

5. CLINICAL STUDIES WITH "ESSENTIAL" PHOSPHOLIPIDS 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).

<%Tab. 28: Serum transaminase levels and clinical symptoms (154)%>

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/l.

The trend towards liver cell necrosis seen with combined antituberculous medication is obviously reduced considerably by EPL. These findings and further data from 8 open studies (125, 151, 218, 274, 310, 392, 399, 653) are borne out by a recent randomized double-blind study performed by B. Marpaung et al. (468). 120 patients with active tuberculosis received, in relation to body weight, 450-600 mg rifampicin, 3 - 4 tablets Intam 6 (containing 250 mg ethambutol HCl, 100 mg isonicotinic acid hydrazide and 6 mg pyridoxine HCl) daily together with 3 x 2 capsules of Essentiale forte (= 1800 mg EPL) or placebo, for a period of 3 months. 101 patients completed the treatment. Only 1 patient had still a positive sputum test at the end of the treatment period. Improvement of the clinical and radiological pictures was consistent with this finding. In 38.5 % of the patients of the placebo group treatment with anti-tuberculous agents provoked an increase in AST levels, while 25 % registered elevated ALT values. In the patients receiving EPL these reactions were observed in 16 % and 8.2 %, respectively ($p < 0.05$, fig. 25).

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 clearly 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).

<Fig. 27: Results of the variables of effectiveness of a double-blind, placebo-controlled study on HbsAg+ CAH in 50 patients. Therapy: over 1 year daily 6 capsules Eseeintiale forte or placebo; significant difference: $2p < 0.01$ - $2p < 0.001$ >

<Fig. 28: Improvement of histological variables; percentage of patients with diminished intralobular necrosis and portal inflammation and with general improvement of the liver score after 12 months. The results are based on liver biopsy findings at the beginning and after the end of 12-month treatment; significant difference: $2p < 0.001$ >

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 α_2 and β -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).
<%Tab. 30: Laboratory variables before and after treatment with EPL (552)%>

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.Toouli 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).

<%Tab. 31: Clinical Studies: EPL and the Regeneration of the liver%>

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 (pallor 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 160ml/24 h in 1 case, was increased by 64%. Albumin in urine was 0.5 to 10g% 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% phospholipid 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 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. <Fig. 29a: Behaviour of ultrafiltration (ml/day) in 5 patients on CAPD with low ultrafiltration over 15 days after intraperitoneal EPL (50 mg/I). Means and SD. UF = Ultrafiltration (149)%>

<Fig. 29b: Changes in ultrafiltration (ml/day) after intravenous EPL (250 mg/day) during CAPD in 6 patients with low ultrafiltration and in 4 patients with normal ultrafiltration. * $p < 0.05$ versus basal. Means and SD. UF= Ultrafiltration (149)%>

A. De Vecchi 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, spasmolytics, 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): malondialdehyde 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 preeclampsia 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).

<%Tab. 32: Application of EPL during pregnancy-related diseases%>

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-diphosphate 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 1g of EPL/dH. 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% ($p < 0.001$) (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.

<Tab. 33: Response of total cholesterol in serum determined in 9 double-blind trials [lla = type Ila according to Fredrickson]>

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% 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 pathological 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 $13.4 \pm 1.3\%$ to $20 \pm 2.3\%$ 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, 153, 172, 288, 314, 336, 349, 462, 640, 686, 687, 691, 700) expressed mean HDL cholesterol in mg/dl or mmol/l. 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/l to 1.42 ± 0.07 mmol/l (+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 %). He demonstrated a significant rise in HDL3 as well as HDL2 ($p < 0.01$).

Mean HDL cholesterol increases of 10 % 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).

<Fig.30: Increase of serum HDL-cholesterol in patients ($n=13$) with hypercholesterolaemia type IIb and IV after a 14-day and a 42-day double-blind treatment with 1.8g EPL/day in comparison with controls ($n=15$). $**=2p < 0.01$, $***=2p < 0.001$ (Wilcoxon matched pairs signed rank test)(288)>

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 mg/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 EPL 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).
<%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 malonedialdehyde 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 malonedialdehyde 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.

<%Fig. 31: Phosphatidylcholine as the substrate for the formation of cholesterol-esters catalyzed by the LCAT-enzyme%>

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.

<%Tab. 38: Presentation of the relative reaction rate of purified LCAT with phosphatidylcholine substrates which contain identical fatty acids in 1- and 2-position

The highest transacylation rates were seen with 1,2-dilinoleoylphosphatidylcholine (according to 28)%>

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 $\mu\text{mol/I/h}$ to 54.4 $\mu\text{mol/I/h}$ 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 promotor

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).

<%Tab. 37: Influence of the type of phospholipid on the lipolytic activity in adipose tissues and post-heparin plasma of 9 healthy subjects. The dependence of the degree of fatty acid saturation is presented as an index of activity relative to EPL = 100 Phospholipid concentration: 0.35 μ mol/ml medium (according to 145)%>

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 hyperlipoproteinaemia 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 shifting 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-PGF₁? (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-PGF₁? 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 8 μ 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 $\geq 25 \mu\text{l}$ tended to stagnate after a transient initial rise. Larger initial volumes ($> 25 \mu\text{l}$) 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 ($n = 9$).
<%Fig. 32: Total plaque volume during 15-month treatment with 2.7 g/day of EPL in 15 patients with a total of 57 plaques (589) (8 patients with an initial plaque volume > 25 μ l; [broken line]; 7 patients with an initial plaque volume \leq 25 μ l)%>

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 for 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).

<%Fig. 33: Mean incidence of angina attacks per week and consumption of nitroglycerin per week for patients with diminished coronary blood-flow rates before and during a 6-week treatment with EPL ($n = 507$). Arithmetical means (264)%>

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.Leutschaft (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. <Fig. 84: Mean walking time in min of patients (n=282) before and during treatment with EPL for 6 weeks. Arithmetical means (264)>

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).

<%Fig. 35: Indomethacin plasma levels in 9 healthy volunteers after a single dose of 50 mg indomethacin alone or in combination with 200 mg EPL (mean a SEM)(453)%>

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).

<%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".

<%Fig. 36:%>

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).

<%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. <%Fig. 37:%>

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 joint 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 afore-mentioned study pre-treatment and post-treatment gastroscopies were performed in 3 patients. In 2 patients 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
- fat embolism-induced pathological changes in the brain

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 - phosphatidylcholine 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 gliacytic 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 (Sclerosis)

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 Involuntional 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 inmedicable 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 involuntional 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 involuntional 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.Borronei 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.

<%Tab. 42: Kind of diseases and number of studies/patients with EPL in neurological diseases%>

<%Tab. 43: Studies with volunteers on "neurology"%>

<%Tab. 44.1.1: Cerebral circulatory disorders (sclerosis)%>

<%Tab. 44.1.2: Cerebral circulatory disorders (sclerosis)%>

<%Tab. 44.1.3: Cerebral circulatory disorders (sclerosis)%>

<%Tab. 44.1.4: Cerebral circulatory disorders (sclerosis)%>

<%Tab. 44.5: Multiple sclerosis%>

<%Tab. 44.2.2: Involuntional dementias%>

<%Tab. 44.3: Friedreich's ataxia%>

<%Tab. 44.4: Mania%>

<%Tab. 44.2.1: Involuntional dementias%>

<%Tab. 44.6: Muscular dystrophy of Duchenne%>

Finally the trial on neurotoxicosis 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 neurotoxicoses 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 neurotoxicosis 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).

<%Fig. 38: Possible pathway of lung surfactant through pneumocytes type II. M = monolayer of phospholipids; TM = tubular myelin; LB = lamellar body; G = golgi;

ER endoplasmic reticulum; I = pneumocyte type I, II = pneumocyte type II (according to 228)%>

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 PA. 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.Tovstanovskaya (666) found in gestation weeks 28-31 reduced phospholipid levels (particularly 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 Dimphosphon (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 malonedialdehyde 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 sulphonamide 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.

<%Tab. 45: Investigations carried out with EPL%>

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.

Also G.Giss (225) described marked trends towards healing of the skin eruptions already after 2-3 months of oral plus parenteral application of EPL (n=26); in some cases the eruptions were reduced so that only minor plaques could still be seen.

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. Mashkilleyson 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 Mashkilleyson 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.

<%Tab. 46: Fatty acid contents in the serum of psoriatic patients which were significantly improved by the treatment with Esaentiale forte (1)%>

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 desquamations, 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 α -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% α -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%.
<Tab. 47:%>

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).