

4. PHARMACOLOGICAL INVESTIGATIONS WITH EPL IN DIFFERENT DISEASE MODELS

4.1 Effect in Liver and Kidneys

4.1.1 Toxic Liver Damage

The suggested hepatoprotective 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 CCl₄ 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: CCl₄, 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 CCl₄.

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, centrilobular 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 CCl₄ intoxication for 9 months, rats showed severe cirrhotic alterations of the liver. The animal group which in addition to CCl₄ 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 diminished; 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. <Fig. 15: Sequential development of alcoholic liver injury in baboons fed ethanol with a normal (a) and with an EPL-supplemented diet (b) for up to 8 years (449)>

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.Keppeler 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, angioneclerosis 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 b₅, 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 centrilobular 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 dithiocyanate 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. Centrilobular 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 dose-dependent 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 ligation, 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 ligation) 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 (auto-immunological) antibody-dependent cell-mediated cytotoxicity (ADCC: fig. 16 and mitogen-induced lymphocyte cytotoxicity.
<%Fig. 18: Schematic representation of ADCC%>

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. 17 b).

<%Fig. 17: a) Lymphocyte cytotoxicity to isolated hepatocytes from control and EPL-treated rabbits (% = number of destroyed hepatocytes compared to baseline value); b) Direct cytotoxicity of untreated and mitogen-stimulated lymphocytes to isolated hepatocytes from control and EPL-treated rabbits. (PHA = Phytohaemagglutinin)>

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) and 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).

<%Tab. 15: EPL and ionising radiation%>

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):

<%Tab. 16: Pharmacological studies: EPL: and the regeneration of the liver%>

21 studies during the period of 1962 to 1991 were carried out in mice, rats or dogs on different models of induced liver damage: CCl₄, 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 salicylate 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/l 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 an 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 E P L therapy%>

<%Tab. 18.2.1: Influence of E P L on the enzyme activity in the aorta (in the serum)%>

<%Tab. 18.2.2: Influence of E P L on the enzyme activity in the aorta (in the serum)%>

<%Tab. 18.3.1: Lowering effect of E P L on serum lipid values%>

<%Tab. 18.3.2: Lowering effect of E P L on serum lipid values%>

<%Tab. 18.3.3: Lowering effect of E P L on serum lipid values%>

<%Tab. 18.3.4: Lowering effect of E P L on serum lipid values%>

<%Tab. 18.4.1: Influence of E P L on lipoproteins%>

<%Tab. 18.4.2: Influence of E P L on lipoproteins%>

<%Tab. 18.4.3: Influence of E P L on lipoproteins%>

<%Tab. 18.5.1: Antiatherogenic effect of E P L%>

<%Tab. 18.5.2: Antiatherogenic effect of E P L%>

<%Tab. 18.5.3: Antiatherogenic effect of E P L%>

<%Tab. 18.5.4: Antiatherogenic effect of E P L%>

<%Tab. 18.6.: Celebrovascular effect of E P L%>

<%Tab. 19.1: Effect of EPL on human enzymes, lipoproteins, plasma, cells and tissues%>

<%Tab. 19.2: Effect of EPL on human enzymes, lipoproteins, plasma, cells and tissues%>

The topics "Influence of EPL on the enzyme activity in the aorta and serum" and "Influence of EPL on lipoproteins" are described in chapters 3.3.2/5.4.6 and 3.4 so that the more detailed description will be on parts 1, 4 and 5. Point 6 will be a part of chapter 4.4.

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 acyltransferase, 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 atherosclerotically 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 radiolabelled tracers (125I-polyvinyl-pyrrolidone, 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 atherosclerotically 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 myocardium the 20% cholesterol diet caused fibrous changes and depositions of fat droplets. The fibrous changes in the myocardium 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 myocardium 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 animal 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 atherogenic 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, 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) particles 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 HDL 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).

Summary

- Phosphatidylcholine micelles/liposomes bind free cholesterol at their surface as HDL does, in dependence of the linoleic acid content and the kind of the electric surface charge.
- In plasma they can accumulate endogenous proteins, such as apo A1, and thus largely resemble HDL with respect to composition, density and function.
- Polyenylphosphatidylcholine micelles/liposomes are more efficient than other PC-particles as to forming complexes with cholesterol. The plasma clearance of these PC-particles, which is usually fast, appears to be slowed down when binding to apo A1 or apo HDL.

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).
<%Tab. 22: Pharmacological assessment of the effectiveness of EPL in fat embolism (survey)%>

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.

<%Tab. 23.1: Pharmacological investigations into the effectiveness of EPL in fat embolism%>

<%Tab. 23.2: Pharmacological Investigations into the Effectiveness of EPL in Fat Embolism%>

<%Tab. 23.3: Pharmacological Investigations into the Effectiveness of EPL in Fat Embolism%>

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 administration 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 gastrotoxicity 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 gastrototoxicity 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 gastrototoxicity 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 gastrototoxicity of piroxicam at the highest dose (tab. 25). The changes were dose-related. Administration for 3 days of diclofenac and EPL (1:1 molar ratio) produced a significant reduction in the gastrototoxicity 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 gastrototoxicity 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 gastrototoxicity 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 gastrototoxic 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 gastrototoxicity, 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 o 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 gliacytic 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 gliocytes, regeneration of neurons, influence of the response of receptors as well as increased activity of membrane-bound enzymes;
- carrier of unsaturated fatty acids;
- antlatherosclerotic 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. (493- 495). 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 noradrenalin 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 ug 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).

<%Fig. 24: Camera lucida drawings of basilar dendritic trees from layer V pyramidal cells with Sholl analysis overlay. Left: 24-month-old control (purina chow). Right: 24-month-old EPL- enriched (EPL***). Rapid Golgi stain (486)%>

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 (anatomic-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.