

2. THE 'ESSENTIAL' PHOSPHOLIPIDS (EPL)

2.1 Chemistry

The term of "essential" phospholipids indicates the highly purified extract of the semen of *Glycine max* (Linne Merrill) with a standardized content of 76 % or 94 % (3-sn-phosphatidyl) choline in the oral preparations and of 94 % in the intravenous forms.

The extract with 76 % (3-sn-phosphatidyl) choline contains beside phosphatidylcholine molecules further phospholipids, in detail 7 % phosphatidylethanolamine, 0.5 % phosphatidylinositol, 11.5 % other lipids such as glycolipids and 5 % oil. The (3-sn-phosphatidyl) choline purified to 94 % presents no more detectable quantities of other phospholipids, only 3 % of other lipids and 3 % of oil.

The word "essential" phospholipids results from the characteristic fatty acid composition of the phospholipids of the soya bean which is distinguished by its particular high content of polyunsaturated fatty acids, predominantly linoleic acid (approx. 70 %), linolenic acid and oleic acid.

According to D. LeKim and H. Betzing (404) the fatty acid composition of these phosphatidylcholine molecules is in mol %:

<Table 5:>

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

<Figure 8: 1,2-Dilinoleoylphosphatidylcholine>

The atomic formula is: $C_{44}H_{80}N_0O_8P \times H_{20}$, the molecular weight: 800.

According to J.M. Fox (195) typical analytical data of the phosphatidylcholine molecules are:

Phosphorus content: 3.8 %

Choline content: 14.9 %

Fatty acid content: 69.0 %

with the following molecular proportions:

Phosphorus:choline = 1.00

Fatty acids:choline = 2.02

Fatty acids: phosphorus = 2.00

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

2.2 Nomenclature

Thanks to the development of modern, patented techniques of separation it has become possible to constantly improve the chemical purity of the (3-sn-phosphatidyl) choline fractions from the soya bean, up to the phosphatidylcholine of 94% today.

This historic development and country-specific peculiarities are the reasons why various names have been used in literature. The terms such as "EPL - Special" (= up to 82 % PC), "EPL - Purissimum" (= 94% PC), polyenylphosphatidylcholine (PPC) or polyunsaturated phosphatidylcholine (PUPC), or polyunsaturated lecithin (PUL) (=76 % PC or 94% PC)

designate the same group of preparations with a very high content of phosphatidylcholine molecules with predominantly polyunsaturated fatty acids. According to the knowledge on the human membrane structures and functions (chapter 1 and 3) it is undisputed that phosphatidylcholine is a very important active ingredient. For this reason the soya-lecithins are standardised to the content of phosphatidylcholine. In this thesis, therefore, the generally known term of "EPL" is used to express the terminologically identical names of PPC, PUPC and PUL, phospholipon 100 (and with restriction LethiconR with a standardized content of 55 % (3-sn-phosphatidyl)choline).

2.3 EPL-Containing Preparations

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

1. EssentialeR capsules:

1 capsule contains:

- 175 mg of EPL (76 % 3-sn-PC) . ' .
- 3 mg of thiamine nitrate (vit. B1)
- 3 mg of riboflavin (vit. B2)
- 3 mg of pyridoxine-HCl (vit. B6) .
- 3 ug of cyanocobalamin (vit. B12)
- 3.3 mg of -tocopherol acetate (vit. E) _
- 15 mg of nicotinamide

2. EssentialeR forte capsules:

1 capsule contains:

- 300 mg of EPL (76 % 3-sn-PC) .
- 6 mg of thiamine nitrate (vit. B1)
- 6 mg of riboflavin (vit. B2)
- 6 mg of pyridoxine-HCl (vit. B6)
- 6 ug of cyanocobalamin (vit. B12) . " .
- 6 mg of -tocopherol acetate (vit. E)
- 30 mg of nicotinamide

3. EPL capsules:

1 capsule contains:

- 250 mg of EPL (76 % 3-sn-PC)

[used only in Japan]

4. EssaheoanR capsules:

1 capsule contains:

- 450 mg of EPL (94 % 3-sn-PC)

5. EssentialeR for i.v. injection

1 ampoule of 5 ml contains:

- 250 mg of EPL (94 % 3-sn-PC)
- 2.5 mg of pyridoxine-HCl (vit. B6)
- 10 ug of cyanocobalamin (vit. B12)
- 1.5 mg of sodium-D-pantothenate
- 25 mg of nicotinamide

6. EssentialeR for infusion

1 ampoule of 10 ml contains:

- 1000 mg of EPL (94 % 3-sn-PC)
- 5 mg of pyridoxine-HCl (vit. B6)
- 15 ug of cyanocobalamin (vit. B12)
- 3 mg of sodium-D-pantothenate
- 100 mg of nicotinamide

(The EssentialeR preparations are also available without vitamins)

7. LipostabilR capsules:

1 capsule contains:

- 175 mg of EPL (76 % 3-sn-PC)
- 1.5 mg of pyridoxine-HCl (vit. B6)
- 1.65 mg of -tocopherol acetate (vit. E)

(The preparation is in some countries also available with 30 mg of ethophylline.)

8. LipostabilR forte capsules:

1 capsule contains:

- 300 mg of EPL (76 % 3-sn-PC)

(The preparation is in some countries also available with 50 mg of ethophylline.)

9. LipostabilR for i.v. injection

1 ampoule of 5 ml contains:

- 250 mg of EPL (94 % 3-sn-PC)

1 ampoule of 10 ml contains:

- 500 mg of EPL (94 % 3-sn-PC)

(The preparation is also available with pyridoxine hydrochloride (vit. B6) 2 mg (4 mg), nicotinic acid 1 mg (2 mg), adenosine-5-monophosphate 1 mg (2 mg).)

EPL is the main active ingredient of all these preparations. This is proven by the mode of action of EPL which is described in chapter 3.

2.4 Pharmacokinetics

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

2.4.1 Absorption in Animal Experiments

The absorption rate determined by D. LeKim and E. Graf (405) following oral administration of radiolabelled EPL to rats was higher than 90 % (fig. 9).

<Fig. 9: Absorption of radioactivity following oral administration of double labelled EPL to conscious rats>

This confirms the results of J. M. Fox et al. (196) who found that EPL is almost completely taken up from the intestinal lumen within 24 hours. S. Parthasarathy et al. (537) also obtained concordant results. Almost 100 % of the administered dose of EPL is hydrolysed to 1-acyl-lysophosphatidylcholine during absorption (fig. 10). Of this, about 50 % is reacylated to intact EPL in the intestinal mucosa (405).

<Fig. 10: Phosphatidylcholine metabolism after intestinal absorption. The framed compounds were measured following lymph cannulation (406)>

This finding is important insofar as studies by M. Whyte et al. (743) indicate that, in mammals, free unsaturated fatty acids given orally are not effective in changing the fatty acid moieties of endogenous phosphatidylcholine so as to produce molecules containing polyunsaturated fatty acids in the 1- and 2-positions. In mammalian systems phosphatidylcholine is esterified preferentially with a saturated fatty acid in the 1-position.

2.4.2 Distribution in Animal Experiments

EPL enters the liver via the lymph and the blood pathways. In the liver, it is partly taken up by cell membranes and subcellular membrane fractions. In the blood, phospholipid fractions of the lipoproteins exchange with EPL, the latter being taken up preferentially by the high-density lipoproteins (HDL) (403, 404, 584, 680, 764, 766-768).

Studies in rats and mice given EPL i.v. showed that approximately 80 % of the injected dose was eliminated from plasma after 15 minutes, approximately 92 % after 75 minutes and approximately 100 % after 10 hours (282, 283).

<Fig. 11: Rates of incorporation and breakdown of EPL (0.5 mg/g body weight) administered i.v. to male and female rats (403)>

After a single oral dose (350mg/kg b.w.) of $^3\text{H}/^{14}\text{C}$ -labelled EPL in rats, maximum concentration of radioactivity in the liver reached 24.5% after 24 hours of application and slowly declined to 4% within 8 days. Significant quantities of radioactivity were found in the striped muscles: 5.8% of the dose after the first 6 hours, an increase of up to 25% being observed within the first 8 days of dosing. Lower amounts of radioactivity were distributed in the kidneys, the lung and the myocard: 2.7%, 0.9% and 0.3% resp. of the administered dose within 24 hours. Following single oral administration of labelled EPL, radioactivity

was detected in the plasma only for 48 hours. After 96 hours approx. 22%, and after 8 days up to 54% were absorbed from the cells (105). After a 5-day repeated oral administration of ³H/¹⁴C-labelled EPL to rats, significant concentrations of the ¹⁴C- radiolabel were detected in the liver (18.6 %), the depot fat (7.0 %), the striped muscles (31.4 %) and the bones (5.6 %). Lower concentrations of C-radioactivity were located in the lungs, kidneys, testes, gastrointestinal tract, blood and plasma. Six hours after the 5th administration of labelled EPL 22.9 % of the dose were accumulated in the liver. This value declined to 2.3 % after 16 hours. ¹⁴C-radioactivity in the striped muscles rose to 36.9 % within 4-day. Little radioactivity was measured in the bones and the depot fat: a maximum of 4.7 % and 5.6 % resp. of the dose 24 hours after the 5th application. Kidneys and adrenals presented 1.3 % and 1.7 % resp. of ¹⁴C after 6 hours. The ¹⁴C- radiolabel in the blood showed a fairly homogeneous distribution in blood cells and plasma throughout the 4-day dosing. Eight days after the 5th dosing up to 65 % of the total radioactivity were concentrated in the blood. After another 8 days already 76 % of the ¹⁴C- radiolabel was detected in the cells (105).

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

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

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

Analytic examinations of the incorporated EPL radiolabel by means of cell fractionation of the liver homogenate after 6 hours of oral application of ³H/¹⁴C-EPL clearly showed that the radioactivity was located to a quantitatively high degree in the membrane-containing fractions, the distribution being ubiquitous in all liver cell fractions (405). This result is in accordance with former investigations on i.v. application of EPL (403).

Hydrolysis by phospholipase A2 showed that 6 hours after application of the radiolabel, the phosphatidylcholine isolated from the liver still contained 96 % of the labelled fatty acids in the 1-position, whereas there were only 4 % in the 2-position. Moreover, the determination of the expired CO₂ after oral application of differently labelled 14C/3H-dilinoleoyl-phosphatidylcholines showed that a high concentration of the 14C-radioactivity was present only when the fatty acid in the 2-position of the phosphatidylcholine molecule was labelled with 14C (403).

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

2.4.3 Pharmacokinetics in Man

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

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

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

<Fig. 12: Absorption kinetics of 3H and 14C in man following oral administration of 3H/14C-PC (n = 5); per cent dose per total blood volume is plotted against time (h) (767)>

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

2.5 Toxicology

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

2.5.1 Acute Toxicity

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

<Tab. 6:>

For reasons of volume, obviously, no higher doses could be applied. As no animal died within the 7-day follow-up period, it can be affirmed that the highest tolerable dose, as used in the mentioned species and application forms, corresponds to the given doses or is even higher. From the clinical viewpoint,

only sporadically slight symptoms were observed, mostly none at all. Only in subcutaneous application, the section showed sporadically rests of substance at the site of application.

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

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

<%Tab. 7:%>

2.5.2 Toxicity in Repeated Administration

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

<%Tab.8:%>

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

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

2.5.3 Teratogenicity and Embryotoxicity of EPL

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

<%Tab. 9:%>

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

2.5.4 Perinatal and Postnatal Toxicity, Fertility and Reproductive Performance

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

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

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

2.5.5 Mutagenic and Carcinogenic Potential

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

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

2.6 Safety Pharmacology and Pharmacodynamic Effects of EPL

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

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

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

In table 10 are summarized the results.

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

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

<%Tab. 10: Survey of results of safety pharmacology and pharmacodynamic effects in different standard models with single or short term administration of EPL