectoris (stages II-IV; n=104). Improved resistance of erythrocyte membranes was observed as a consequence of inhibited lipid peroxidation.

In a 12-week study S.Takahashi (690) demonstrated that the levels of baseline malondialdehyde and their reduction rate were directly proportional.

V.S.Gurevich et al. (246) found a close interrelation between an increasing microviscosity of the platelet membrane - as a consequence of enhanced lipid peroxidation - and an increase in platelet activity, when investigating patients with unstable angina pectoris. The observation that lipid peroxidation was inhibited, therefore, was of particular importance because it suggested the possibility of a favourable effect on platelet activity which is intensified in this condition.

Together with a reduction of the atherogenic lipoprotein fractions in serum, the inhibition of lipid peroxidation following stimulation of protective factors by EPL provides a possibility for interrupting the progression of atherosclerotic changes in the vascular wall.

5.4.6 Effects on Enzyme Activity

5.4.6.1 LCAT
Lecithin: cholesterol-acyltransferase (LCAT) - an enzyme that is synthesized in the liver and circulates in plasma - derives overall significance from the catalysis of the esterification of free cholesterol in plasma. In this way free cholesterol on the surface of lipoproteins, erythrocyte membranes or in cells can be taken up by the HDL, be esterified and eventually eliminated from
plasma (595-597). Increased esterification of free cholesterol leads to a marked enhancement of its transportation in the HDL core. Qualitative and quantitative changes in the lipoprotein substrate (including a rise in the content of phospholipids with predominantly saturated fatty acids) cause diminishing LCAT activity in plasma. The supply of EPL rich in unsaturated fatty acids on the other hand, activates the LCAT reaction (28, 63, 65, 66, 219, 462, 595-597, 640, 687, 690).

Hereditary LCAT deficiency may entail excessive cholesterol content in erythrocyte membranes of up to 90% as compared with healthy controls and may interfere negatively with membrane rigidity and fluidity (Norum and Gjone quoted in 597).

LCAT catalyses the transfer of fatty acids in the 2-position of phosphatidylcholine to free cholesterol (fig. 31). This reaction takes place in or on the HDL which are the preferred substrate of LCAT (65,66). V.Blaton et al. (66) observed that the role of cholesterol transesterification was promoted by enriching the HDL with EPL. This trial involved 93 patients with hyper-LDL-aemia or hyper-VLDL-aemia who received EPL injections (1g of EPL/d) for the first 14 days; treatment was then continued for 69 subjects on an oral dosage scheme of 1.8 g of EPL/d. The authors observed a close interrelation between LCAT activation and the EPL-related percentage increase in plasma cholesterol esterified with linoleic acid as well as the relative increase of these esters in HDL and LDL. The increase in cholesterol- linoleic acid esters was mainly localized in the HDL since HDL is the preferred substrate of LCAT. This indicated increased LCAT activity.

According to G.Assmann et al. (28) who investigated different phosphatidylcholines, including dilinoleoylphosphatidylcholine (tab. 36), the mechanisms of LCAT activation remain to be established. In their opinion the formation of an LCAT/substrate complex and hence cholesterol esterification are facilitated by an increased fluidity of the PC substrate due to unsaturated fatty acid chains in the 1- and 2-position of the molecule, as present in 1,2-dilinoleoylphosphatidylcholine.

U.Svanberg et al. (687) also considered the proper functioning of the LCAT reaction to be an elementary precondition for the catabolism of triglyceride transporting lipoproteins.

In the clinical studies summarized below, changes in LCAT activity represented just one of the parameters applied to measure the therapeutic success of EPL. A significant increase in LCAT activity (p < 0.01) was demonstrable under controlled test conditions (462). A.S.Blagosklonov et al. (63), who used EPL in 83 cases in connection with hemabsorption to correct disturbances in lipid metabolism, considered the observed increase in HDL cholesterol to be related to EPL-induced intensification of LCAT activity and an enhanced mobilisation of cholesterol from vascular walls. V.K. Serkova (640) supported this view. In the cases investigated, the tendency towards an increase in LCAT activity was most pronounced when baseline values were lowest (690).

In controlled studies G.Salvioli and his group (595-597) pursued the study of LCAT behaviour in liver disease and its possible activation by EPL. After 5 days of EPL infusions of 2 g/d, LCAT activity increased from 31.2 μmol/I/h to 54.4 μmol/I/h on average. The activation was reflected in a reduced cholesterol content of erythrocyte membranes and a reduced cholesterol/ phospholipid ratio. The authors considered the EPL-induced elevation of the linoleic acid content in the HDL as favourable for an improved fluidity of the particles, thereby facilitating the deposition of apoprotein A-1 and hence supporting the promoter
function of this apoprotein for the formation of the LCAT/substrate complex. These results derive substantial confirmation from another trial (219) involving patients with chronic liver disease. After 2 weeks of oral EPL treatment (1.8 g/d) the increase in LCAT activity correlated with an improved liver function in these patients.

On the whole the results concerning EPL-associated activation of LCAT seem very promising. Further controlled investigations are to follow.

5.4.6.2 Lipases

Lipoprotein lipases (LPL) as well as hepatic triglyceride lipases (HTGL) lyse the triglycerides in chylomicrons and very low density lipoproteins (VLDL). They thus initiate the transition of the VLDL into lipoproteins of higher density, that are crucial for the uptake and transport of cholesterol. Their enzymatic activity is governed by apoproteins and phospholipids (145) the unsaturated fatty acid content of which is of decisive importance in this context as has already been pointed out by V.Blaton and his team (64) in 1974. C.Desreumaux et al. (145) isolated lipases from the tissue of healthy volunteers and incubated them in-vitro with substrates of different phospholipids. Activation was highest when the EPL and HTGL had been incubated in an EPL-containing substrate, while the stimulation produced by phospholipids containing saturated fatty acids alone, was much weaker (tab. 37).

According to V.K.Serkova (640) the lipolytic action is further enhanced by the promotion of the dispersion of lipid macro-aggregates under EPL treatment. In several trials I.Zulic et al. (771–773) have investigated LPL activation by EPL in comparison to placebo. During a 6-week treatment period 80 patients with hyperlipoproteinemia received 1.05g of oral EPL/d; another study included 45 patients who were given 1.8g/d of oral EPL for a period of three weeks. In the treatment groups the activity of EPL increased by 25% and 40%, respectively, while no change was observed in the control groups. V.G.Kukes et al (382, 383), who had administered 1.8 g/d of EPL to 55 patients over a period of 30 to 50 days, also reported a significant increase (p < 0.001) in the activity of heparin-dependent lipolytic enzymes. The stimulation of lipoprotein lipase was most noticeable when initial values were low.

The results obtained in the controlled studies including 180 patients with hyperlipoproteinaemia, provide essential evidence for a lipase-stimulating effect of EPL which is to be seen in the context of the EPL-related reduction of serum triglycerides described in chapter 5.4.4.

5.4.7 Influence on Platelets and Red Blood Cells

5.4.7.1 Investigation into the Influence of EPL on Increased Platelet Aggregation

M.Yoritsune and T.Mozai (759), among others, have described a close relationship between high lipid levels in serum and an increased tendency to adhesion and aggregation of platelets. Platelet aggregates are considered to be one of the factors contributing to atheroma formation in the vascular wall.

Deposits enhance the sensitivity of the vascular wall towards substances that are released from the platelets after their aggregation and which lead to an increase in vessel wall permeability. This, in turn, encourages and accelerates the accumulation of further plasma constituents - such as lipids - in the injured wall. A leading role in stimulation and migration of smooth muscle cells from the media to the intima is attributed to a growth factor that is synthesized and released by the platelets (platelet derived growth factor = PDGF).
Apart from a reduction of serum lipids under EPL treatment, the authors of the trials summarized below also observed a favourable effect on platelet membranes. Such investigations mostly involved patients suffering from coronary heart disease or diabetes, since the question of a possible effect on increased platelet aggregation is of particular interest in these diseases.

Over a period of 14 days V.A. Almazov et al. (12) administered infusions of 500 mg of EPL/d to 24 patients with angina pectoris. During this relatively short observation period they achieved a reduction in relative platelet aggregation by approx. 60% in comparison with baseline values (p<0.02). Both the rate of primary and secondary aggregation and the interval until the aggregation peaks were reached were clearly diminished. Microscopic examination revealed a reduced number of aggregates and within them a reduced number of platelet conglomerates.

The authors explained this change in platelet activity by a quantitative and qualitative improvement of serum lipids due to the supply of EPL, by a reduction of the cholesterol content in the platelet membrane, and by the exchange of membrane phospholipids with EPL.

A significant inhibition of platelet adhesion to glass as well as an inhibition of platelet aggregation also have been described by S. Coccheri et al. (114) who administered 500 mg/d of intravenous EPL to 25 patients either as a single administration or for a period of 15 days.

S.S. Belousova et al. (47) also attributed the decrease in platelet aggregation observed with EPL to the shfiting of cholesterol from the platelet membrane into the EPL-enriched HDL. Platelet aggregation was shown to slow down, which tallied with the results of V.G. Almazov et al. The optical density of the aggregates decreased. These changes did not vary during the 3-month follow-up phase after EPL treatment. Reduced platelet sensitivity towards substances provoking aggregation (e.g. collagen) became evident.

A similar phenomenon was described by O. Fakhri et al. (170). They used the relative dispersion of light transmission fluctuations as a parameter and measured platelet aggregation by means of electron optical analyser. 10 days treatment with 1 g i.v. EPL/d and 30 days of daily oral administration of 1.8 g of EPL clearly reduced the sensitivity of thrombocytes to ADP (also ref. to 74). This was related to an inhibitory effect on the ADP-induced rise in Ca++ in the platelets. Moreover, the authors observed an inhibition of PAF-induced platelet aggregation both in-vitro and in-vivo (tab.19.2).

In accordance with Y.G. Almazov et al. C. Galli et al. (216) described a definite improvement in the composition of thrombocyte membranes: 6 weeks after the onset of treatment 7 healthy volunteers receiving 10 g of EPL/d showed a reduction in total lipid content and cholesterol content, which was significant as compared with baseline values, while the phospholipid/total lipid ratio increased and a higher rate of esterification with linoleic acid in platelet phospholipids was observed. The authors regarded this as an indication of an exchange of phospholipids between cell membranes and the plasma compartment.

According to the authors, the incorporation of EPL into biological membranes like those of platelets, red blood cells and arterial walls might lead to an improvement of membrane fluidity and cellular function.

In association with reduced platelet aggregation V.G. Kukes et al. (382, 383) observed an improvement of rheographical findings in their patients with chronic heart disease (see also 46, 74).

R. Merchan and his group (483) arrived at similar results when administering i.v. injections of 250 mg/d of EPL over a period of 30 days in cerebral insufficiency of the elderly. Fifteen and 22 days after the beginning of treatment the intensified spontaneous blood coagulation was found to decrease distinctly, while the thrombo-elastogram showed fibrinolytic activity to increase. Hence the platelet-related disturbance of the coagulation balance was being checked. These findings were confirmed later by S.S. Belousova et al. (47).

Some authors have associated the reduction in spontaneous platelet aggregation under EPL with a favourable influence on endogenous prostaglandin synthesis (94, 520).
The study of T. Numano et al. (520) involved 11 patients receiving 1.5 g of oral EPL over 16 weeks. The authors observed an elevation in serum 6-keto-PGF1α (stable metabolite of the antiaggregatory and vasodilatory prostaglandin PGI2) which was particularly noticeable in the 8th week, and a drop in thromboxane level (TXB2). The significant reduction (p < 0.05) of the TXB2-/6-keto-PGF1α ratio was interpreted as a cytoprotective effect of EPL.

The available reports show that EPL reduces the enhanced tendency towards platelet aggregation resulting from disturbed lipid metabolism. This may provide a starting point for the inhibition of the progression of angiopathies associated with chronic heart disease, diabetes mellitus and cerebral insufficiency.

5.4.7.2 Investigation into the Influence of EPL on Red Blood Cell Fluidity

Structural changes in the red blood cell membrane resulting from an increased accumulation of cholesterol, i.e. a pathological cholesterol/phospholipid ratio, impair the fluidity and functioning of the membrane and limit the red blood cell (RBC) deformability. These changes obstruct the passage through the narrow lumen of capillaries and promote RBC aggregation thus adversely affecting the viscosity and flow properties of blood. The resulting disturbances in microcirculation may contribute to a progression of pathological processes, especially when coronary heart disease, angina pectoris, retinopathy, and impaired cerebral or peripheral circulation are concerned.

A. M. Ehrly and R. Blendin (167) obtained evidence for an improved filtration of RBC through an 8μ capillary filter after a single injection of 750 mg of EPL given to healthy volunteers; they suggested that this was due to improved RBC deformability. Fifteen and 45 min after the injection, both the filtration rate as well as the number of red blood cells per mm of the filtrate were higher than initial values, the same applies to the total number of the red blood cells filtered. Hematocrit as well as blood and plasma viscosity remained unchanged in these tests.

When patients with chronic occlusive arterial disease were examined under similar test conditions, the highest number of filtered red blood cells was detected 60 min after an i.v. injection of 750 mg of EPL (p < 0.05); 30 min later counts almost equalled the initial values. The exchange of membrane phospholipids containing saturated fatty acids with EPL was considered a possible cause for the facilitated filtration of RBC and their improved deformability.

A. S. Blagosklonov et al. (63) confirmed an improved passage of red blood cells through microfilters (Nucleopore, USA) and the normalization of RBC aggregation in their patient group. Parallel to hemabsorption their patients had received i.v. injections of 500 mg of EPL and after that had taken 1.8 g of EPL for 3 months.

The cholesterol/phospholipid index of RBC membranes dropped by 28% to normal values. Contrary to A. M. Ehrly et al. (167) the authors observed a normalization in hematocrit and blood viscosity and an associated statistically significant rise in capillary flow.

The favourable influence on rheological findings and on lipid parameters correlated with an improvement of the clinical picture: depending on the severity of the coronary condition involved, these favourable changes persisted for up to 12 months after withdrawing EPL.

In controlled studies involving patients with coronary heart disease (659) who received doses of EPL between 0.6 and 1.2g/d, the rates of platelet and RBC aggregation did not reach those detectable in healthy volunteers. They were however markedly lower than those of untreated controls 1 month after the start of treatment.

Atherosclerotic patients also were subjected to a 4-week EPL therapy (1.5g/d orally) under controlled conditions (759). In addition to other parameters, again the index of red blood cell deformability was improved and blood viscosity reduced.
These findings were substantiated by the trial results of R. Merchan et al. (483) who administered 250 mg/d of EPL for 4 weeks.

G. Salvioli et al. (596, 597) carried out extensive controlled investigations on the type and incidence of morphological RBC changes in liver disease. According to their report the cholesterol increase in RBC membranes following a reduction in LCAT activity, provokes expansion and rigidity of the membranes with changes in RBC morphology in the form of uneven contours. The authors infused 2g of EPL/d over 5 days. As a consequence of the EPL-related LCAT activation the cholesterol content in the RBC membranes was lowered and the cholesterol/phospholipid ratio decreased; at the same time membrane phospholipids were exchanged for EPL which increased the content of linoleic acids in the membranes.

The changes in red blood cell morphology receded together with the reduction of the cholesterol/phospholipid ratio.

The study reports discussed substantiate the improvement in the fluidity of red blood cell membranes under EPL treatment. This is the result of a normalized membrane cholesterol content and/or the result of a relative increase in membrane phospholipids that are rich in linoleic acid (EPL). In combination with other parameters on which EPL exerts a positive influence, improvement in the fluidity of red blood cell membranes represents an essential contribution to inhibition of the progression of atherosclerotic changes in the vascular wall.

5.4.8 Investigation on the Progression and Symptoms of Atherosclerosis

After evidence had been obtained showing that EPL lowers raised serum lipids, which constitute the no. 1 risk factor for atherosclerosis in man it was clear that evidence for changes in the formation of atherosclerotic plaques in the vessel wall was required.

The results of animal experiments or studies on isolated tissue samples are promising, but are not fully applicable to man (tab. 18.5). There are still no reliable models applicable to reversal of atherosclerosis in man. For a few years now it has been technically possible to observe atherosclerotic plaques on a long-term basis, to measure them and register their growth behaviour. Hence evaluation of a possible therapy-induced retrogression of atherosclerosis in man is no longer based on the subsidence of atherosclerotic symptoms alone. In the studies available, EPL was used in atherosclerosis patients in order to study the effects described before in combination with further measures, when the severity of the disease required this.

In the majority of studies attention was focused on the serum lipid pattern under EPL treatment with a reduction or normalization of values serving as indication of a possible lessening of the atherosclerotic risk for the patient in question. Results suggest that this may very well be feasible with prolonged administration of EPL. Moreover, additional evidence for a possible inhibitory effect on the progression of atherosclerosis has been established via a favourable influence on the flow properties of blood. In addition, the influence of an EPL-related improvement in serum lipid levels and the flow properties of blood on the given atherosclerotic symptoms was assessed, provided that sufficient numbers of large patient groups were available displaying a relatively homogeneous localization of the atherosclerotic lesions.

5.4.8.1 Measurement of the Size of Atheromas in Human Vessels

For 18 months a pilot study kept track of the size of plaques by means of a real-time scanner covering sections of the superficial femoral artery as well as the carotid, iliac and popliteal artery (589).

Fifteen patients with asymptomatic atherosclerosis (stage 1) were participating in whom at least 1 atheroma had been diagnosed at one of the sites mentioned. The participants took 2.7g/d of oral EPL for at least 1 year. At the end of the observation period of more than 12 months the majority of the initial plaque volumes \( \leq 25 \, \mu l \) tended to stagnate after a transient initial rise. Larger initial volumes \((> 25 \, \mu l)\) stagnated in most cases or showed a downward trend at
the end of the 12-month observation period fig. 32). The I b means of orthogonal polynomials showed the tendency to regression clearer in the total plaque volume and the femoral artery (p < 0.05; n = 15) than at the carotid artery (n = 9).

5.4.8.2 Effects on Impaired Coronary Circulation

On the basis of objective findings and subjective symptoms patients with coronary heart disease (various stages of angina pectoris) or postmyocardial infarction conditions were assessed or a possible improvement of their condition.

Encouraging results from investigations on rats (163) have shown a protection by phosphatidylcholine of reperfused ischaemic hearts. Untreated isolated hearts subjected to low-flow ischaemia recovered 15% contractility only (as compared to time control hearts) following reperfusion, whereas contractility significantly enhanced to about 61% (as compared to control hearts), if phosphatidylcholine was added 10 or 20 min before ischaemia occurred. In addition, the incidence of arrhythmias during ischaemia and subsequent reperfusion was reduced.

ECG:

A number of studies have included ECG diagnostics (12, 13, 34, 301, 312, 382, 460, 488, 523, 573, 635, 639).

Depending on the severity of the disease, the EPL dosage and the duration of therapy, an improvement of ECG findings could be achieved in many cases. Among others this was reflected in a dose-related reversal of pathologically changed terminal segments. S-T depressions were found to disappear; previously negative T-waves were reversed to positive. These favourable changes indicated a relief of stenocardiac complaints. Exercise tolerance as tested on the bicycle ergometer improved. The phase until S-T depression occurred became longer, with the depressions themselves being less distinct (12, 13, 382, 383).

Incidence of Anginal Attacks, Nitro-Consumption:

All authors reported a decrease in anginal attacks (12, 13, 264, 301, 312, 382, 508, 635, 639, 659).

The investigations of V.A.Almazov et al. (12) included 34 male patients suffering from ischaemic heart disease and angina pectoris (stages III and IV); they received 500 mg/d of intravenous EPL for a period of 14 days. 20 of the 34 patients reported an absence of anginal attacks at the end of the first/beginning of the second week of treatment. The other 14 patients experienced a reduction of attacks from 8 to 10 within 24 h to 1 to 3 attacks within 24 h, with the severity decreasing as well. Daily nitro-consumption, therefore, could be reduced to 2 to 5 doses as well.

V.K.Serkova (639) who treated 42 patients with stable angina on exertion (stages II to IV) for 30 days on an oral daily dosage of 1.8 g of EPL, observed a 50% reduction in the nitro-consumption of her patients. Corresponding results have already been described by G.Hevelke et al. (264) in a multicentre study comprising 507 patients (fig. 33).

Subjective Symptoms:

For the patients, the EPL-related subsidence of subjective complaints was of particular significance. In a number of cases patients experienced an increase in their exercise tolerance without pain after prolonged treatment.

In the trial group of V.A.Almazov et al. (12) the walking distance without stopping or requiring nitroglycerin was extended from 30-50 m to 3000 m.
In a controlled trial by L.D. Itkina et al. (312) geriatric patients with atherosclerosis suffered from fatigue, decrease in vitality, disturbed sleep, sensation of constriction in the heart region, retrosternal pain, palpitation. On completion of the EPL treatment 88 of 94 patients reported a decrease in complaints and an increase in vitality. These changes were more pronounced after 2 months of treatment than after 1 month. Six of the patients did not experience any improvement due to the severity of the disease. An increase in the physical and mental activity of their patients after EPL treatment was also observed by S.M.Idu et al. (301).

In summary it is safe to say that the capability of EPL to exert a positive influence on the coronary heart disease is determined by the stage of the pathological process. EPL may then serve as an adjuvant to be administered in combination with cardio-active measures.

According to V.K.Serkova (639) a cardioprotective action of EPL is to be inferred, however, from the inhibition of lipid peroxidation and the improvement of energy-supplying metabolic processes especially in the heart muscle. Results from studies on pharmacology suggest a stimulation of prostacyclin synthesis and an increase in glutathione concentrations in the vascular walls.

5.4.8.3 Effects on Impaired Peripheral Circulation

As with impaired coronary flow the stage of the disease will determine whether an improved permeability of vessels can be achieved.

In a controlled study comprising healthy volunteers and patients in stages I + II as well as III + IV of the disease (according to Fontaine) J.Klemm (352) demonstrated an improvement of blood flow in the muscles of the lower extremities after a 30-day treatment with 1.8 g/d of oral EPL. This increase concerned both reactive hyperaemia as well as blood supply at rest. Flow velocity was raised as well, though slightly less in patients in stages III + IV due to longer collateral pathways.

The author assessed these changes in the light of a reduced blood viscosity, i.e. in association with an EPL-induced amelioration of flow properties rather than with a directly vaso-active influence.

S.Luczac and R.Leutschacht (460) employed the oscillometric index as a measure of therapeutic success in occlusive vascular changes, using it to determine the patency of major vessels in 200 elderly male patients. During the first 2 weeks the patients received 1 g/d of intravenous EPL plus 1.35 g orally; this was followed by 500 mg/d of intravenous EPL plus 1.35 g orally for another 6 weeks and a subsequent maintenance therapy of 1.35 g/d of oral EPL covering 18 months. An improvement of the oscillometric index and the walking distance (from 0-200 m to 1500 m) was observed in 35 patients. The withdrawal of EPL resulted in a shortened walking distance.

In a cross-over trial H.Pristautz (574) investigated the influence of high doses of EPL (1.8 g/d orally) on rheographic and oscillographic findings as compared with the influence of low doses of EPL (1.05 g/d orally) and placebo. A dose-related, distinct increase in the oscillographic index ( > 0.8 mV) indicated improved vascular passage. Rheographic findings also were characterized by a dose-related improvement. For instance a clear decrease in vessel wall rigidity was observed under the high doses of EPL together with a marked increase in flow rate. The age of the patients was of no account for these findings.

In a comprehensive trial involving 808 patients G.Hevelke et al. (264) confirmed the above results. After a 6-week treatment with EPL, 198 patients with intermittent claudication and 505 with pain at rest reported complete relief. Mean pain-free walking time was extended from 9.8 to 21.3 min on average (fig. 34). About one-third of the patients showed improved pulse recordings. According to the authors the therapeutic success of EPL in impaired peripheral circulation largely depends on its long-term administration. In
conclusion, few results have been collected as yet to document the action of EPL in impaired peripheral circulation. Those available suggest the earliest possible treatment with EPL in order to arrest the progression of the disease at an early stage.

5.4.9 The Use of EPL in Fat Embolism
Numerous clinical trials involving a high number of patients have been conducted to provide evidence of the prophylactic and therapeutic effect of EPL. The following survey (tab. 38) lists 23 studies representing the experience gathered with EPL in the prevention and treatment of fat embolism according to case number, groups and criteria of success and stratified for therapy or prophylaxis; reference is made of the pertaining EPL literature.

<%Tab. 38: Results from prophylaxis and therapy of fat embolism with EPL as against other therapeutic regimens not including this substance%>

The results of the papers 59, 90 and 259 were not adjusted for the therapeutic failures that were due to faulty dosing (too low dosing; early discontinuation; late onset of therapy), but charged the occurring deaths and cases of fat embolism to the account of EPL treatment or prophylaxis. The 3 studies (400, 479, 480) were evaluated only once, since they seem to describe the same patient population.

Following fracture or accident, fat embolism occurred in 19 out of 4485 patients (0.42%) who had received prophylactic treatment with EPL; while 29 cases of fat embolism (0.73%) were registered in 3952 patients who had not received this prophylactic medication. The investigators (259, 709) drew special attention to the fact that fat embolism developing despite the prophylactic regimen was less serious and that a lethal course was less frequent than in the group without prophylaxis.

Moreover it had been possible to perform osteosynthesis under the protection of EPL even after pre-existing fat embolism (87, 400, 463).

The studies covered 221 cases of fat embolism treated with EPL. 202 patients recovered, 19 (8.6%) died. In the retrospective control groups, who had not received EPL, 58 out of the 83 patients with fat embolism died (70%) and 25 survived. Even though the clinical trials presented here were conducted as open studies without controls or in retrospective comparison and a statistical analysis was not performed, the mere comparison of figures points to the life-saving and life-protecting action of EPL therapy.

5.5 Gastrointestinal Inflammation
In chapter 2.5 was described that EPL is a non-toxic preparation.
Acute toxicity studies of diclofenac-Na and ASA with or without EPL were carried out in order to ascertain whether the combination of an NSAID with EPL causes changes in the toxicological properties of the anti-inflammatory drug: there were no differences with regard to clinical symptoms, body weight, autopsy findings or LD50 values. In acute toxicity studies in mice it could be shown, however, that the toxicity of indomethacin is markedly reduced by the simultaneous administration of EPL (tab. 39).

<%Tab. 39: Acute toxicity of indomethacin alone and in combination with EPL in mice (446)%>

No systemic intolerance was observed.
Based on this safety and on the results of the pharmacological investigations (see chapter 4.3) clinical trials with volunteers and patients were performed.

5.5.1 Effect of EPL on the Pharmacokinetics of NSAIDs
The effect of EPL on the bioavailability of indomethacin was studied in 9 healthy volunteers in an open, randomized cross-over trial (453). Indomethacin (AmunoR) was administered p.o. at a dose of 50 mg either alone or in combination.
with 200 mg EPL. The absorption of indomethacin was slightly retarded in combination with EPL (fig. 35).

The relative bioavailability of indomethacin/EPL was 117.4% which was not significantly different from indomethacin alone. Side-effects (dizziness) were comparable.

In another open, randomized crossover investigation, which included a 14-day wash-out phase, 500 mg EPL + 50 mg diclofenac were compared with 50 mg diclofenac in 8 healthy volunteers (630). The 8 male healthy volunteers took on an empty stomach 2 combination capsules, each containing 250 mg EPL + 25 mg diclofenac, or two 25 mg sugar-coated tablets of diclofenac.

In accordance with the areas under the concentration time curve, the geometric mean of the ratios was 1.16 (tab. 40).

In volunteer no. 6 practically no blood levels were measured under the reference drug. If this volunteer is excluded a ratio of 0.99 will result.

In the present study the relative bioavailability ranged from 0.42 to 3.46; similarly wide variations have been published previously (489).

5.5.2 Studies in Healthy Volunteers for Objective Proof of Efficacy

In a first randomized double-blind cross-over study in healthy volunteers the effect of EPL on daily blood loss in the stool after indomethacin versus indomethacin + EPL was investigated (716).

Six men over 50 received a thrice-daily dosage of 1 capsule containing either 50 mg indomethacin + 224 mg EPL or 50 mg indomethacin alone for 7 days. Each treatment period including the last one was followed by a 7-day wash-out phase. Fecal blood loss was determined over a period of 30 days using 51Cr-labelled red blood cells. Direct comparison of the above preparations showed a significant difference in fecal blood loss in favour of the EPL combination (fig. 36).

In the 7-day wash-out phase following administration of the EPL combination this effect was partially offset by higher blood loss, probably as a result of a "rebound effect".

Gastric microbleeding was checked in a second randomized single-blind cross-over study with EPL + indomethacin versus indomethacin alone (250).

Seven male and 2 female healthy volunteers received in separate capsules 111.75 mg EPL or an equivalent amount of placebo plus 25 mg indomethacin 4 times daily for 7 days. The cross-over phases were separated by a 14-day wash-out phase. The gastric microbleeding rate was determined on the 3rd and 8th day of treatment.

The study was designed as a single-blind investigation. The investigator yet requested the random code only after the study and its evaluation had been concluded so that it was performed in a double-blind manner. The microbleeding rate increased in the reference group by 0.43 ml/day after 3 days and by 0.20 ml/day after 8 days. The corresponding values with EPL + indomethacin were 0.28 and 0.15 ml/day (tab. 41).

AM = arithmetic mean;
SEM = standard error of the mean;
D = arithmetic mean of the individual differences;
MPSRT matched pairs signed rank test;
n.s. = not significant;
*= 0.05? 2p< 0.01; += 0.10 ? 2p< 0.05

The comparison of treatments by means of the Wilcoxon matched pairs signed rank test revealed a tendency to significance on day 3; 2p = 0.0645.
5.5.3 Studies with EPL in Gastroduodenal Damage, Especially Due to Administration of NSAIDs.

An open pilot study was carried out in 20 inpatients with drug-induced gastrointestinal complaints (631). After a 5-day treatment without EPL they were given, 4 capsules of 450 mg EPL daily together with the dfU9S provoking gastrointestinal irritation for 10 days.

Variables of effectiveness included 7 subjective parameters (hiccup, heartburn, epigastric distress, loss of appetite, nausea/vomiting, feeling of fullness, constipation) associated with mucosal damage in the upper gastrointestinal tract. Semiquantitative assessment was based on symptom intensity.

18 out of the 20 patients registered an improvement of symptoms with EPL treatment. 15 of them were very much better. Therapeutic benefits noticed most by the patients were relief from pain and nausea and improved evacuation of the stomach and bowels. As a rule, the favourable effect occurred during the first days of treatment and was complete in about a week.

11 female and 9 male patients with rheumatoid arthritis and NSAID-induced epigastric complaints, aged 44-79 years, were given in another open clinical study a daily dose of 2.7 g EPL for an average period of 13.7 days, in addition to their medication consisting of indomethacin, diclofenac, piroxicam, ASA and tiaprofenic acid or phenylbutazone (287).

During this period subjective complaints could be reduced by 65- on an average. Pre-treatment and post-treatment gastroscopies, in some cases biopsies, were performed in 13 patients with a mean time interval of 23 days. In 80% of cases an improvement or even complete healing was observed.

A third pilot study including 19 patients was performed to evaluate the effect of EPL on piroxicam-induced gastrointestinal disturbances (20). The patients received a daily dose of 2 capsules of EPL (2 x 450 mg EPL) 3 times daily 1 hour before meals, concurrently with piroxicam as far as possible. Preliminary examinations were carried out on the day before starting the 10-day treatment course.

The following 11 subjective variables of effectiveness were studied: hiccup, heartburn, epigastric distress, loss of appetite, nausea/vomiting, feeling of fullness, constipation, headache, disorders of sight and hearing, occult gastrointestinal bleeding, hypersensitivity reactions. As in the study described previously (631), the variables of effectiveness were evaluated on a semiquantitative basis using a scale ranging from + (mild) to +++++ (pronounced). The clinical pictures of the patients studied were classified as follows:

- Painful osteoarthritis 13 out of 19
- Abarticular pain 3 out of 19
- Inflammatory rheumatism 3 out of 19

All patients complained of heartburn, epigastric distress, loss of appetite and feeling of fullness.

The investigators noted clear improvement in 15 out of 19 patients whose symptoms disappeared completely or nearly so. EPL was assessed as very good by the patients. Subjective improvement generally occurred after a few days (< 4).

Four patients reported normal bowel evacuation with EPL.

In order to obtain information about the dose-effect ratio, in an open trial 30 outpatients with at least 3 GI side-effects related to the administration of diclofenac for degenerative joint disease received EPL capsules 10 minutes prior to diclofenac in 3 different diclofenac:EPL ratios: 1:1, 1:3 and 1:10 wt/wt (19). The diclofenac dose varied between 75 mg and 150 mg/day. The degree (low, mild, moderate, severe) of GI disturbances was assessed before and at each visit for approx. 1-3 weeks, and the therapeutic effect was evaluated at the end of treatment.

EPL relieved from all symptoms in 5 of 12 patients at the low 1:1 dose, in 4 of 8 at the mean 1:3 dose, and in 8 out of 10 at the high dose (fig. 37).

In 4 of 5 patients who showed no response to EPL the increase of the dose resulted in marked improvement or disappearance of symptoms. It is striking that 6 of 7 patients with a gastroscopically diagnosed history of ulceration reported
subjective relief from gastric disturbances with EPL. From 2 cases, in whom gastroscopy could be repeated, one improved and the other remained unchanged.

Parallel to the dose-finding study with EPL + diclofenac, 2 open studies were performed in which 2 capsules of a combination preparation, each containing 50 mg EPL + 25 mg diclofenac, were administered 2-3 times daily to 20 patients (each trial involving 10 patients) for 21 days. The variables of effectiveness included rheumatological and tolerance parameters.

The results emerging from the 2 clinical studies were similar.

In (146), pain at rest was relieved in 2 cases, increased in 2 cases and remained unchanged in 5 cases; pain on effort and on pressure was alleviated in 5 and 4 cases resp. unchanged in 3 and 5 cases, and worsened in 1 and 0 patient, respectively. The flexibility of the affected joints improved in 2 cases and showed no change in 7 cases. In (408), pains at rest, on effort and on pressure were relieved in 6 cases, joint flexibility being improved in 1 case; no aggravation occurred.

In both studies a relief was noted also in some cases with regard to daytime and nocturnal pain. Since various rheumatic diseases, various drug treatments and physical regimens were allowed, the assessed rheumatological variables of effectiveness only permit the conclusion that EPL caused no reduction of the effectiveness of diclofenac. Of the 10 patients included into the study (146), 2 (after 13 and 15 days resp.) had to discontinue the combination preparation due to complaints in the epigastric region increasing with the treatment. These complaints were associated partly with pain on pressure and burning as well as with feeling of repletion. The patients involved in this clinical study were pretreated with diclofenac, all of them complaining of epigastric distress, pain on pressure, sensation of fullness, and in some cases of heartburn on admission. After terminating the study, it was found that only 2 of these patients, who were initially selected because of epigastric distress after diclofenac intake, still reported these symptoms with the combination preparation. 8 patients had no epigastric complaints; however, constipation, sensation of repletion and in 1 case a marked loss of appetite were noted, but should not be overrated. None of the complaints required discontinuation of treatment.

The results from study (408) are similar. 1 patient discontinued medication after 3 days due to persistent stomach pain, sensation of repletion, and nausea/vomiting. Another patient had merely gastric pain which, however, increased steadily; this aggravation was confirmed by endoscopy. 1 patient had 2, another 5 symptoms, which disappeared after 3 and 6 days, respectively. 6 out of 10 patients showed up to 9 symptoms which could be relieved in some cases during the 3-week treatment period: slight (2), moderate (1) or clear (3) remission.

In the aforementioned study pre-treatment and post-treatment gastroscopies were performed in 3 patients. In 2 patient 3 small prepyloric ulcers and 2 bulbar ulcers were diagnosed on the last day of treatment, in 1 patient the condition barely changed, in another patient healing of corpus and antrum gastritis and of a florid duodenal ulcer was established on the last day of treatment.

After completing the treatment with EPL + diclofenac the investigator (146) assessed the changes in comparison with the initial condition as follows: improvement in 8 cases, no change in 2 cases. The assessment for study (408) was: considerable improvement in 2 cases, improvement in 3 cases, no change in 3 cases, aggravation in 1 case.

This group of pilot-studies was finished with the results of Josenhans et al. (355). Here the patients received 1.35 g EPL together with an NSAID for 14 days following 1 day without therapy. On the average, the complaints were reduced by 78%. Patients and doctors judged the outcome compared to the status before therapy as much better in two cases, better in nine cases and unchanged in four cases. Two patients stopped therapy after aggravation of symptoms.

Finally, in September 1988 E.A.Zhukova et al. (763) presented their observations of children with duodenal ulcer disease. 41 children (25 male, 16 female), aged 6-15 years, received as basic treatment diet, antacids, sedatives and
spasmolytics. 19 of them were treated additionally with Essentiale (3x1 capsule daily) for 3-4 weeks on an in-patient level and for another 2-3 months on an out-patient level. The remaining 22 patients served as control.

In the Essentiale group pain, dyspeptic and astenovegetative symptoms disappeared. The concentration of pepsin in the gastric juice fell significantly, whereas in the control group the proteolytic activity remained elevated. The activity of lipid peroxidation was normalized with Essentiale only and corresponded to the values in healthy children. The rate of recidivation was reduced by the factor of 1.7 in comparison with the control group.

5.6 Neurological Disorders

Until today 14 experimental studies have been performed (chapter 4.4): EPL is taken up to a small extent into the brain, endogenous phospholipid synthesis is stimulated, and the following positive influences have been described for the:
- choline content in the brain
- dopamine and noradrenaline concentration
- growth of the dendritic tree
- detoxifying systems
- cellular immunological reaction (EAE model)
- prolongation of revival time after asphyxia

The first positive impressions have to be compared now with the results from 35 clinical investigations into 1968 patients (tab. 42). Furthermore, 3 studies with 31 volunteers were performed.

There are some major problems limiting the value of these trials:
- There are no real dose-effect studies showing the most efficient dose of EPL; as a consequence, very different EPL dosages were used (from 250 mg i.v. up to 35 g [45 g] orally).
- The majority of neurological diseases are of multifactorial origin, so that EPL was given as an adjuvant in addition to other therapeutic measures.
- The mode of application, the duration of application, and the used galenic preparations varied very much.

A description of these studies is worthwhile only under consideration that effects are seen which are based on the mode of action of EPL as a carrier for choline/polyunsaturated fatty acids, and as a membrane therapeutic.

The so-called cholinergic hypothesis is on the basis of classic deliberations on the neurologic relevance of phospholipids, which are biologic precursors to acetylcholine. This hypothesis holds that memory decline and impaired cognition in old age and in dementia are related to a deficit of central cholinergic transmission (43, 134, 160, 161). The rate-limiting factor in the regulation of acetylcholine synthesis is the bioavailability of choline as a substrate for cholinacetyltransferase (39). The dependence of the cerebral acetylcholine level on plasma choline concentrations is deduced from experiments into rats (385, 507).

The increased cerebral bioavailability of acetylcholine by administration of the precursors - phospatidylcholine or choline - is of importance in neurology, for example to treat the following syndromes:
- Tardive dyskinesia
- Gilles de la Tourette's syndrome
- Friedreich's ataxia
- Alzheimer's disease
- Dementia of Alzheimer's type
- Mania

The property of EPL to provide polyunsaturated fatty acids is of special interest in multiple sclerosis, as already mentioned in chapter 4.4. The influence of EPL on glialytic and neuronal membranes was mentioned as well as the positive effect to be expected in atherosclerotic changes of the brain vessels.
In table 43 EPL studies in volunteers are summarized. Two of the 3 studies were aimed at the EPL effect on the choline level in plasma and erythrocytes. The third study was a double-blind trial versus placebo with a very high daily EPL dose of 26 g for 5 weeks; the variable of effectiveness was memory decline in the aged volunteers. While the choline levels increased in relation to the dose, EPL failed to produce effects on the memory of the test persons.

5.6.1 Cerebral Circulatory Disorders (Scierosis)

As has already been seen from the experimental investigations, EPL definitely helps in these disorders. Table 44 at the end of this chapter summarizes study design, number of patients, mode and duration of treatment and the results of the individual studies of this and the following indications.

There is a steadily increasing number of publications showing that EPL improves oxygen supply and consumption in the brain, cerebral blood flow, microcirculation, vessel resistance and blood coagulation, lipid values, anti-peroxidative processes, and subjective symptoms, such as headache, vertigo, concentration, fatigability, memory, speech and irritability. The studies were performed with lower doses of EPL ranging from 250 mg i.v. to 3 g orally, from a single dose up to a treatment of 2-14 years. The results on the reduction of the cerebral blood flow time of the open studies were confirmed by a double-blind study by R.Felix and J.P.Hedde versus placebo.

5.6.2 Involutional Dementias

The psychopathological syndrome of dementia is characterized by a damage to previously intact intelligence, lack of drive and initiative, or loss of the ability to cope with activities of daily life. This leads to a progressive dedifferentiation of the personality. Since the origin of the condition may be quite different, dementia does not present a uniform picture. According to K.Foerster and F.Regli (185) the following forms are distinguished:

1) demential syndrome in known immedicable primary disease, e.g. spinocerebellar degeneration;
2) demential syndrome in known and medicable primary disease of vascular or metabolic origin (see chapter 5.6.1), in vitamin deficiency states, chronic intoxication etc.
3) demential syndrome in unknown primary disease, i.e. senile dementia of Alzheimer's type with onset of the disease after the age of 65, and presenile dementia (the actual Alzheimer's disease) with onset of the disease before the age of 65.

Modern classification of dementias distinguishes between primary and secondary dementias (397).

Primary demential processes:
1) dementias of Alzheimer's type (60-70% of primary dementias)
2) multi-infarction dementias or dementias of the vascular type (22-22.5% of primary dementias);
3) mixed forms of both types (12-13.6% of primary dementias).

Secondary dementias:
1) irreversible forms, such as Pick's disease, Huntington's chorea or Creutzfeldt-Jakob disease;
2) reversible forms, such as communicating hydrocephalus or chronic alcoholism.

A central characteristic especially of Alzheimer's dementia is the pronounced deficit of cholinergic transmission due to the depletion of cholinergic neurons; however, not only the transmission of an individual transmitter is impaired, but also a number of systems, such as noradrenergic, serotonergic and peptidergic systems. The activity of cholinacetyltransferase is slowed down. Acetylcholinesterase, i.e. the decomposing enzyme, was also found to be reduced.
in Alzheimer patients in both cerebral cortex and liquor cerebrospinalis (568, 710).

From this brief description can be seen the heterogenicity and complexity of involutorial dementias.

Correspondingly, the impression of the 8 EPL studies into this field is quite varied. As with other preparations, a valid conclusion about the effects cannot be drawn yet (table 44.2). The studies were all performed in the first half of the eighties when decisive progress was expected from the influence on the cholinergic system by increasing the choline level, and a certain success of EPL seems to be possible. The durations of treatment of some weeks to some months, however, are too short and the parameters are too subjective or too vague. This deficit, unfortunately, also applies to the 4 double-blind trials; the study designs are insufficient (number of patients, duration of treatment, dose, etc.). Only objective parameters and duration of treatment over several years might allow clearer statements.

The double-blind study by S.D. Brinkman et al. can be considered as representative; the authors tested 3 different EPL doses; only 1 patient with less pronounced Alzheimer's disease out of 10 patients showed clear improvements. It might be possible that a certain subgroup of patients with involutorial dementia responded to EPL treatment. Maybe intensive research into neuronal membranes could clarify the picture.

5.6.3 Friedreich's Ataxia

In literature has been described that the administration of choline chloride and lecithin produced improvements in patients with Friedreich's ataxia (38, among others). These improvements, however, were not so pronounced as to decisively improve the patients' way of living.

The existing EPL studies do not provide further essential knowledge. It seems that in prolonged application the pathological process might be stabilized. The patients included in the one and only double-blind study, unfortunately, were treated only for 4 weeks with high-dose oral EPL As could be expected, no beneficial results were obtained.

5.6.4 Mania

Two controlled studies, one of them double-blind, from the beginning of the eighties are available. They were carried out in the same trial centre. The results are interesting, but the number of treated patients is far too low (8 and 6, resp.). Further must be taken into account that in all cases basic therapy was given.

5.6.5 Multiple Sclerosis

Four publications are available, one of them being a double-blind trial. The applied EPL doses ranged from 500 mg i.v. + 800 mg orally to 6–8 g orally. In 2 studies i.v. EPL was given in a first time, and then oral medication. The duration of treatment of 5.7 to 23.8 years in the study by A.R. Borromei et al. is quite striking. The results have been confirmed by the double-blind, long-term study of W. Autenrieth and I. Neu.

The obtained results are in accordance with the described findings from animal experiments (chapter 4.4) in allergic encephalomyelitis (651). Therefore, an interesting therapeutic approach can be presumed for EPL in this condition, and further studies appear to be justified.

5.6.6 Other Diseases

An open study by I.B. Islamova and L.P. Grintso of 1989 is available on muscular dystrophy of Duchenne. Despite the low dose of 250 mg Essentiale i.v. for 2 weeks and 900 mg Essential forte orally administered for 1.5 months to 12
patients, improvements of the cholesterol/phospholipid ratio both in plasma and red blood cells were reported as well as improved motor function and memory.

The double-blind cross-over study into 6 patients with Gilles de la Tourette's syndrome, in contrast, yielded no discernible benefits. In this study high doses of 45 g Lethicon were administered for periods of up to 4 weeks.

\[\text{Tab. 42: Kind of diseases and number of studies/patients with EPL in neurological diseases}\]

\[\text{Tab. 43: Studies with volunteers on "neurology"}\]

\[\text{Tab. 44.1.1: Cerebral circulatory disorders (sclerosis)}\]

\[\text{Tab. 44.1.2: Cerebral circulatory disorders (sclerosis)}\]

\[\text{Tab. 44.1.3: Cerebral circulatory disorders (sclerosis)}\]

\[\text{Tab. 44.1.4: Cerebral circulatory disorders (sclerosis)}\]

\[\text{Tab. 44.5: Multiple sclerosis}\]

\[\text{Tab. 44.2.2: Involutional dementias}\]

\[\text{Tab. 44.3: Friedreich's ataxia}\]

\[\text{Tab. 44.4: Mania}\]

\[\text{Tab. 44.2.1: Involutional dementias}\]

\[\text{Tab. 44.6: Muscular dystrophy of Duchenne}\]

Finally the trial on neurotoxosis in young children should be mentioned (302). Lipids, lipoproteins and lipid peroxidation products were determined in the serum, erythrocytic membrane and cerebrospinal fluid (CSF) in 110 patients, aged from 1 month to 3 years, with infective neurotoxosis associated with acute viral respiratory infections, pneumonia or intestinal infections. There was a considerable fall in total phospholipids and their fractions in the serum and erythrocyte membranes, and elevations in the CSF, as well as an enhanced process of lipid peroxidation in the serum and CSF. Essentiale was given together with vitamin E and lipoic acid. Essentiale was administered i.v. at the rate of 1 ml/kg b.w. daily in 5% glucose solution, while vitamin E and lipoic acid (0.5 to 1 mg/kg b.w. in saline) were given by i.m. injections. The daily dose was divided in 2-3 applications. The 28 patients receiving this triple medication showed generally quicker improvement, and the manifestations of neurotoxosis disappeared on an average 2 days earlier than in the untreated patients. Total plasma phospholipids and phosphatidylcholine increased significantly, phosphatidylethanolamine to a lesser extent, and lysophosphatidylcholine decreased significantly. Sphingomyelin remained normal throughout the whole trial period.

The authors discuss that the success of the treatment was not only related to the enhanced detoxification and liver synthesis, but also to the direct supplementation of the phospholipid component in the neuronal membrane.

5.7 Lung Diseases

The number of pharmacological studies with EPL in this field is so small that they were not described separately in chapter 4 but are integrated in the following clinical part.

There are primarily three fields in which the phospholipids and their supramolecular organisations, i.e. their role in membrane systems, are involved in pathological processes:

1) The lack of surfactant plays a major role in the etiology of shock lung in premature babies (IRDS) and adults (ARDS). The surfactant, which is synthesized by pneumocytes type II, is excreted by lamellar bodies into the alveolar lumen, where it forms a monomolecular coating. This coating inhibits the alveolar collapse during expiration, the formation of edema, and hinders sticking together of the alveoli; it is further responsible for transport functions towards the bronchi, constitutes a protection against dehydration, and favours the phagocytosis of bacteria through alveolar macrophages by opsonisation. The composition of the surfactant is as follows: approx. 10% apoproteins, 7-12% phosphatidylglycerol, and 73-81% phosphatidylcholine (primarily with saturated fatty acids in both positions, e.g. dipalmitoyl-PC) (454).

\[\text{Fig. 38: Possible pathway of lung surfactant through pneumocytes type II. M = monolayer of phospholipids; TM = tubular myelin; LB = lamellar body; G = golgi;}\]
2) As all inflammatory diseases, also pulmonary inflammation is associated with impaired function and progressing destruction of the membranes, i.e. of phospholipids. In such situations, the affected tissues exhibit an increase in lipid peroxidation products, raised phospholipase activity and, as a consequence, reduced phosphatidylcholine (PC) and increased lyso-PC levels.

3) Also the capillary system in the lungs can be affected by atherosclerotic changes of the vessels. The impaired oxygen uptake of the blood is aggravated by modifications of the rheological properties of the blood, and particularly by the modified flexibility of erythrocytes which, in turn, is determined by the cholesterol/phospholipid ratio in the erythrocyte membranes.

The therapeutic effectiveness of "essential" phospholipids (EPL) in these conditions was demonstrated in 3 animal experiments and in 8 clinical studies.

5.7.1 Animal Experiments

P.A. Chizov (111) applied intravenous adrenalin (1:5000) to induce pulmonary edema in rats. The author explains the formation of edema by adrenalin-induced activation of phospholipase A and subsequent increase in lyso-PC. As a result, membrane permeability is increased. Adrenalin also enhances lipid peroxidation. 1 hour before the application of adrenalin (1 ml/kg b.w.) one group of animals received a prophylactic dose of 6.6 ml/kg b.w. Essentiale (= 330 mg EPL/kg b.w.). EPL produced a significant reduction of mortality (1/8 in contrast to 6/8 without EPL) and of edema, measured as significant increase of the lung dry weight (p < 0.05). Also the phospholipid concentration in both plasma and lungs increased significantly.

After induction of acute pneumonia in rabbits by bronchial obstruction P.A. Kazaryan et al. (338) determined lipid peroxidation and phospholipase activity in the blood and in the lungs of the animals. Curative doses of Essentiale i.v. (50 mg EPL/kg b.w. and day) were administered for 7 days. EPL produced a reduction of lipid peroxidation and of phospholipase activity.

B. Balicco et al. (35) studied the influence of hyperbaric oxygen on the pulmonary architecture. The destructive processes in the tissue were largely inhibited when the animals were given 100 mg/kg b.w. EPL i.p. before hyperbaric O2 administration.

5.7.2 Clinical Studies

In pregnant women showing an IRDS risk for the baby G.K. Stepankovskaya and V.A. Tovstovanvskaya (666) found in gestation weeks 28-31 reduced phospholipid levels (particulary of phosphatidylcholine, but also of sphingomyelin and phosphatidylethanolamine) in the blood of mother and fetus and in the placenta, caused by surfactant deficiency. Unlike the conventional prevention therapy with glucocorticoids, thyroxine, ethyl alcohol etc., involving the risk of side-effects, these women received a substitution treatment with Essentiale. The phospholipid values, especially phosphatidylcholine were found to have increased again in both mother and fetus, and reached normal levels. Also the values of the hormones favouring the surfactant synthesis in the fetus (oestriol, oestradiol and placental lactogen) increased.

For 7-12 days M.M. Vainberg and L.A. Nikulin (717) treated 27 children, aged 0.8 to 3 years, suffering from acute pneumonia with a combination of Essentiale and Dimephosphon (Soviet product, vasodilator with phospholipase-inhibiting properties). The authors saw the origin of the pathology and the associated hypoxia in the lack of surfactant (i.e. of phosphatidylcholine), in reduced capillary circulation in the lungs and in the membrane damages caused by raised lipid peroxidation and phospholipase activity and subsequent phospholipid deficiency. In contrast to standard treatment, the additional combination treatment led to a shortened duration of the disease, to improved oxygen uptake and to a normalization of the surfactant composition. In both erythrocytes and
bronchial lavage was found a significant increase in the PC content, and a significant reduction of the phospholipase C activity and of the malonatedaldehyde content.

Further studies into acute pneumonia with Essentiale alone confirm that EPL is able to reduce lipid peroxidation and phospholipase activities with simultaneous increase of the PC/lyso-PC ratio. These biochemical changes were associated with an improvement of the pathological picture, and with a shortened duration of the disease (338, 345, 367, 369). In 54 patients with chronic lung disease O.V.Aleksandrov and S.S.Markin (10) found atherosclerotic changes, and an increase of blood viscosity, of erythrocyte aggregation and of the cholesterol/phospholipid (C/PL) ratio in these cells with concomitant reduction of flexibility. After a 4-week Essentiale therapy the C/PL ratio was found to have decreased and erythrocyte flexibility to have increased.

I. Seri (638) administered for several months Essentiale as adjuvant in addition to standard therapy to 104 patients with chronic lung diseases. He observed an increase of sulphamamide tolerance, reduced azotaemia, raised serum albumin levels and increased effects of various antibiotics.

These studies confirm the application fields of EPL in acute and chronic diseases of the lung. The therapeutic possibilities consist first of all in the prophylactic substitution of PC in the decisive pregnancy weeks to support the formation of surfactant in the unborn child, in compensating membrane damages in the presence of inflammatory processes in the lung, and in improving atherosclerotically changed blood flow properties and erythrocyte flexibility.

5.8 Psoriasis

Psoriasis ranges among the most common dermatoses all over the world. 2-3% of the total population of the United States and Europe are affected, and this trend is increasing.

Family history of psoriasis is common and it seems to be an inherited disorder.

No clear knowledge has been obtained as yet about the pathogenesis of psoriasis. Several theories explaining the origin of this disease are discussed. Some authors do not exclude viral origins, others interpret the symptoms as a response to intestinal fungus infection. The presence of activated lymphocytes, keratinocytes with various surface antigens, and the proliferation of lymphokines in the psoriatic plaques back up the theoretic approach of a disturbed regulation of the immune system, i.e. an immunological disease. Hyperlipoproteinemia and disturbed fatty acid patterns, finally, presume rather a metabolic disease.

No causal therapy of psoriasis exists to date. The usual symptomatic treatment is determined by
a) the kind of the clinical form, psoriasis vulgaris with its manifold manifestations being the most common form. Further variants are p. arthropathica and p. erythrodermica;

b) the severity of the clinical picture;

c) the kind of the psoriasis-induced accompanying diseases (such as liver disease, hyperlipoproteinemias).

The following therapeutic methods - individually or in combination - are usually employed today:
- topical application of glucocorticosteroids
- exposure to UVB or UVA light in combination with oral methoxypsoralen treatment (PUVA)
- administration of retinoid derivatives

New, apparently promising measures have been added recently to the therapeutic possibilities.

One of these measures, the clinical importance of which has been demonstrated in several studies, is the oral or parenteral administration of EPL
alone or in combination with PUVA, thalassotherapy or with indifferent topical preparations, such as Cold Cream or Vaselinum album.

R.Kageyama and Y.Morita (330) reported already in 1959 about experiences with oral EPL administration for 1-5 months in 4 patients with psoriasis vulgaris. Whereas 1 patient did not respond to the therapy, the other 3 showed improved clinical symptoms already 2-3 weeks after the beginning of therapy. After 4 months the skin manifestations disappeared completely in 2 of them, and were considerably improved in the 3rd patient.

EPL treatment led to a reduction of serum cholesterol values in all 4 patients. With EPL administration (n = 6) J.B.Entigknab et al. (168) observed a stabilization of the serum lipid values, which were within normal already at the beginning of the study, whereas these values were fluctuating in the control group (n = 4) receiving placebo. Because of the low number of patients no statistically significant improvements of the clinical symptoms were found in this randomized double-blind study.

J.Borowski (69) who treated besides psoriatics (n=15) also patients with neurodermitis and seborrhoic eczema with EPL, partly over 2 years, increased the dose to 2.3 - 4 g EPL/day. Recidivations occurring in 10 of the 15 patients during the observation period were described by the author as very little pronounced. Good results were achieved also in the other dermatoses.

Another EPL therapy alleviated quickly the 5 cases of recidivation. The author observed another group of 327 patients with psoriasis over 5 years. The treatment consisted of a combination of keratolytic ointments, diet, sun bathing, and oral/parenteral EPL administration. At the end of the 5- year observation period 291 of the 327 patients had no recidivations for 1-4 years. In the remaining 36 patients recidivations were little pronounced and disappeared quickly when the medication was repeated.

In the investigations carried out since 1983 EPL administration has mostly been combined with PUVA radiation (A.I.Abramovich 1984, 1989 (1-3); G.I.Pagava 1983 (535); Y.A.Khalemin 1987 (343); T.A.Glavinskaya 1987 (226); A.L. Mashkillelyson et al. 1990 (477)). In some cases EPL was given in addition to sea-salt baths and exposure to sunlight over several weeks (A.I.Abramovich 1989 (2); N.Kirjakova 1991 (348)).

According to the authors the significance of EPL administration in addition to conventional therapeutic measures resides in - an earlier onset and more complete remission of the skin manifestations after few weeks of treatment already;
- reduced number of required PUVA sessions and thus reduction of the radiation dose;
- reduction of recidivations, i.e. sustained improvement.

Patients with clear disorders of the lipid metabolism had their serum cholesterol values reduced, and the phospholipid/cholesterol ratio in membranes and lipoproteins was normalized (I.V.Chichenina 1987 (107, 108); T.A.Glavinskaya 1987 (226)).

In their report on psoriatic patients treated with EPL Mashkillelyson et al. (477) found improvement of liver function parameters, improved cell membrane structure and function, and a reduction of the hyperproliferation of epidermic cells.

Finally, Abramovich (1-3) studied the spectrum of 23 fatty acids in serum by means of gas-liquid chromatography.
With EPL therapy he observed a decreasing tendency for the values of palmitic acid (p < 0.05), palmitoleic acid (p < 0.001), oleic acid (p < 0.05) and docosohexaenoic acid (p < 0.01); these values approached the normal range, whereas the mean value of linoleic acid increased significantly (p < 0.05) without however reaching normal within the observation period. The author presumed that deficiency states of linoleic and linolenic acid might be at the origin of the syndrome which is characterized by a general lack of polyunsaturated fatty acids in the organism. It appears that psoriatic patients present a disturbed synthesis of arachidonic acid from linoleic acid. In animal experiments deficiency of linoleic acid provoked primarily skin desquamation, which are typical for the condition of psoriasis.

5.9 Tolerance of EPL

From the toxicological investigations (chapter 2.5), from safety pharmacology, and from the general pharmacodynamic effects of EPL (chapter 2.6) it is evident that this extract is safe and non-toxic. It is easy to believe this fact since EPL corresponds with endogenous phospholipids and differs only by its polyunsaturated fatty acids in the 1-position of the phosphatidylcholine molecules (chapters 2.1 and 2.2).

There is no risk that oxygen autoxidizes the polyunsaturated fatty acids during storage after the manufacturing process: G.-Sh. Wu et al. (752) showed that 1 Mol ?-tocopherol is enough to efficiently protect 2,000-20,000 Mol linoleic acid from oxidation with atmospheric oxygen. To substitute the naturally present vitamin E, which is lost during manufacturing, 0.3% ?-tocopherol are added to the EPL during the production process. This quantity guarantees sufficient protection of the unsaturated fatty acids in the phospholipids.

The "essential" phospholipids (EPL) were marketed for the first time under the name of "Essentiale" in 1952. Meanwhile, it has been further purified, different galenic forms were brought into the market, and it has been registered in 54 countries.

In the following will be described the clinical tolerance of EPL on the basis of the existing studies into liver disease and lipid metabolism disorders as well as on records of adverse drug reactions. A differentiation will be made between oral and intravenous application forms. In addition, long-term studies with orally administered EPL will be summarized apart.

5.9.1 Oral Application of EPL

Data on the tolerance of daily doses of 700-2700 mg EPL are available from 1705 patients who participated in 39 clinical studies on lipid metabolism disorders (230). Only undesired drug reactions affecting the gastrointestinal tract have been reported. In 20 out of 1504 patients undergoing EPL therapy, and in 5 of 201 placebo-treated patients, complaints such as slight, unspecific gastric disorders, soft stool and diarrhea were observed (tab. 47). In 1 case only the investigational medication had to be interrupted because of diarrhea (589). The incidence of side-effects in these clinical studies was 1.3%.

Abnormal values with respect to haematology, blood chemistry and urine analysis, or interactions with other substances have not been reported.

Besides the undesired drug reactions recorded in clinical studies, 7 spontaneous records were made in Germany between 1978 and 1989, 2 about stomach complaints and 2 about allergic reactions. Three records about arrhythmias are from the time before 1988, when the formulation of Lipostabil still contained etophylline.

With the very slight gastrointestinal complaints the intolerance might also be attributable to the gelatine contained in the capsule mass.
According to a rough estimation a total of 225 million daily doses of Lipostabil were sold in Germany between 1954 and 1989 (230). No manifestations of intoxication or overdosing have been reported. There are neither contraindications nor precautions to be observed in the administration of the preparation. From the animal experiments on fertility and reproduction as well as from the hitherto obtained clinical results no restrictions for the application during pregnancy and lactation can be inferred. The same data also apply for the use of EPL in the field of liver diseases (388).

5.9.2 Oral Clinical Long-Term Application of EPL

Eight studies on chronic hepatitis and 4 on raised lipid values in the blood, over a duration of treatment of at least 1 year given to a total of 751 patients (1669.2 patient years), are at the basis of this survey (27, 32, 156, 265, 275, 303-305, 321, 576, 589, 646).

In study 305, for 3 of 25 EPL-treated patients with chronic HBsAg positive hepatitis not specified subjective discomfort was recorded.

In study 589 diarrhea was recorded for 1 of 12 patients; 1 patient had withdrawn EPL medication after 11 months, but no reason was given.

Since no severe side-effects have been described, an incidence of 0.30% of slight adverse reactions was calculated for the indication of chronic hepatitis, and 1.2% for the indication of raised lipid values in the blood.

5.9.3 Intravenous Application of EPL

The significance for adjuvant and mono-therapy with EPL i.v. in liver disease was evaluated in a total of 3,499 patients (245). Doses of up to 5000 mg/day for periods of up to 3 months, in some cases for longer periods, were administered (e.g. 372, 550). Despite the relatively high doses in some cases the incidence of undesired drug reactions was low.

Diarrhea and abdominal complaints occurred only in 7 patients with fatty liver (505, 682). After dose reduction the manifestations subsided in 5 of these patients.

In another study (451) increased intestinal movement was observed in 3 of 30 patients with cirrhosis of the liver; 1 patient complained of fever.

One patient with chronic persisting hepatitis (373) developed general weakness, nausea, tachycardia, reddening of the skin and fever at the beginning of the therapy with 10 ml (1000 mg EPL i.v.); the symptoms disappeared when the preparation was given as slow drip infusion.

In another study (110) a patient with chronic active hepatitis complained of headache and chills with i.v. EPL therapy; the investigator, however, described this patient to be very nervous.

Six of 17 patients reported temporary pain after intravenous injection of EPL in another study (565). The unusual high incidence of undesired side-effects in this study may be attributable to the fact that the quality of the solution for injection was impaired due to inappropriate storage conditions. Also in another study (637) slight exacerbation of pain in the right hypochondrium was observed in 3 of 75 patients.

V. Martinez-Llinares and co-workers (475) described aggravated gingivitis in a patient with cirrhosis of the liver after intravenous EPL administration; the medication had to be discontinued.

Hypersensitivity reactions of the skin, finally, were reported in a total of 4 patients with liver disease (235, 732). These effects are probably related with the benzyl alcohol contained in the intravenous preparation; the medication had to be stopped.

Summarizing can be said that in the existing study reports undesired drug reactions occurred in a total of 27 of 3,499 patients, i.e. an incidence of 0.77%. Only in 5 of these cases the medication had to be discontinued. Dose reduction, slow injection or discontinuation of the preparation produced in all patients complete disappearance of the undesired symptoms. The incidence of
side-effects recorded in clinical studies is conclusive with the incidence of spontaneous records of undesired drug reactions made by doctors between 1979 and 1989. The incidence amounts to 0.0018% to 0.00056% of the used ampoules (245).

Similar good tolerance has been described for the intravenous administration of EPL even in even and often fatal fat embolism where shock and injury add to the compromised general condition of a patient and make the application of a drug to a prime necessity (632).
6. DISCUSSION OF THE PHARMACOLOGICAL AND CLINICAL RESULTS

6.1 EPL as a Membrane Therapeutic

The mentioned studies on EPL on various indication fields constitute a representative selection under the angle of membrane therapy. When all publications and reports, as far as known, are taken together their number will amount to over 2330.

It would not be admissible, however, to conclude that in the mentioned indications and others EPL was a preparation to be used in any case. In various fields detailed investigations are not yet available, e.g. on the possibility to influence membrane structure and function for many years, or large-scale double-blind studies to give clinical evidence of the hitherto observed properties, such as improved membrane fluidity and activation of membrane-dependent enzymes.

Yet it cannot be excluded that in the long run the organism adapts itself to membrane changes, e.g. by shifts in the phospholipid pattern, enhanced cholesterol incorporation, changed protein spectrum, or simply by metabolisation of the incorporated phosphatidylcholine molecules. It is true that membranal changes, such as increased fluidity, are admitted by the organism to a certain extent only in order to maintain normal functions like membrane passage, barrier function, information transfer etc. This observation has already been described in the case of erythrocytic membranes (526).

A general statement on EPL as a membrane therapeutic is hampered also by the following facts:

1) an interaction takes place between the incorporated polyunsaturated phospholipids and other membrane components, which is still largely unknown;
2) it is not yet possible to clarify the effects of lipid composition on membrane function according to a consistent pattern; again the available information on membranes is still too fragmentary, and there is too much diversity in the existing data to permit generalizations (658);
3) the physiological content in linoleic acid, not to speak about other components, which is bound to the phosphatidylcholine is very different in membranes of different parts of the body (tab.48):

<table>
<thead>
<tr>
<th>Tab. 48: Percentage of linoleic acid contained in the phosphatidylcholine in different parts of the human body (742)</th>
</tr>
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</table>

The membrane composition is dependent of a fine regulation permitting only therapeutic "windows" for "essential" phospholipids.

The uncertainty about the actually obtained effects on the membrane level is still bigger if one considers that the liver alone has a membrane surface of approximately 33,000 square metres (389), and that the various compartments of the body take up phospholipids from blood circulation to different degrees. In principle, bioavailability could be demanded for each compartment. However, this is impossible not only because of the high number of compartments or of inconvenience to carry out such investigations in humans (an ethical and analytical problem), but it is doomed to failure also because there is no marker substance to control metabolization. The labelling must take account of the fact that the main active ingredient in EPL, 1,2-dilinoleoylphosphatidylcholine, is very similar to the large pool of endogenous phospholipids, otherwise, it cannot be detected any more in the organism.

At the moment another bioavailability study is ongoing in 3 probands with 1-[d6]-linoleoyl-2-linoleyol-phosphatidyl-[Me-d9]-choline. Only for the manufacturing of this substance 2 new steps for the synthesis were required, which will be patented.

How is it possible then to judge if and, if yes, when the application of EPL as a membrane therapeutic is advisable?

We find a key to the answer when we make an overall assessment of the existing knowledge on mode of action, pharmacological models and clinical applications. A second approach would be to combine the huge number of findings (the total picture) with the results described in literature:

The most detailed investigations on membranes were carried out with blood cells, especially with erythrocytes, which are most easy to obtain and to analyse. In patients with liver disease, for example, abnormalities in the
composition of the plasma lipoproteins are associated with corresponding changes in the erythrocyte membrane lipid composition which can affect its shape and deformability and can decrease its permeability to sodium (531).

Apart from the observation that there are disease states in which the chemical composition of membrane phospholipids is altered significantly (761), also losses of phospholipids have been reported: e.g. K.R.Chien et al. (109) found out that the interruption of the blood supply to rat liver produces a progressive loss of phospholipid from the ischemic cells; whole homogenates and post-mitochondrial supernatants from liver, ischemic for 3 hours, showed a 40% and 55% decrease in phospholipids, respectively. Phosphatidylcholine and phosphatidylethanolamine were predominantly affected. It was suggested that the loss of phospholipids from the microsomal membranes results from the activation of endogenous, membrane-bound phospholipases.

Not only loss of phospholipids, but also loss of polyunsaturated fatty acids in phospholipids has been described, e.g. in severe chronic liver disease (323, 482, 597, 629).

Also the influence of a disease on changes in the membrane lipid composition of other organs has been described. It has been shown, for example by direct measurements, that membrane lipid abnormalities occur in the kidney of rats with experimental liver disease (337): the changes seen, cholesterol accumulation and arachidonic acid depletion, might well interfere with sodium transport and renal prostaglandin metabolism.

The observed abnormal lipid composition of the serum may reach a level which can override the intracellular homeostasis mechanisms and lead to a chronic change in all membrane lipid composition and fluidity (649). Therefore, a secondary outcome of various diseases and disorders, in which the composition of serum lipids is changed, could be a change in the lipid fluidity of all membranes. In most of these cases, the cell membranes become more viscous mostly due to an increase in cholesterol/phospholipid ratio or decrease in lecithin/sphingomyelin ratio. One example is an increased microviscosity of the lung surfactant as a characteristic of premature newborns (227, 648) or of allergic alveolitis in adults (356).

If membrane fluidity is decreased, there may be interference with a number of cellular processes. These include the inward and outward transport of various compounds including water, urea, electrolytes etc., the cellular response to drugs and hormones, the capacity of the cell for phagocytosis, endo/exocytosis and for membrane recycling and so on (482, 531).

According to M.Shinitzky (694) the obvious candidate for membrane fluidization both in vitro and in vitro is lecithin from natural sources, which can fluidize membranes either by extracting excess cholesterol or by incorporation into the membrane, either passively or by exchange.

The general examples given in literature on the behaviour of membrane changes in different diseases thus coincide with findings on EPL and confirm the therapeutic value of EPL.

In this context it has to be underlined that the processes at the membranes led gradually to taking into account the whole organism (holistic approach). The scientific interest in EPL has developed a dynamic which is reflected every year in many publications on various approaches of the mode of action and on a large field of application.

The continued scientific interest in EPL will now be shown with 3 examples:

1) It is very likely that the well-known drug withdrawal syndrome in addicts originates from a hyperviscous state of the brain membranes (261). On the other side, upon chronic intake of pharmacologically relevant doses of ethanol, anaesthetics and other drugs, a tolerance is developed. The tolerance is characterized by at least partial restoration of lipid fluidity by increase in cholesterol and saturated fatty acid content. In both situations the question rises whether EPL is able to attenuate withdrawal symptoms. A first indication can be seen in the recently published investigation by M.I.Khodzhaeva (346).

2) According to M.Shinitzky (649) the "fortification" of "weak" antigens, by manipulation of the membrane microviscosity, has the advantage of being non-
toxic, non-adverse and maintaining the overall texture of surface determinants; diagnostically, this method could facilitate the detection of buried antigens like the cold agglutinin in human erythrocytes; clinically, it opens up a new approach for augmentation of antigenicity as for cancer active immunotherapy, or for suppression of antigenicity as for tissue transplantation. According to the same author another interesting aspect of passive antigenic modulation relates to autoimmune diseases which originate from over-exposure of normal membrane constituents; as a result of an abnormal membrane microviscosity, haptenic residues which are naturally masked by the membrane become exposed and faultily recognized as non-self.

In this context has to be mentioned also the study by J. Neuberger et al. (513), who probably due to the EPL pretreatment of rabbits succeeded in changing the antigenicity of the subsequently isolated hepatocytes in a way so that the antibody dependent cell-mediated cytotoxicity did not exceed normal levels any more.

2) Also as a consequence of aging naturally masked haptenic residues become more exposed, when cell membranes are progressively rigidified. As this rigidification correlates well with increase in cholesterol, it is, therefore, expected that these antigens would become less mobile. These changes are not limited to antigenicity, but are also related with changes in accessibility and capacity of membrane receptors or certain enzyme activities (649). As in the case of antigen exposure, e.g. receptor exposure may increase and it may become vulnerable to enzymatic degradation or even to shedding. Therefore, the apparent increase in the number of accessible receptors may be actually associated with a decrease in total receptor pool stores in the membrane.

These changes in lipid composition with aging concern especially the relative increase of cholesterol, sphingomyelin, glycosphingolipids and saturation and EPL might be basically useful in counteracting these changes. The influences of EPL on decreased activities of membrane-dependent enzymes in the age has already been described (a.o. 49, 50, 257, 353). Ongoing studies into these approaches are recommended and extended to receptors and immune expression.

It should also be studied until which age limit damages induced by membrane changes are reversible, or at which stage of age the protein population is altered irreversibly as a result of shedding, enzymatic degradation, (per-)oxidation, crosslinking or genome aberration (649).

It can be expected in any case that refluidization of older or aging membranes by EPL is of valuable help in gerontology and in geriatrics when the frequent diseases in old age, such as gastrointestinal inflammation, liver diseases and atherosclerosis are present.

6.2 Outlook on the Further Development of EPL
Every year appear about 30 new publications on EPL. Steadily increasing knowledge on membrane function and the special effects of EPL extent and diversify the potential for its application as a membrane therapeutic (see also chapter 6.1).

These facts make demands on the preparation with respect to strategic trials, dose schemes adapted to each pathologic condition, galenic formulation, and composition, which will be discussed more in detail below.

6.2.1 The dosage
The majority of the existing clinical studies with EPL were carried out with daily doses of 1.5 g to 3 g orally, and 500 mg to 2.0 g intravenously. In isolated cases higher doses were applied (up to 45 g orally (566), see table 41.1.1), especially on the basis of deliberation such as that - not sufficient quantities of the phospholipid substance will pass the blood brain barrier;
the major part of the applied material will be captured by other tissues or organs before reaching the target site (404).

No dose-finding studies are available which would indicate the optimum dose in a specific indication field under consideration of factors such as body weight, sex, age, activity and duration of the disease etc. Neither can such studies be expected because of the complicated nature of indications like liver disease, neurologic diseases or disorders of the lipid metabolism including atherosclerosis.

The mere fact that a large variety of liver diseases exist reflects the necessity of individual dosing:
- this indication field covers conditions ranging from simple disorders of liver function to hepatic coma and liver cell carcinoma;
- it may be of viral, toxic, metabolic, autoimmune or other origin;
- it is often associated with biliary, hemorrhheologic or other accompanying diseases;
- and - let alone mixed forms - pure hepatoses, such as fatty liver, fibrosis or cholestasis have to be distinguished.

Under the consideration that EPL as a membrane therapeutic favours the regeneration of the liver (to stick to our example) we could take the regenerative potential of the organ as basis for the dose; this attempt, however, will fail as long as no adequate variable will have been found.

Neither do help us experimental data since very different doses of EPL yielded beneficial results. Individual studies with increasing dose ranges are available which, as a general trend, describe a better therapeutic success with higher doses. From these results, however, cannot be deduced definite dose schedules for humans (see also tables 14 and 18).

Conversely, J.F.Flood et al. (184) stated in their publication that the dose-effect relation of cholinergic substances is similar to an inverted U-curve. For EPL this would mean an optimum dose effect. One or the other clinical study suggests such a phenomenon (62 and comparing the studies in table 41.2), being insufficient, however, to give evidence of this presumption.

Knowing about this dilemma, the EPL doses will be oriented also in future along the clinical experiences collected by doctors and along the results obtained so far in trials. Evaluating the existing experimental and clinical studies, the optimum dose range amounts to 1.5 to 3.0 g orally (possibly even up to 4.5 g) and to 1.0 g to 2.0 g intravenously.

The Lipostabil and Essentiale products on the market have an EPL content of 175/250/300 mg in capsules, in the liquid form of 5%, (500 mg/10 ml), and in the parenterals of 5% (250 mg/5 ml, 500 mg/10 ml) and 10% (1000 mg/10 ml).

The recommended daily dosage is 3 times 1 to 2 capsules (= up to 1.8 g), and 1-2 - in severe cases up to 4 - ampoules for injection or infusion (= up to 4.0 g).

### 6.2.2 The Galenic Preparation

Two main new galenic preparations are under investigation (164):

a) Chewing tablets which allow to apply a daily EPL dose of up to 2.7 g (or even 3.0 g) in one unit;

b) EPL lyophilisate consisting of 500 mg EPL as active ingredient and of 2000 mg maltose for cryoprotection, to be dissolved in 8.30 ml aqua dest.

The development of the chewing tablets has the advantage of allowing higher daily doses (see 6.2.1) with simultaneous reduction of the number of units to be taken. Compliance can thus be improved and the dosage can be chosen more individually by the physician according to the kind and the severity of the disease. This formulation was possible by mixing a melt of carbohydrate (palatinite = isomalt) with EPL, which results in a solid hard non-sticking material at room temperature. By freeze-milling and after adding some usual tableting excipients the EPL/carbohydrate powder can be compressed to tablets. These tablets show good chewing properties, they do not stick to the teeth and have a pleasant taste.
The reason for the development of EPL lyophilisate in vials was to obtain a parenteral EPL form without cholic acid as solubilizer and with improved stability. While the EPI ampoules nowadays available on the market have to be stored in the refrigerator, the new lyophilisate can be stored at room temperature. As long as the freeze-dried product remains dry it can be stored, most probably, for over 3 years. One pilot study by E. Kuntz (390) has already shown positive effects with these EPL vials in severe liver insufficiency, which are similar to those obtained with Essentiale ampoules. Moreover, the vials can also be used for the preparation of drugs with liposomes for parenteral application.

6.2.3 Future Studies

The research areas mentioned in chapter 6.1 indicate possibilities for EPL application but have not yet reached the planning stage. In two cases however, more concrete steps for studies have been made:

- Experimental studies which are aimed at providing further insight into the mode of action of EPL as a membrane therapeutic and, in the second run, studies which would confirm these experimental results, formulated as work hypothesis, in pharmacological trials.
- On the clinical level two strategic approaches are focussed: a large-scale, multi-centre, international double-blind study to find out whether EPL, apart from dietary effects, is able to positively influence hypercholesterolemia, also in the long-term application (total cholesterol > 250 mg/dl, LDL cholesterol > 175 mg/dl); and a second large-scale, multi-centre double-blind study to find out whether in long-term application EPL was able to inhibit or stop alcoholic fibrosis, or to reduce existing alcoholic fibrosis.

On the experimental field the following studies are presently ongoing:

- on the influence of the peroxidation processes of endothelial cells to prevent atherosclerotic changes;
- on modifications of fluidity in the erythrocytic monolayer by means of monolayer photometry and computer modelling;
- on modifications of the properties of immunocytes with EPL;
- on the chylomicron features after high-fat meals with and without EPL;
- on the activation of delta-6-desaturase, the key enzyme to catalyze the chain prolongation of the precursor acids to arachidonic acid and thus to eicosanoids.

Furthermore, a series of experimental trials are discussed which will be described more in detail below:

On platelets:

As already described, EPL has been used in coronary heart disease to reduce the platelet cholesterol content and hence to normalize their hyper-reactivity and hyper-aggregability. In cirrhotic patients, however, the situation is opposite: the platelets are unreactive and difficult to aggregate, which may contribute to the major clinical problem of variceal bleeding. However, the conclusion that EPL-induced removal of platelet cholesterol might actually be detrimental is too simple for three reasons:

1) EPL infusion raises the arachidonic acid content of erythrocyte membranes (597); the low 20:4 content of cirrhotic patients may be responsible for impaired aggregation and override the stimulating effect of the high cholesterol/phospholipid ratio (C/PL) (530).

2) Laffi et al. (396) provided evidence that defective aggregation of cirrhotic platelets in vitro may partly reflect prior activation in vivo. This is an intriguing possibility since a raised platelet C/PL ratio may play an important role in this activation: by enhancing thromboxane formation (due to promoted release of arachidonate or its precursor fatty acids) and perhaps by prolonging spleenic residence time through rigidifying the membrane. If so, the ability of EPL to counteract or reverse membrane cholesterol deposition as new platelets enter the circulation may be very important.
3) Abnormal apo E-rich HDL particles have recently been identified as a potent antiplatelet agent in cirrhotic plasma (144).

On HDL and LCAT:

The just mentioned abnormal HDL particles have direct apolipoprotein-mediated effects on cells in vitro which are not seen with normal HDL and which do not involve lipid transfer: they interfere with receptor-mediated endocytosis of LDL by cultured cells (532), they transform normal erythrocytes (discocytes) into echinocytes (533), they impair against induced aggregation of normal platelets (144), they inhibit mitogen-induced lymphocyte proliferation (289) and they fail to provide adequate protection against infarction by T.b. brucei (224).

We also know that HDL and other lipoprotein abnormalities in liver disease largely coincide with an LCAT deficiency.

Therefore, EPL infusion may improve HDL functioning in two ways: by stimulating endogenous LCAT activity, and by improving abnormalities in HDL lipid composition and thereby potentially reversing any deleterious alterations in apolipoprotein orientation or confirmation.

On renal dysfunction:

Renal cortical brush-border membranes in the biliary obstructed rat are cholesterol-enriched (and so have reduced membrane fluidity and an enhanced ability to cotransport Na⁺/glucose).

Because such abnormalities are secondary to LCAT deficiency and raised plasma lipoprotein C/PL (306), it is believed that kidney membranes in jaundiced patients will be similarly cholesterol-enriched and of reduced fluidity. If so, EPL infusion may lead to an improvement in renal functioning by normalizing the cholesterol content of kidney epithelial cells.

Finally, also clinical studies on pathological syndromes are planned. Besides secondary hyperlipoproteinemias and hepato-renal syndromes also multimorbidity of aged persons will be included (see also chapter 6.1); this multimorbidity is characterized, for example, by atherosclerotic changes of the vessels accompanied with senile dementia, liver fibrosis and reduced gastrointestinal mucosa. All these manifestations present membrane involvement.

6.2.4 Changes of the Composition

At the moment, EPL preparations under the name of "Essentiale" with and without vitamins of the B complex are on the market for the field of liver diseases. The presence on each market depends on the requirement of the national authorities to give evidence of the synergistic effect of the components of the preparation. This requirement on the individual B vitamins combined with EPL can hardly be met: it is principally extremely difficult to give evidence of the effectiveness of a mono-preparation in liver diseases when strict scientific criteria are taken as a basis.

Since on an international scale the authorities tend to make stricter demands on the approval of medical preparations and to require evidence of the synergism, the Essentiale combination forms will increasingly be reduced to EPL mono-forms. Vitamin E is an exception: it will be maintained either as a component or as adjuvant for antioxidative purposes.

This also applies to the use of EPL in disorders of lipid metabolism. The components of nicotinic acid (1 mg), vitamin B6 (2 mg) and adenosine-5-monophosphate (1 mg) contained in each ampoule, and 1.5 mg vitamin B6, 1.65 mg vitamin E-acetate and 30 mg theophylline in the Lipostabil simplex capsule, as well as 50 mg etophylline in the Lipostabil forte capsules will be taken out gradually. In addition to the argument of the lacking evidence of synergism also the underdosing of these components has to be taken into account; it has not been studied whether this underdosing is compensated in part by the carrier function of EPL.

From these changes in the composition will result that Essentiale and Lipostabil forms will be registered which will contain exactly the same
quantities of the actual active ingredient - EPL - and which will differ only by
their names. This fact is conclusive with the fact that EPL is a membrane
therapeutic.

Nevertheless, there are deliberations to give evidence of the synergism
between EPL and other preparations. In Italy studies are being performed with a
combination of silybin (the main active component in Sylimarin®) and
phosphatidylcholine (IdB1016) in patients with chronic liver disease (93). Such
investigations are justified by the fact that both liver preparations have
different but complementary modes of action.

Similar reasons apply to the combination of EPL and glycyrrhizinic acid (23,
448) since the latter is a reputed liver therapeutic in Asia and since may it
may serve as solubiliser for EPL.

Finally, a large-scale, double-blind study with Interferon + EPL has been
initiated to give evidence of the synergism of effects, and to reduce the side-
effects of Interferon.

Similar considerations let us think of studies (a) with lipid-lowering HMG-
CoA reductase inhibitors (EPL might prevent their aggregation-inducing effects
(247)) or (b) with tetrahydroaminoacridine (inhibitor of acetylcholinesterase
activity) combined with EPL in the treatment of Alzheimer's disease (220).

In any case, combinations of EPL with one or several other preparations are
of interest when the disease is caused by or related with membrane-associated
damages.

Finally, the changes in the phospholipid composition of EPL should be
pointed out. There are thoughts to enrich the fatty acids of the
phosphatidylcholine molecules with polyunsaturated ones, which are expected to
produce therapeutic effects, for example on the triglyceride level, such as
eicosapentaenoic acid or docosahexaenoic acid (393). Other considerations aim at
admixing other phospholipids, such as phosphatidylserine, to extend the range of
action (60, 100, 410, 699).

For the time being however, neither of these 2 projects will be realized
because of the increased manufacturing costs and of the demands of the
authorities to document suitability and synergistic effects.
Phospholipids - Structural Elements

Phospholipids are essential components of all cellular and subcellular membranes. Phosphatidylcholine and phosphatidylethanolamine predominate quantitatively, substantially constituting the typical bilayer configuration. Phospholipids belong to the amphipathic molecules with a water-soluble and a fat-soluble component. In the bilayer configuration the hydrophilic groups are arranged at the outer and inner side of the membrane towards the surrounding medium; the lipophilic groups, in contrast, face each other at the inner side of the bilayer configuration.

Further important constituents of biological membranes are cholesterol, glycolipids as well as peripheral and integral proteins. The latter, in particular, act as receptors for biologically active substances and as transport proteins or enzymes. The basic structure of biological membranes is thus a series of recurrent unities of lipid-protein-complexes. The membrane is asymmetric. The function of the external (cellular) and internal (subcellular) membrane systems depends on their composition and on the integrity of their phospholipid structure.

In addition to their presence in cell membranes, phospholipids constitute structural and functional elements of the surface monolayers of lipoproteins and of surfactants.

Fluidity

Of utmost importance for the function of biological membranes is their fluidity, which is decisively influenced by phospholipids. Besides the content of cholesterol, proteins and the nature and charge of the polar headgroups of phospholipids in the system, membrane fluidity depends on the length of the chains of the fatty acid residues in the phospholipid molecule as well as on the number and the type of pairing of their double-bonds.

"Essential" Phospholipids

The term of "essential" phospholipids indicates the highly purified extract derived from Glycine max. with a standardized content, as a rule, of 76% or 94% (3-sn-phosphatidyl)choline in the oral preparations, and of 94% in the intravenous forms.

"Essential" phospholipids supply the organism with non-toxic (3-sn-phosphatidyl)choline molecules with a high content in polyunsaturated fatty acids, in particular linoleic acid. Approx. 15 kg of soya beans are required to obtain the recommended daily dose of 1.8 g EPL. The main active ingredient is 1,2-dilinoleoylphosphatidylcholine, which represents about 52% of the applied mixture of phosphatidylcholines.

1,2-dilinoleoylphosphatidylcholine is not physiologically present in the human body. Endogenous phospholipids are partly substituted by these "essential" phospholipids which are incorporated in all membrane-containing fractions and thus improve their fluidity.

Functions of "Essential" Phospholipids

With the administration of "essential" phospholipids the following functions of phosphatidylcholines in general are combined with those of dilinoleoylphosphatidylcholine in EPL:

1) They are high-energy, basic, structural and functional elements of all biological membranes, such as of cells, blood corpuscles, lipoproteins and of the surfactant.
2) They are indispensable for cellular differentiation, proliferation and regeneration.
3) They maintain and promote the biological activity of many membrane-bound proteins and receptors.
4) They play a decisive role for the activity and activation of numerous membrane-located enzymes, such as sodium-potassium-ATPase, adenylate cyclase and lipoprotein lipase.
5) They are important for the transport of molecules through membranes.
6) They control membrane-dependent metabolic processes between the intracellular and intercellular space.

7) The polyunsaturated fatty acids contained in them, such as linoleic acid, are precursors of the cytoprotective prostaglandins and other eicosanoids.

8) As choline and fatty acid donators they have an influence in certain neurological processes.

9) They emulsify fat in the gastrointestinal tract.

10) They are important emulsifiers in the bile.

11) They co-determine erythrocyte and platelet aggregation.

12) They influence immunological reactions on the cellular level.

These multiple tasks of phospholipids are always related to the morphology of biological membranes; in each point this list of the multiple tasks of phospholipids, which is not exhaustive, is based on the incorporation into biological membranes, and thus on the intact character of the structures.

**EPL as a Membrane Therapeutic**

With its special ingredient dilinoleoylphosphatidylcholine EPL is theoretically of importance in all those diseases in which damaged membrane structures, reduced phospholipid levels and/or decreased membrane fluidity are present. This is a hypothesis which is supported by experimental and clinical investigations on various membrane-associated disorders and illnesses.

**Active Principle and Indications**

Studies on the active principle of EPL as well as pharmacological and clinical trials are available on the following disturbances and diseases related to membrane damages:

- In liver diseases the hepatocyte structures are always damaged, for example by viruses, organic solvents, alcohol, medicaments, drugs or too fatty food. As a consequence, membrane fluidity and permeability are disturbed, membrane-dependent metabolic processes are impaired as well as membrane-associated enzyme activities. Immunological and receptor properties may be changed. This may considerably inhibit the metabolism of the liver.

- In hyperlipoproteinemia with or without atherosclerosis various pathomechanisms, such as lipid peroxidation, decrease of lipid- metabolizing enzyme activity and modification of lipoprotein structure and function interact and provoke a rise in serum cholesterol and triglyceride levels and a subsequent accumulation of fat in the peripheral tissue, avoiding the receptor-mediated uptake of cholesterol. As it is relatively lowered, serum HDL takes up and transports less cholesterol from the periphery back to the liver.

- One of the hemorheological disturbances is an elevated cholesterol/phospholipid ratio in the membranes of platelets and red blood cells (RBC) with concomitant changes in membrane function. This leads to an increased tendency of platelets and RBC to aggregate, which in turn influences blood flow properties and microcirculation.

- In neurological diseases the reduction of choline - a precursor of the neurotransmitter acetylcholine -, the deficiency in unsaturated fatty acids, or increased rigidity of neuronal membranes may influence metabolic processes and functions of the nerves.

- In gastrointestinal inflammation the mucosa quality, membrane structures, membrane-dependent immunological reactions and the local prostaglandin synthesis are altered.

- In lung diseases, such as infant or adult acute respiratory distress syndrome, the fatal outcome of the disease is triggered by a phospholipid deficiency in the pulmonary alveoli (surfactant).

- In kidney diseases phospholipid deficiency in the membranes is present involving impaired excretion and reduced prostaglandin synthesis.

- In chronic ambulatory peritoneal dialysis (CAPD) and in peritonitis the sharp fall of surface-active phospholipid material is striking. Disorders in the peritoneum and a reduction in ultrafiltration are the consequences.
In the multitactorial picture of gestosis disorders of the lipid metabolism and lipid peroxidation as well as impaired liver and kidney function can be observed.

In skin diseases, such as psoriasis, the pathological mechanisms seem to be favoured, among others, by alterations of cell structures and of the fatty acid and phospholipid composition.

In aging patients we are often faced with a combination of age-linked physiological changes and diseases, e.g. degenerative liver damage or atherosclerotic changes of the vascular wall associated with other degenerative or not degenerative diseases.

All these very different diseases may have comparable membrane disorders in common. With "essential" phospholipids such disorders may be positively influenced, eliminated or even improved beyond normal due to the high content in polyunsaturated fatty acids, for example:

1) HDL particles enriched with EPL are able to take up more cholesterol from LDL and tissues. More cholesterol can be transported back to the liver. This action on the reverse cholesterol transport is unique. All other lipid-lowering agents reduce either the cholesterol absorption in the body, or the cholesterol synthesis in the liver and its distribution to the periphery. These substances, however, do not physiologically mobilize the cholesterol already present in the periphery. Moreover, intravenously administered EPL micelles absorb apoprotein A-1 and may, to a certain extent, behave like HDL particles and take up cholesterol.

2) The stimulation of lipolytic enzymes, such as lipoprotein lipase and hepatic triglyceride lipase, favours the break-down of triglyceride-rich lipoproteins.

3) The cholesterol/phospholipid ratio in membranes, platelets and red blood cells decreases and membrane function is improved. Aggregability and blood viscosity decrease, microcirculation and life-span of the mentioned blood corpuscles increase.

4) Peroxidative reactions are reduced, damaged hepatocyte membrane structures restored, membrane fluidity and function stabilized, immunomodulation and cell protection improved, and membrane-associated liver functions enhanced.

5) With the normalization of the cholesterol/phospholipid ratio also the bile is stabilized.

6) Due to its specific property as a surface-active emulsifier EPL solubilize fat in the gastrointestinal tract, and in risk and treatment of fat embolism.

7) The substitution with polyunsaturated fatty acids and choline may have a cytoprotective effect in the brain and activate neuronal processes.

8) Liposomes with polyunsaturated phosphatidylcholine molecules may act as drug carriers, such as of vitamin E.

Liver Disease

Experimental and clinical results support the assumption that the therapeutic application of EPL has protective and even curative and regenerative effects on the biological membranes of sinus endothelial cells and hepatocytes. The cytoprotective effect of EPL has been corroborated in 7 in vitro and in vivo experiments, in which 20 different models with 5 different animal species were used. Types of intoxication which are known to play a role in the etiology of liver disease have mostly been applied: chemical substances, medicaments, alcohol, cholestasis, immunological phenomena, exposure to radiation, etc.

The hepatoprotective effects of EPL have been confirmed as compared to the controls and were the more pronounced the earlier EPL was administered:

1) Structures of membranes were normal or largely normalized;

2) Fatty infiltrations and hepatocyte necrosis could be diminished or even eliminated;

3) Corresponding data were found for lipid peroxidation, transaminase and cholinesterase activity, and for serum lipids; liver cell metabolism increased;

4) The increase of RNA and protein synthesis and of the liver cell glycogen content indicated a stimulation of the liver cells;
5) Reduced collagen production, collagen/DNA ratio and liver hydroxyproline indicated a reduced formation of connective tissue. Until end of 1989 78 open, 35 single-blind and 13 double-blind clinical trials in altogether 8334 patients were carried out; 74 of these studies were based on 3 groups of criteria, 41 on 4 groups and 5 on 5 groups of criteria (including electron microscopy). In 45 studies histological controls were performed. The dosage of EPL ranged from 525 mg to 2700 mg/day when administered orally, and from 500 mg to 3000 mg/day in intravenous application. The duration of treatment lasted from some weeks to up to 30 months. The main liver indications were: acute hepatitis, chronic hepatitis, fatty liver, toxic liver damage, and cirrhosis of the liver.

The clinical findings, showing the efficacy of EPL, can be summarized generally as follows:

1) accelerated improvement or normalization of subjective complaints, of clinical findings and several biochemical values;
2) a better histological result as compared to the control groups;
3) a shortened duration of hospitalisation.

Two pharmacological, 11 open and 1 double-blind trials have shown the effectiveness of EPL in hepatotoxicity of anti-TB agents, which is the only representative clinical model of liver damage to assess effectiveness of a liver therapeutic in intoxication.

Renal Disorders and Chronic Ambulatory Peritoneal Dialysis
Eight pharmacological and 23 clinical studies give a first impression of EPL and its influence on renal disorders. A significant rise of creatinine, urea and sodium clearances, correction of disorders of lipid metabolism, disappearance of proteinuria and hypoalbuminemia, and decrease of lyssolecithin excretion range among the effects mostly seen. Signs of intoxication could be improved in particular in nephrotic forms. As main effects of EPL are considered the stabilization of renal cell membranes and a positive influence on cytoprotective prostaglandins. At least in patients with diminished ultrafiltration EPL provoked a reincrease of the ultrafiltration rate in chronic ambulatory peritoneal dialysis after intraperitoneal and intravenous administration. Quality improvement of the peritoneal surfactant is discussed as a possible mode of action.

Gestosis
A total of 684 patients in 13 studies received as adjuvant treatment 250-1000 mg EPL i.v. and/or 1.8 g EPL orally per day.

In these patients suffering from early or late gestosis the subjective symptoms, such as hyperemesis gravidarum, clearly improved or disappeared. This positive effect was also seen with respect to accompanying disturbances, such as lipid peroxidation, renal disorders, pathological liver function and hyperlipidemia.

Hyperlipoproteinemia/Atherosclerosis
To date, the influence of "essential" phospholipids on the lipid metabolism has been studied in 14 in vitro and in 95 pharmacological investigations, in which EPL was applied in different models or diets in 11 different animal species by the intravenous, oral, subcutaneous, intracardial or intraperitoneal route, prophylactically, simultaneously or curatively. The results of these studies, reflecting the effects of EPL in lipid metabolic disturbances can be summarized as follows:

1) Increase of polyunsaturated fatty acids in cholesterol esters, phospholipids, triglycerides and lipoproteins, in serum and aorta.
2) Influence on enzyme activities in serum and aorta, such as on LCAT, HTGL, LPL, ACAT and phospholipase.
3) Lowering effect on serum lipid values.
4) Influence on lipoprotein structure and cholesterol content, especially on HDL-C/LDL-C ratio.
5) Antiatherogenic effect in prophylactic and therapeutic use.
6) Decrease of (lipid) peroxidation and platelet aggregation together with improved hemorrheology.

Until end of 1989 205 clinical trials, 12 out of them double-blind, and 53 controlled in a total of 7606 patients (save fat embolism) were carried out.

In the mean, EPL reduced total serum cholesterol by -8 to -30%, LDL cholesterol by -10 and -31%, triglycerides by -12 and -58%, and it increased HDL cholesterol by +10 to +45%.

In a pilot study in 15 patients with hyperlipoproteinemia and high-cholesterol plaques signs of reduced growth rate of minor plaques and a reduction of the size of larger high-cholesterol plaques were found.

Eleven studies demonstrated the influence of EPL on the erythrocyte morphology including improved cholesterol/phospholipid ratio in membranes, filterability and flexibility of red blood cells, erythrocyte aggregation, blood viscosity and capillary blood flow.

In 20 studies it was shown that platelet membrane composition improved, and that platelet susceptibility to ADP, PAF or collagen, and the thromboxane/6-keto-PGF1α ratio of thrombocytes were normalized.

According to some authors, injected EPL micelles or liposomes simulate HDL function and improve reverse cholesterol transport.

Finally, in 18 experimental studies and in 23 clinical trials in altogether 4485 patients WB8 demonstrated that EPL has a prophylactic and therapeutic effect in fat embolism.

Gastrointestinal Inflammation

The following experiments, among others, were carried out:
- whole-body autoradiographs of rats after an oral dose of radioactively labelled 1,2-dilinoleoylphosphatidylcholine, visualizing the high EPL concentration in the gastric and intestinal mucosa even 24 hours after administration;
- mucosal PGE2 synthesis and release experimentally reduced by indomethacin application reincreased significantly after 60 and 120 minutes of simultaneous EPL administration.

Correspondingly, NSAID-induced gastric mucosal damage was reduced or prevented in experimental studies, when EPL was applied concomitantly.

Similar results were found in orientating clinical trials in gastroduodenal damage, especially due to NSAIDs, which, however, were dose-related.

These findings underline the significance of the surface-action of phospholipids for the hydrophobic properties of the gastrointestinal surface.

Neurology

Fourteen experimental studies have shown that:
EPL is taken up to a small amount intact into the brain endogenous phospholipid synthesis is stimulated – positive effects, such as raised choline content in the brain, improved cerebrovascular circulation and cerebral enzyme antioxidant system, and favourable actions on the dendritic material have been demonstrated.

35 clinical investigations in 1968 patients and 3 in 31 volunteers have also given clinical evidence of some positive effects in certain diseases, such as involutional dementias and multiple sclerosis. Improvements of subjective well-being, such as headache, dizziness, memory, concentration, endurance, irritability, insomnia, angina attacks, and walking time have been reported. However, final conclusions cannot yet be drawn since, for example, neither the exact EPL dose to be applied in different neurological diseases is known nor is the classification of the diseases with their sub-types clear enough.

Lung Surfactant

From 3 experimental and 8 clinical studies can be inferred that the therapeutic possibilities of EPL in this indication field consist first of all
in the prophylactic substitution of phosphatidylcholine molecules in the
decisive pregnancy weeks to support the formation of surfactant in the unborn
child. Another possibility is the compensation of membrane damages, e.g. in the
presence of inflammatory processes in the lung, or in atherosclerotically
changed blood flow properties and erythrocyte flexibility.

Psoriasis
Thirteen studies in 915 patients, treated for 1 month up to several years
demonstrated the value of EPL as adjuvant therapeutic application in this
condition. The positive effect is probably based on the correction of lipid
values in the skin, primarily of fatty acid levels.

Geriatrics
In geriatrics, where age-related physiological changes in the organism are
often combined with specific diseases, the membrane therapeutic EPL may prove to
be useful. The effects described so far for various indications can be
summarized as follows: enhanced memory performance of the aging brain, promotion
of gastrointestinal function by mucosa restoration, activation of the liver
metabolism and detoxication, activation of renal function, influence on the
lipid metabolism and on atherosclerosis by cholesterol mobilization, improvement
of the coronary, peripheral and cerebral blood flow and, finally, correction of
the increased cholesterol/phospholipid ratio in cellular membranes in general.
Since often multimorbidity is present in old age and the elderly mostly have to
take different drugs, it is essential that geriatric disorders be treated with a
preparation that does not provoke additional side-effects or which even
alleviates the adverse reactions of the accompanying medication. "Essential"
phospholipids are a phytotherapeutic product without noteworthy side- effects
even in long-term application, and without contraindications.

Membrane Therapeutic
On the basis of the available data on EPL and its different modes of action,
of pharmacological investigations and of a broad range of clinical trials it can
be summarized that EPL acts primarily by its influence on membranes. As membrane
changes and damages occur in many disturbances and diseases the therapeutic
approach with EPL in man is a holistic one.
The possibilities of application reside, on the one hand, in the kind and
severity of membrane damage and, on the other, in the influence on membrane
fluidity and thus on membrane-dependent metabolic processes by the incorporation
of the special molecule 1,2- dilinoleoylphosphatidylcholine. In addition, the
membrane pool of substrates for the endogenous metabolism is increased.
The application of EPL as a membrane therapeutic seems to be limited by the
attempt of the cell to maintain normal membrane homeostasis which allows only
certain variations, although the cell is also active to eliminate damages.
From this we can infer that EPL can be applied (in general as adjuvant
medication) in membrane-associated damages and diseases, and, in specific cases,
to enhance membrane-related physiological processes.
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