PLAQUEX

SUMMARY & REFERENCES TO LITERATURE PERTAINING TO Essential Phospholipids (EPL)

CONTENTS

1. Safety Studies

1.1 Intravenous

1.1.1 About the effect of cholin-phospholipd pure fraction on mineral metabolism in humans

SUMMARY: Until now the question is unanswered whether lipid soluble acids have a general biological significance for Kation transport through the cell membrane. We tested the acute effect of intravenously administered cholin phopspholipids on water metabolism, kidney function and mineral metabolism. Solutions were used containing 10% of a very pure phospholipid fraction containing high amounts of essential fatty acids with cholin in the basic molecule part. Test subjects included 8 females and 12 men. As a result renal potassium excretion was reduced by 0.5439 mg/min (17.8 %) median. Phosphorous excretion increased by a median of 0.0566 mg/min (18.4%). The reason was a change of the tubular reabsorption quota for these 2 substances. The renal excretion of sodium, calcium, magnesium and chloride was unchanged. Plasma concentration of potassium increased by 0.415 mg% (2.2%), that of phosphorous increased by 0.37 mg% (3.5%). Plasma magnesium decreased by 0.055 mg% (2.6%). The parenteral adminstration of cholin phospholipids causes a change in the water and mineral metabolism within 60 minutes which can be attributed to the influence of phospholipids on ionic transport through cell membranes. The total excretion of sodium, potassium, calcium, magnesium and phosphorous in a 24 hour urine sample was unchanged after 20 cc of a 10% phospholipid administration.

1.1.2 Excerpt of: 5.1 The "essential" phospholipids in hepatology – 50 years of experimental and clinical experiences: *Kuntz E, Z Gastroenterol (Suppl 2) 1991: 29:7-13*

…….. The fundamental knowledge on the pharmacokinetics of EPL (intestinal absorption, distribution in plasma, metabolism, enterohepatic circulation) is largely supported by over 15 experimental studies. Toxicity of EPL can certainly be excluded on the basis of 20 experimental in-vitro and in-vivo studies. This is also true when excessive doses are given both in short term and long term use. There is no fetal toxicity or any mutagenic potential. A carcinogenic potential can be ruled out.
EPL entered the German market in 1952 and, in the meantime, it has been registered in 53 countries. Within these 38 years, 126 clinical studies with a total of 8'334 patients including a phase-IV multi center study with 2'862 patients have been carried out…….

2. Effects of EPL on Blood Lipids

2.1 Oral Application

2.1.1. Effects of dietary polyenylphosphatidylcholine on metabolism of cholesterol and triglycerides in hypertriglyceridemic patients: *Kesaniemi YA et al, Am J Clin Nutr 43:98-107, 1986.*

SUMMARY: This study was carried out primarily to determine whether the feeding of lecithin (polyenylphosphatidylcholine) has systemic effects on metabolism of cholesterol and triglycerides in patients with endogenous hypertriglyceridemia (type 4 hyperlipoproteinemia). Ten patients were studied during control periods and lecithin feeding. In the former period, 7 g safflower oil were added to the diet to balance the addition of 10 g of lecithin in the latter period. Lecithin feeding had no influence on levels of plasma cholesterol and triglycerides, or lipoprotein-cholesterol, transport of VLDL-triglycerides, or total steroid balance. However, lecithin feeding did significantly increase the molar percent of bile acids and decrease the molar percent lecithin in gallbladder bile suggesting that it has a systemic effect. In addition, it had a small but significant inhibitory effect on intestinal absorption of cholesterol.

2.1.2 Hypolipidemic effects of alisat and EPL in patients with diabetes mellitus: *Mel'chinskaia EN et al, Terapevticheskii Arkhiv. 72(8):57-8, 2000*

Abstract
AIM: *To* study a hypolipidemic action *of* alisat and EPL in patients with non-insulin-dependent diabetes mellitus (NIDDM) .MATERIAL AND METHODS: Changes in blood lipids were studied in 121 NIDDM patients aged 36-66 years with compensated or subcompensated carbohydrate metabolism an sugar-reducing therapy. The latter consisted of a 52-week course of alisat (600 mg/day) or EPL (900 mg/day) in baseline levels of total cholesterol (TC) under 6.5 mmol/l and above 6.5 mmol/l, respectively. RESULTS: Alisat and EPL treatments reduced TC levels from 5.4 (+/- 0.25) to 4.77 (+/- 0.12) mmol/l and from 7.07 (+/- 0.24) to 5.92 (+/- 0.30), LDLP cholesterol from 4.0 (+/- 0.31) to 2.98 (+/- 0.15) mmol/l and 5.54 (+/- 0.25) to 4.04 (+/- 0.34) mmol/l, respectively.

EPL changed LDLP cholesterol and triglycerides from 0.51 (+/- 0.05) to 0.33 (+/- 0.03) mmol/l and from 2.54 (+/- 0.25) to 1.66 (+/- 0.15) mmol/l, respectively, while HDLP cholesterol rose from 1.22 (+/- 0.10) to 1.55 (+/- 0.07) mmol/l. Alisat did not change significantly.
CONCLUSION: Adjuvant EPL is recommended in combined treatment of NIDDM in marked dyslipidemia, alisat in moderate dyslipidemia.

2.1.3 Correction of dyslipoproteinemia in middle-aged and elderly patients with atherosclerosis using EPL in combination with adebit: *Korkushko OV et al, Vrach-Delo. 1989 Aug (8): 19-22. Russian*

A combination of EPL and adebit was used in the treatment of 62 patients (age 60-89 years) with stable stenocardia. EPL normalizes the cholesterol metabolism, adebit normalizes the carbohydrate metabolism. Results of the treatment indicate that elderly and old, in particular, showed an improvement of the clinical condition of the disease, normalization of the lipid metabolism.

2.2 Intravenous and oral administration

2.2.1 A contribution to the therapeutic effect of oral and intravenous administration of „essential“ choline phospholipids

SUMMARY: 19 patients aged between 41 and 78 years (av. 60,5 years) with very high serum cholesterol (above 250 mg%) and total blood fat of over 600 mg% were chosen. They were initially put on a diet, whereby the 50% of the total fat intake consisted of plant fats. 4 weeks after the start of the diet, therapy with EPL (NOTE: Equivalent to Plaquex) was begun.
16 patients received received 2x2 cps. 1 hour before the meal, 3 patients received EPL intravenously at a dose of 1 ampoule per day.
12 patients suffered from angina pectoris, whereby 4 of them have already suffered a MI, 2 came because of advanced cerebral sclerosis, 3 because of high blood pressure, 1 because of generalized atherosclerosis and 1 because of rheumatoid arthritis. 3 of the patients had diabetes in addition. The patients were treated between 1 and 9 months (median 2,5 months orally, 1.5 months i.v.). The patients were examined every 3-4 weeks. The median total dose was 300 capsules or 20 ampoules respectively.
Before treatment the median serum cholesterol level was 342,5 mg% with a maximum of 480 mg% and a minimum of 278%. The total blood lipids had a medium of 996 mg% with a maximum of 1300 and a minimum of 638 mg%.
After the treatment the median cholesterol was 252 mg% (max. 366,min. 139 mg%) corresponding to a median decrease of 90 mg%, which is 26.5 % of the initial value (max. 58%, min. 0%).
The total blood fat was only measured in 7 patients. The median was 702 mg% (max. 1146, min. 500 mg%). The average decrease was 294 mg% or 29,5 % of the initial value (max. 58.4%, min. 0%). After stopping the treatment the levels started to rise again despite continuation of the diet. Newly applied EPL decreased the values again. The intravenous therapy worked faster and more intensively.
During the first few weeks of oral EPL administration and 10 – 15 days intravenous administration the serum cholesterol level may rise due to mobilisation of pathological deposits from the walls of blood vessels. A decrease of serum cholesterol below normal values is not to be expected.
EPL was also used to dissolve fatty embolus after a car accident. The paralysis of the limbs completely disappeared after 5 days with 2 – 4 ampoules per day.

The patients complaints of angina pectoris, claudication, hypertension, nephrotic syndrome and fatty embolus disappear.

2.2.2 Essential phospholipids versus nicotinic acid in the treatment of patients with type IIb hyperlipoproteinemia and ischemic heart disease: *Klimov AN et al, Cardiovasc Drugs Ther 9(6):779-84, 1995*

SUMMARY: In patients with moderate, dietary noncorrigible hyperlipoproteinemia type IIb and ischemic heart disease, treatment with nicotinic acid is limited by the side effects of the drug. In 100 patients, 6-month treatment with nicotinic acid (n = 50) or essential phospholipids (EPL) (n = 50) indicated comparable efficacy for both substances: Significant ($p < .001$) reduction of serum total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride values were similar in both groups while nicotinic acid increased highdensity lipoprotein (HDL) cholesterol significantly ($p < .01$) better than EPL. A detailed analysis of ultracentrifugal lipoprotein profiles, hydroperoxide concentrations in LDL, and cholesterol-accepting properties of HDL in a small number of EPL- and nicotinic acid-treated patients revealed favorable shifts in the lipoprotein profile, significant ($p < .05$) reductions of LDL hydroperoxides, and favorable increases of the most antiatherorgenic HDL2b subfraction only in the EPL-treated group. Clinically both medications reduced the intensity and number of angina pectoris attacks per week ($p < .05$), but only EPL-treated patients significantly ($p < .05$) increased their working capacity in the veloergometric test. Since in the nicotinic acid-treated group dropouts (nine patients, eight related to the drug) and side effects [14] exceeded those in the EPL-treated group (two dropouts not related to the drug, no side effects), it is suggested that EPL is a preferable alternative in the treatment of patients with moderate, dietary noncorrigible hyperlipoproteinemia IIb and ischemic heart disease.

FULL TEXT: In patients with moderately elevated serum lipid levels and ischemic heart disease (IHD), treatment produces rather controversial results. On the one hand, the use of strongly acting lipid-lowering drugs, such as HMG CoA reductase inhibitors, can be effective but is not reasonable in cases of slightly elevated serum cholesterol levels. On the other hand, IHD patients with signs of angina pectoris need an effective, long-term or intermittent treatment, including a reduction of lipid values, in order to control the process of atherogenesis.

One of the drugs widely used in St. Petersburg, Russia for these patients is nicotinic acid, a well-known and effective medication. Unfortunately, high doses and long-term application of this drug raise the problems of tolerance and compliance. Therefore, we compared the laboratory and clinical efficacy of nicotinic acid with "essential" phospholipids (EPL). EPL is a highly purified phospholipid fraction extracted from soybean and containing phosphatidylcholine (PC) as an active ingredient, with polyunsaturated fatty acids as acyl residues in the 1- and 2-positions of the PC molecule. PC is the main component of lipoproteins and biomembranes constituting monolayers and bilayers. EPL is incorporated into different lipoprotein particles and, due to its unsaturated nature, which differs from human PC, it is capable of modfying lipoprotein properties, thus leading to a lipid-lowering effect [1].

Patients and Methods

One hundred patients (90 males, 10 females; mean age 51.2 ± 1.1 years) with type llb hyperlipoproteinemia (Fredrickson) and evidence of angina pectoris, according to a standard WHO questionnaire and S-T depression on ECG of at least 1 mm (0.08

seconds) on the bicycle ergometry test, were selected for this study and were randomly assigned to treatment with either "essential" phospholipids; n = 50) or nicotinic acid (n = 50). In 32 cases the clinical and ECG data indicated previous myocardial infarction. Patients with secondary hyperlipidemia and severe concomitant diseases (arterial hypertension, liver and renal disorders, diabetes melitus, malignant, hematological, and neurological diseases, etc.) were not entered into the study.

About 4 weeks prior to the start of the trial, all medications with any lipid-lowering action were discontinued and patients received a low-fat, cholesterol- free diet in accordance with European Atherosclerosis Society recommendations. During the diet and trial periods, the basic treatment (long-acting nitrates, calcium channel blockers) was not changed. The patients in the EPL group were given 2 ampules of 5 ml iv per day (= 0.5 g/day EPL) for 2 weeks, and for the following 5.5 months were given 6 capsules per day (= 1.8 g/day EPL). Nicotinic acid was used at a dose of 0.5 9 tid (= 1.5 g/day). The daily dose of nicotinic acid did not exceed 1.5 g since a further increase of the drug dose led to a significant increase in the frequency of side effects (mainly gastrointestinal). We have also found indications in the literature that nicotinic acid at a dose of 1.5 g/day was more reasonable, since, in addition to a significant reduction of LDL cholesterol, there was a low dropout rate due to side effects [2].

All patients were hospitalized for the first and last month of the treatment period. Both groups were basically comparable in the beginning of the study. However, total cholesterol (TC) and LDL cholesterol (LDL-C) were slightly but significantly higher in the nicotinic acid group, while manifestations of angina pectoris attacks were significantly more pronounced in the EPL group after the diet period (Table 1).

At day 14 and at the end of each month of treatment until month 6, the main lipid values [TC, triglycerides (TG), HDL-C, LDL-C (Friedewald formula), and atherogenic index (TC -HDL-C)/HDL-C)J were measured (Technicon AA-II autoanalyzer). Determination of lipid in plasma was made parallel with carrying out the CDC-NHLBI Lipid Standardization Program, which allowed us to attain the required degree of measurement precision. Additionally, at the second and sixth month a detailed physical examination was performed, including ECG at rest, veloergometry according to Bruce, as well as evaluation of the frequency and intensity of angina pectoris attacks (the frequency of anginal attacks was measured per week; the intensity was measured as follows: 0- missing, l-slight, 2-moderate, 3-severe).

In eight patients (two nicotinic acid and six EPL treated) changes in the structure of lipoprotein particles were analyzed by ultracentrifugation (Schlieren profile) [3] and conjugated dienes in plasma as weIl as by measuring the hydroperoxide content of apoB-containing lipoproteins spectrophotometrically [4]. The cholesterol-accepting properties of patients' HDL (four nicotinic acid- and three EPL- treated patients) were estimated using gradient gel electrophoresis before and after in vitro incubation of HDL with celite cholesterol [5,6]. Statistical analysis of data was performed by analysis of variance.

Results and Discussion

The data confined the results of preliminary reports of the trial [7,8]. In the nicotinic acid-treated group, dropouts (nine patients, eight related to the drug) and 1 side effects (14; skin rash, gastrointestinal pain) were more frequent than in the EPL-treated group (two dropouts not related to the drug; no side effects). Therefore, the overall evaluation

of tolerance after 6 months of treatment showed a significant ($p < .01$) advantage for the EPL treatment regimen (Table 3).

Clinically, in both groups the intensity of angina pectoris attacks was slight to moderate in the beginning and could be further reduced to a similar extent by both medications. Also the number of attacks week, although higher ($p < .05$) in the EPL group before treatment, was reduced and identical after 6 months in both groups. Only EPL led to an increase of physical tolerance in the veloergometrical test (Tables 1 and 3). These slight clinical advantages of EPL were not completely consistent with the laboratory findings of lipid values.

Lower TC and LDL-C values ($p < .05$) in the EPL group in the beginning of the treatment persisted for the first 14 days. EPL was given intravenously, while nicotinic acid patients received the final dosage of 1.5 g/day for the first time at day 10 of treatment (Tables 1 and 2). After 6 months both groups showed reductions of TC, LDL-C and TG ($p < .001$). Reduction of TG was more pronounced in the nicotinic acid-treated group ($p < .05$). HDL-C increased significantly in each group, but was higher in the nicotinic acid-treated patients ($p < .01$). The atherogenic index was reduced within 6 months in both groups ($p < .001$; Table 3) but was reduced more significantly in the nicotinic acid-treated group ($p < .05$).

To elucidate the slight discrepancies between clinical and laboratory findings, the structure and properties of lipoprotein particles were studied in detail in a limited number of patients of each group. Analytical ultracentrifugation revealed a decrease in VLDL in nicotinic acid- and EPL-treated patients (Fig. 1) that corresponds to the decrease of TG in either of the groups. HDL isolated and separated into subfractions by gel electrophoresis before and after in vitro incubation with celite cholesterol showed a favorable shift from HDL2a and HDL3a to the most antiatherogenic HDL2b especially in the EPL-treated patients, as had been suggested earlier [9] (Figure 2). This was also confirmed by the changes in the HDL Schlieren profile after EPL treatment, which showed an increase of the total HDL fraction and a shift to a higher flotation rate ($F_{O1,20}$; Figure 1). Since atherogenesis is closely related to the oxidative modification of lipoproteins like LDL, patient sera were investigated with respect to the lipid peroxidation product content, such as conjugated dienes and hydroperoxides. Neither nicotinic acid nor EPL influenced the plasma conjugated diene content, but hydroperoxides in apoB-containing lipoproteins were reduced by EPL after only 2 weeks and 3 months of treatment ($p < .05$; Tables 2 and 3).

In summary, both treatment regimens were regarded as comparable in this special kind of patients with moderate, dietary noncorrigible type IIb hyperlipoproteinemia. However, the absence of side effects with EPL is of importance in clinical practice, and a possible increase of HDL2b by "essential" phospholipids as well as a reduction of hydroperoxides in LDL offer reasonable hints that this drug exerts a favorable effect on atherogenesis by increasing reverse cholesterol transport and reducing LDL modification.

3. Effects of EPL on Atherosclerosis

3.1 Oral Application

3.1.1 The antihyperlipemic and anti-atherogenic effectiveness of essential phospholipids (EPL) in the pharmacological test. *Leuschner F et al, Arzneim Forsch (Drug Res) 26 (9A) (1976) 1743-1772*

SUMMARY: To prove antilipemic and antiatherogenic effectiveness several animal species were given essential phospholipids (EPL), during different experimental procedures. The following actions were studied:

1. Effect of EPL-substance after prophylactic and therapeutic oral administration (daily dose: 50, 150, 450 mg/kg bodyweight) in rats with acute and subacute hyperlipemia induced by triton.
2. Effect of EPL-substance after prophylactic and therapeutic oral administration (daily dose: 50, 150, 450, 1800 mg/kg bodyweight) in rats with diet induced hypercholesterolemia.
3. Effect of EPL-substance after daily oral administration (dose: 50, 150, 450 mg/kg bodyweight) on the development of coronary and aortic atherosclerosis and various biochemical parameters in cholesterol-fed cockerels.
4. Effect of EPL-substance after daily oral administration on subacute triton-hyperlipemia in mini pigs (dose: 50, 150, 450 mg/kg bodyweight).

Triton administration causes a greater or smaller increase in all parameters of the lipid metabolism measured. EPL treatment decreases these parameters during therapeutic and prophylactic administration in some cases even reaching normal values. The effect was clearly dose dependent. EPL inhibit the increase in total lipids in dietetic hypercholesterolemia (rat) during therapeutic as well as prophylactic administration. The effect was clearly dose dependent in all doses, being statistically significant at the highest dosage level.
In cockerels EPL were effective at all dose levels in counteracting the development of coronary atherosclerosis while the effect in atherosclerosis of aorta was less distinct. Except for non-esterified fatty acids, EPL reduced all biochemical parameters measured.

3.1.2 Hyperlipoproteinaemia and atherosclerosis in rabbits fed low-level cholesterol and lecithin: *Hunt-CE, Duncan-LA; Br-J-Exp-Pathol. 1985 Feb; 66 (1): 35-46*

Dutch-Belted rabbits were fed for 18 months an atherogenic semipurified gel diet containing 14% hydrogenated coconut oil and 0.06% cholesterol (approximately 0.15 mg/kcal) or a non-atherogenic basal gel diet containing the same ingredients but with no coconut oil or cholesterol. Rabbits fed atherogenic diet developed hypercholesterolaemia (means 733 mg/dl at 16 months) and plasma lipoprotein (LP) distribution shifted from a pattern in which high density lipoproteins (HDL) predominated to one in which very-low-density lipoproteins (VLDL) were predominant. Total cholesterol /triglyceride ratio in d less than 1.006 LP changed from 0.3 to 1.8. Plasma cholesterol and LP distribution returned to normal in rabbits fed atherogenic diet for 18 months followed by atherogenic diet plus 3% soya lecithin for an additional 4 months. Rabbits fed atherogenic diet for 18 months had extensive, usually full circumference fibromuscular plaques in main branches of coronary arteries and all portions of aorta which comprised lumen area by almost 50 %. These lesions were modified in rabbits fed atherogenic diet plus lecithin. The plaques lacked foam cells and

cholesterol clefts, were less cellular with a distinct fibrous surface and occupied less space.
Animals fed basal diet did not develop hypercholesterolaemia (means 86 mg/dl at 16 months) although distribution of plasma LP shifted slightly in favour of increased low-density lipoporoteins (LDL) and decreased HDL compared with rabbits fed standard commercial diet. Basal diet rabbits had no coronary atherosclerosis and only minimal focal foam cell lesions in proximal aorta. Liver injury including fatty change cholangitis and portal fibrosis occurred in animals fed atherogenic diet. Thus rabbits fed appropriate diets low in cholesterol accumulate cholesterol-enriched LP in their plasma and develop lesions in abdominal aorta and main branches of coronary arteries which are similar to those in man. Also, in this experimental model, dietary lecithin promotes a return to normal of the LP distribution profile and removal of lipid from established atherosclerotic plaque.

3.1.3 Early detection and modification of preclinical arteriosclerosis

Rudofsky G, Vasa Suppl. 1990;30:21-4. German

With the combination of pulsed doppler and real time echo-sonography early changes in arterial walls can be detected. The carotid, iliaca, femoral and popliteal artery as well as the aorta were examined in 211 patients for a time period of 15 months to 4 years. The patients were examined with the real time scan every 3 months and the locations and the development of the plaques were documented and their size in volume calculated. The femoral and carotid arteries were favored by plaque formation, followed by the iliaca.
The following substances were used with the patients:
a. placebo
b. ASS at a dose of 1.5 and 1 g/d
c. Naftidrofuryl 900 mg/d
d. EPL
ASS showed an increased plaque growth at the dose of 1.5 g/d. At 1 g/d no difference to placebo was noted. The combination of ASS and Dipyridamol reduced plaque growth compared to placebo (this could not be proven statistically significant due to a small number of patients). Naftidrofurly could significantly reduce – as prooven in other studies- plaque growth, probably through blocking the H2 Serotonin receptors. Even after 4 years further reduction of plaque size was seen. Patients receiving EPL showed a significant decrease in plaque growth after 9-12 months treatment, which continued for 18 months during the period of observation.

3.1.4 Effect of oral polyunsaturated lecithin on the development of atheroma and fatty liver in the cholesterol-fed rabbit: *Adams CW, Baker RW, Morgan RS, Orton CC, J Pathol. 1969 Han;97(1):35-41*

A previous study showed that *intravenous* administration of polyunsaturated lecithin (polyenoic phosphatidyl choline) depresses the formation of aortic atheroma and fatty liver in the cholesterol-fed rabbit (Adams et al. 1967 – “see 3.2.8”). Other investigators have reported that *oral* administration of this polyunsaturated lecithin suppresses atheroma formation in the rat (Dornbusch and Zielke, 1960). The following problems are considered in the present investigation:
(a) Is polyunsaturated lecithin significantly absorbed from the gastro-intestinal tract as an intact molecule ?
(b) Does polyunsaturated lecithin exert an anti-atherogenic effect and prevent fatty liver when administered orally to the cholesterol-fed rabbit ?

(c) Does the anti-atherogenic action of intravenously administered polyunsaturated lecithin depend on the level of this particular phospholipid in the plasma or is it merely due to an increased proportion of polyunsaturated fatty acids therein ?

SUMMARY: Orally administered polyenoic phosphatidyl choline (polyunsaturated lecithin) does not appear to be significantly absorbed in intact state in the normal rabbit; its high content of linoleic (18: 2) acid is distributed amongst the various plasma lipid ester species.
Oral administration of PPC to the cholesterol-fed rabbit does not protect the animal against hypercholesterolaemia, aortic atherosclerosis and fatty liver. Conversely, *intravenously* administered PPC mitigates these three manifestations of cholesterol feeding.
These results indicate that the beneficial effect of intravenous PPC is due to the presence of polyenoic phosphatidyl choline in the serum and not to general elevation of the serum polyunsaturated fatty acid pool. It is suggested that polyenoic phosphatidyl choline in the serum could act as a donor of polyunsaturated fatty acid in lipid transacylation and in cholesterol esterification reactions.

3.1.5 Influence of oral polyunsaturated and saturated phospholipid treatment on the lipid composition and fatty acid profile of chimpanzee lipoproteins: *Rosseneu M et al, Atherosclerosis 32 (1979) 141-153*

The influence of treatment with polyunsaturated lecithin (EPL) and with saturated lecithin on the lipoprotein composition and fatty acid profile was investigated in 4 male chimpanzees. The animals were successively given 3 isocaloric diets containing the same amount of fat with a degree of saturation varying from 1 in the control diet to 0.2 in the diet enriched with polyunsaturated lecithin, to 4 in the diet enriched with saturated lecithin.

The VLDL, LDL and HDL3 fractions were isolated by ultracentrifugal flotation; changes in their lipid and fatty acid composition were followed and their microviscosity was measured.

The treatment with polyunsaturated lecithin increases the cholesterol esters and lysolecithin content in HDL3, presumably via activation of the enzyme LCAT. These modified HDL particles have a more fluid surface and a denser core and are susceptible to act as better cholesterol carriers.

A complementary effect of this treatment is a decrease of the plasma triglycerides and VLDL concentration, an increase in the unsaturation ratio of the triglycerides which might take place via activation of triglyceride lipase.

The saturated lecithin treatment increases the plasma VLDL and LDL concentrations and the triglyceride levels and increases mostly the saturation ratio of the cholesterol esters. These effects are likely to enhance the progression of atherosclerosis.

3.1.6 The effect of bioflavanoids and lecithin on the course of experimental atherosclerosis in rabbits: *Bialecka M, Ann-Acad-Med-Stetin. 1997; 43:41-56. Polish*

Abstract
Atherosclerosis and its clinical manifestations are still one of the most important civilization problems. New questions arise: is it really an inevitable process? Are there any rational methods to prevent the development of
atherosclerotic changes or to facilitate its regression? The aim of the work was to evaluate the influence of bioflavonoids extracted from milk thistle (Sylibum marianum

L), troxerutin (O-(beta-hydroxy-ethyl)-ruozid and lecithin, administered together and as a single therapy, on the experimental atherosclerosis development in rabbits. Sixty male mixed-breed rabbits were randomly assigned to 6 equal groups: I--control, II--fed on fat-rich diet
(FR/DB), III--fed on FR-diet and sylimaryn concentrate (S), IV--animals fed on FR-diet and troxerutin (T), V--rabbits fed on FR-diet and soya bean lecithin (L), VI--animals fed on FR-diet and sylimaryn-phospholipid complex (SF). The whole experiment lasted 12 weeks.
Following tests have been performed: electrocardiographic, biochemical, pathomorphological (including macroscopic and microscopic evaluations of aorta). Biochemical analysis included: cholesterol concentration (total, low density lipoprotein fraction cholesterol and high density fraction cholesterol), triglycerides, b-lipoproteins, phospholipids, fibrinogen, trace elements (calcium, magnesium, zinc and copper) and dimalonic aldehyde concentration. Concentrations of ascorbyl free radical, total cholesterol, triglycerides, P-450 cytochrome and phospholipids in liver have been estimated. Evident normalization of lipid metabolism and inhibition of atherosclerotic changes have been observed in the group of animals fed on SF complex. Concentrations of total cholesterol, LDL-cholesterol fraction, phospholipids and triglycerides decreased in serum. Decrease of serum dimalonic aldehyde was followed by increase of ascorbyl free radicals concentration in
liver. Significant increase of serum zinc has been also noted, which exceeded values observed in control group. Concentration of P-450 cytochrome increased in liver microsomes. Sylimaryn and lecithin showed less anti-atherosclerotic activity, and troxerutin displayed the least anti-atherosclerotic activity (Tab. 1-2, Fig. 1-2) .On the basis of the achieved results the following **conclusions** were drawn: 1) Sylimaryn and lecithin have anti-atherosclerotic activity in rabbits. **2) Sylimaryn-phospholipid complex shows the strongest anti-
atherosclerotic activity**. 3) The achieved results allow us to undertake clinical trials using SF-complex in prevention and treatment of atherosclerosis.

3.2. Intravenous Application

3.2.1 Specificity of the effect of polyene phosphatidylcholine depending on the mode of
administration and animal species: *Tarkhovskaia TI, Khalilov EM, Fortinskaia ES, Kliuchnikova*
ZhI, Rikhter F, Morvinski I,Kliain K, Rassul F, Kiunnert V, Rotch V.

Biulleten Eksperimentalnoi Biologii I Meditsiny. 113(1): 55-8, 1992 Jan.

The effects of polyene phosphatidylcholine (PPC) treatment at oral and intravenous administration to rats and rabbits in hypercholeterolemic diet were studied. No aorta damage was observed in either of rat groups. But fatty liver appeared, and it was the greatest in rats, who received cholesterol and PPC. The result may be attributed to adaptive protection of peripheral tissues due to high experiment duration (18 months) in the state of active reverse cholesterol transport (RChT). No antiatherogenic effect was noted in rabbits at PPC administration (170 mg/kg), while its intravenous injection (50 mg/kg) resulted in marked reduction of plasma cholesterol level, elevation of HDL cholesterol and decrease of the extent of aorta damage. The conclusion is drawn on the ppc high antiatherogenic effect predominantly at intravenous administration, and on advisability of its use in cases of RChT deficiencies, as its activator.

3.2.2 Extraction of cholesterol from biological membranes with positively charged micelles of phosphatidylcholine: *Borodin EA, Lanio ME, Khalilov EM, Markin SS.Torkovskaia TI*

Biulleten Eksperimentalnoi Biologii I Meditsiny 99 (2): 164-6, 1985 Feb

The content of cholesterol in red cell and platelet membranes was lowered in rabbits with experimental atherosclerosis after intravenous injection of positively charged micelles of soybean phosphatidylcholine. That lowering was accompanied by a reduction in membrane microviscosity, rise of the activity of Na,K- and Ca-ATPases of red cells, and a decrease in the rate of the ADP- and collagen-induced platelet aggregation. Injection of phosphatidylcholine gave rise to an increase in the blood serum content of phospholipids and cholesterol in high density lipoprotein fractions, to a reduction in the content of triglycerides and the atherogenicity index, as well as to the lowering of the microviscosity of high density lipoproteins. The aortal area affected by atherosclerotic lesions was 2 times less in the group of animals given phosphatidylcholine.

3.2.3 Atherosclerosis induced in hypercholesterolaemic baboons by immunological injury; and the effects of intravenous polyunsaturated phosphatidylcholine: *Howard AN, Patelski et al, Atherosclerosis 14: 17-29, 1971*

SUMMARY: Groups of 5 – 8 baboons were fed either a control or hypercholesterolaemic diet for 6 months. During the last 90 days, each group was given 5 i.v. injections of bovine serum albumin (BSA) at 16 day intervals or control injections of saline. Only those animals which were both hypercholesterolaemic and injected with BSA developed aortic and coronary atherosclerosis.
An intravenous injection of 1 g polunsaturated soy phosphatidylcholine thrice weekly into animals receiving the atherogenic diet and BSA, reduced the incidence and severity of aortic atherosclerosis but had no effect on plasma cholesterol, phospholipids or the fatty acid composition of the cholesterol esters and lecithin. Compared with controls, animals given the hypercholesterolaemic diet had increased aortic lipase and normal cholesterol esterase activity. Those given the same diet and phosphatidylcholine i.v. had a normal aortic lipase and over 50 % increase in cholesterol esterase acitvity.
It is concluded that immunological injury hastens the onset of atherosclerosis produced by feeding a hypercholesterolaemic diet and that changes in aortic lipolytic enzymes may be the mechanism by which phosphatidylcholine reduces atherosclerosis.

3.2.4 Removal of endogenously labeled lipid from atherosclerotic aortic explants incubated in vitro: *AK Horsch, K Hudson et al,"Atherosclerosi,s is it reversible ?", 1978, 74 – 88*

CONCLUSION: Summary of the data: there appears to be some removal of lipids characteristic of the atherosclerotic process from the arterial wall and EPL seems to promote the elimination of cholesterol esters from tissues. No significant differences in the arterial lipid composition were found. The incorporation of 3H-EPL into cholesterol esters is significantly higher in the atherosclerotic artery. The 3H-EPL cholesterol esters are removed from arterial tissues after 8 weeks of EPL treatment, as are also 14c-acetate, 3H-oleic and 14c-linoleic labeled cholesterol esters. …..

3.2.5 Intravenously administered lecithin liposomes: a synthetic antiatherogenic lipid particle: *Williams KJ, Werth VP, Wolff JA, Perspect Biol Med. 1984 Spring;27(3):417-31. Review*

CONCLUSION: We propose that lecithin liposomes are a hitherto unrecognized type of antiatherogenic lipid particle. They appear to mobilize tissue cholesterol for excretion. Their infusion into animals produces a transient rise in serum cholesterol and a rapid regression of experimental atherosclerosis. Their infusion into humans also produces a rise in serum cholesterol, implying a similar mobilization of tissue stores. Liposomes modify, and are modified by, lipoproteins and red cells in ways that appear to favor extraction and excretion of tissue cholesterol……..

3.2.6 Cinnarizine and phosphatidylcholine in the treatment of cerebral arteriosclerotic pathology and arteriosclerosis of the lower extremities [comparative study]: *Ciammaichella A, Solitro A, Arcuri P, Minerva Cardioangiol. 1975 Mar;23(3):191-200, Italian*

SUMMARY: Two groups of 30 patient with cerebral and lower extremity arteriosclerosis received Cinnarizine and Phosphatidylcholine courses of varying length respectively. The rheographic, thromboelastographic, skin temperature and cyclo-ergometric data showed that Cinnarizine had the better overall effect. Consideration of the differences in the mechanism of action of the two drugs, the positive benefits obtained with Phosphatidylcholine, the multiplicity of pathogenetic factors in atherosclerosis and its anatomopathological course suggest that association of these two drugs can usefully be exploited.

NOTE:Cinnarizine dosage was 50 mg (2 cps.) 3 times daily. Phosphatidylcholine was applied intravenously 250 mg twice daily or 220 mg (2 capsules) 3 times daily orally. The intravenous form also contained 0.5 mg Vitamin E, 2.5 mg Vitamin B6 and 1.5 mg ATP.

3.2.7 Therapy of arteriosclerosis with “essential” phospholipids: *Pupita F, Gagna C, Med Monatsschr. 1969 Nov;23(11):514-5. German*

SUMMARY: 1. The EPL therapy given 31 arteriosclerotic patients caused a statistically significant improvement of all pathological serum lipid values and a clear decrease of arteriosclerotic symptoms.
2. The performance of the adrenal gland (ACTH stimulation) increased in 5 patients treated with EPL.
3. The tendency towards accelerated blood clotting often seen in arteriosclerotic patients was normalized under EPL treatment.
4. Patients with nephrotic syndrome showed the same positive results with respect to the lowering of serum lipids and the normalization of blood clotting as arteriosclerotic patients.

NOTE: Patient group 1 (20 atherosclerotic patients) received EPL orally 2 cps. 3 times daily for 1 year. Patient group 2 (5 atherosclerotic patients) received EPL i.v. 1 ampoule daily for 3 weeks plus 5 capsules daily.

3.2.8 Modification of aortic atheroma and fatty liver in cholesterol-fed rabbits by intravenous injection of saturated and polyunsaturated lecithins: *Adams CW et al, J Pathol Bacteriol. 1967 Jul; 94(1):77-87.* Supported by the British Heart Foundation, the Tobacco Research Council and by USPHS Grant No. HE-06483-04 of the NIH.

ADAMS *et al. (1963b)* and Adams and Morgan (1967) have recently suggested that phospholipids may play a dual anti-atherogenic role by mobilising cholesterol from arteries and by preventing the sclerogenic action of this sterol on connective tissues. The arterial wall and certain other organs react to the presence of cholesterol by synthesising phospholipid *in situ* (Zilversmit and McCandless, 1959-60; Zilversmit *et al.,* 1961; Christensen, 1961, 1962). Such endogenous synthesis of phospholipid may be a local defence mechanism against sterol deposition (Adams, 1964). The present investigation concerns the effect of intravenous administration of either egg lecithin or polyunsaturated lecithin on the development of cholesterol-induced fatty liver and atheroma in rabbits.
…..
RESULTS: *Gross appearances.* The control rabbits fed with the cholesterol-enriched diet without phospholipid injection showed fatty streaks and small atheromatous plaques in the arch and descending thoracic aorta from 4 ½ wk on diet onwards; their livers often appeared grossly fatty from the 5th wk. Cholestero-fed rabbits given intravenous injections of ovolecithin showed more aortic atheroma than the control group, but their livers appeared less fatty. Cholesterol-fed animals given PPC (soy lecithin) showed no macroscopic evidence of either aortic atheroma or fatty liver. The blood plasma in animals receiving injections of PPC appeared relatively clear and translucent whereas that of both cholesterol-fed controls and ovolecithin-injected animals was always opaque as a result of excess chylomicrons.
Histochemical Results. The descending aorta and aortic arch of rabbits given injections of ovolecithin usually show greater intimal thickening and lipid deposition than the corresponding control animals. In contrast the aorta of only one animal given injections of PPC shows a small focus of atheroma in which lipid stains red with OTAN and blue with PAN, indicating that it contains phospholipid and free cholesterol.
The livers of the controls develop more severe fatty change than in ovolecithin-injected rabbits. There is less phosphoglyceride in the livers of the ovolecithin-injected animals than in the controls. PPC injection has a markedly protective effect against the development of fatty liver, even more so than ovolecithin. ……..

3.2.9 Modification of enzyme activities in experimental atherosclerosis in the rabbit: *Patelski J et al, Atherosclerosis 12 (1970) 41-53*

SUMMARY: In rabbits fed an atherogenic semi-synthetic diet, the following were found: in the aortic wall, decreased cholesterol esterase and increased lipase and phospholipase A activities, no changes in malate and lactate dehydrogenase activities, enhanced incorporation of free fatty acid into cholesterol esters, and increased accumulation of mainly esterified and also free cholesterol; increase in plasma cholesterol and serum phospholipase A activity but no change in liver phospholipase A and serum and liver lipase activities. Compared with control animals, those fed the same diet and injected with PPC showed no alterations in the aortic enzyme activities and serum phospholipase A and in the incorporation of free fatty acid into aortic cholesterol esters; serum and liver lipase activities were increased. Compared with atherogenic diet-fed animals injected with saline, the severity of atherosclerosis and the incorporation of free fatty acids into the aortic wall were reduced; there was no change in the elevated plasma cholesterol levels. The mechanism of cholesterol ester accumulation in the arterial wall is discussed in the light of these observations.

3.2.10 Effect of intravenous polyunsaturated phosphatidylcholine in experimental atherosclerosis: *Howard AN, Patelski J, Verh Dtsch Ges Inn Med. 1972;78:1245-8.*

The relationship between the metabolism of phospholipids, glycerides and cholesterol esters in the arterial wall and the development of atherosclerosis has become a subject of considerable interest in recent years. We have shown [1] that in experimental atherosclerosis in rats and in rabbits, there is an increase in lipase and phospholipase activity and a decrease in cholesterol esterase activity in the arterial wall and it is suggested that these might be contributing factors in the production of the arterial lesions.

Since polyunsaturated phosphatidyl choline is known to affect the lipolytic enzymes [2], a study was made of rabbits fed control atherogenic diets, injected with this drug [3]. The experimental animals were all fed a semisynthetic diet which produced hyperlipaemia and atherosclerosis. They were injected every second day via the marginal ear vein with 1 ml (100 mg) EPL. The specific activities of the lipolytic enzymes are presented in Table 1 for 10 and 18 weeks treatment. Animals fed atherogenic diets alone and injected with saline showed increased phospholipase A (after 18 weeks) and lipase activities and decreased cholesterol esterase activities (both after 10 and 18 weeks) compared with normal. The arterial lipolytic enzyme activities of animals fed the atherogenic diet and injected with EPL were not significantly different from normal.

Atherosclerotic lesions could be seen macroscopically in experimental animals fed for 18 weeks but not in those fed for 10 weeks. As shown in Table 2, plasma cholesterol was elevated four-fold in animals fed the semi-synthetic diet but there was no significant difference between those injected with saline or EPL. The extent and severity of the lesions was reduced. The decrease in aortic atherosclerosis we observed with EPL could not be explained on the basis of decreased hypercholesterolaemia but was more likely due to the changes in aortic enzyme activities.

A similar study was also made in hypercholesterolaemic baboons given injections of bovine serum albumin [14]. Groups of 5 to 8 baboons were given either a control or hypercholesterolaemic diet for 6 months. During the last 90 days, each group was given 5 i.v. injections of bovine serum albumin (BSA) at 16 day intervals or control injections of saline. Only those animals which were both hypercholesterolaemic and injected with BSA developed aortic atherosclerosis (Table 3).

An i.v. injection of 1 g polyunsaturated soya lecithin thrice weekly into animals receiving the atherogenic diet and BSA, reduced the incidence and severity of aortic atherosclerosis but had no effect on plasma cholesterol, phospholipids or the fatty acid composition of the cholesterol esters and lecithin.

In the hypercholesterolaemic baboon lipase was found to be elevated but there was no change in cholesterol esterase compared with normal animals (Table 3). Species differences may be an explanation of the conflicting result but it is noted that in our rabbit experiments, the plasma cholesterol in the experimental group was almost double that in the hypercholesterolaemic baboons. Thus substrate inhibition may be of importance. It is of interest that an increase in lipase would facilitate the release of free fatty acids from the triglycerides and favour the esterification of cholesterol and the deposition of cholesterol esters in the arterial wall. Injections of EPL in the hypercholesterolaemic rabbit caused a decrease in aortic atherosclerosis and normalisation of the aortic enzymes. In the baboon experiments, a similar result has been achieved with respect to lipase, but in contrast the activity of cholesterol esterase was increased by over 50 % above normal. The more pronounced effect in the baboon compared with the rabbit may be due to the use of a much higher dose of EPL (5 times

greater/kg body wt). As for the rabbit given an atherogenic semi-synthetic diet, plasma cholesterol was unaffected by the administration of EPL and its effect on atherosclerosis was not mediated by a lowering of plasma cholesterol.
The mechanism by which cholesterol esters accumulate in the arterial wall has not so far been elucidated. Increased esterification of cholesterol or decreased hydrolysis of the esters may both be important. Certainly an increase in cholesterol esterase (hydrolase) activity would disfavour their accumulation. It has been argued that the deposition of esterified rather than free cholesterol is more damaging to the arterial wall because free, but not esterified, cholesterol is easily transported across cell membranes [5, 6]. If this view is correct, an increase in chol- esterol esterase activity should lead to less arterial lipid deposition. The fact that EPL, which elevates cholesterol esterase, also decreases the incidence and severity of aortic sudanophilia, is consistent with this hypothesis.

3.2.11 The use of EPL preparation in the combined efferent therapy of the clinical manifestations of an atherosclerotic process: *Belotserkovskii MV et al, Ter Arkh. 1993;65(8): 32-6. Russian*

This study was made in Russia from 1989 – 1991 with 76 humans aged 39 to70 years, suffering from coronary heart disease, claudication, hypertension and some of them already have had a myocardial infarction. The patients were divided in to 4 groups.
Group 1 included 28 patients, of which 21 suffered from CHD. They were treated twice with plasma phereses with a treatment interval of 4-5 days.
Group 2 included 10 patients, of which 8 had CHD. They were treated with 10 ml EPL intravenously daily for 14 days in 10 ml Glucose 40 % and received plasma pheresis twice. After leaving the clinic the patients continued with 6 cps. EPL per day for 1 month.
Group 3 included 24 patients, of which 19 had CHD. The patients were treated with 1 plasma phereses and 3 semiselective extracorporal hemocorrection (SEH).
Group 4 included 14 patients, of which 12 had CHD. They were treated with 3 SHE, 1 plasma pheresis and 10 ml EPL i.v. per day with 10 ml Glucose 40 % for 14 days. After leaving the clinic the patient continued with 6 cps. EPL per day for 1 month.
All patients were examined before the trial and 4 –7 days after the trial.
RESULTS: In patients of group 4 the total cholesterol was reduced. Patients receiving EPL (group 2+4) had a lowered atherogenic index. The best results in patients with CHD were achieved in group 4. Fibrinogen was not reduced by EPL. This may be due to liver problems in patients with atherosclerosis. EPL together with SEH improves the patients with all forms of atherosclerosis and extends the remission time of the disease.

NOTE: translated from Russian into German by Loreta Hartung and from German into English by Anita Baxas.

4. Studies on the basic functions of phospholipids

4.1 Pleomorphic functions of highly unsaturated phospholipids in biological membranes and serum lipoproteins: *Stoffel W et al, Med Welt 29(4): 124-31, 1978*

This study examined the different physiological functions of phospholipids. They include the following:
a) Phospholipids are the main component of cellular and subcellular membranes and maintain structural integrity of the cells and organelles through the bilayer form.

b) Phospholipids are responsible for transmembrane transport, anchorage of intrinsic proteins and glycoproteins that function as receptors.
c) Phospholipids – in particular high unsaturated phospholipids - are responsible for the activity of membrane bound enzymes (eg. Na/K-ATPase, Ca-ATPase).
d) Phospholipids are used for the prostaglandine synthesis.
e) Phospholipids are important for the structural integrity of serum lipoproteins that are responsible for the transport of triglycerides, cholesterol and cholesterol ester.

80% of the phospholipids involved in chylomicrones, VLDL and HDL and 60% of the phospholipids in LDL is phosphatidylcholine. The study examines the role of the enzyme LCAT (Lecithin-Cholesterol-Acyl-Transferase) that catalyses the transfer of the acyl-rest in the beta-position of the phosphatidylcholine to the 3-beta-hydroxyl-group of the cholesterol molecule.

4.2 Effect of Polyenylphosphatidylcholine on lipid-metabolizing enzymes of the arterial wall: *J Patelski, Int Conf on Atherosclerosis Milan, Nov 09-12, 1977, N.Y. (1978) 525-530*

Different diets are known to produce hyperlipidemia, changes in enzyme activities, and atherosclerosis in experimental animals. Several patterns of lipolytic enzyme activities of the aorta can be distinguished, as indicated by our investigations of experimental atherosclerosis (2,3,7,8,14).
Intravenous injections of soya polyunsaturated phosphatidylcholine (PUPC) in sodium deoxycholate were found to modify the enzyme activities (2-4,8, 14) and reduce the incidence and severity of aortic atherosclerosis (1,2,8). Table 1 shows the effects of the diets and PUPC solution on lipolytic enzyme activities of the aorta.

The effects of PUPC-sodium deoxycholate solution were compared with those of hydro-sols of PUPC and phosphatidylcholine from egg yolk by means of acute experiments in normal rabbits (4). The following results were obtained: egg phosphatidylcholine hydrosol decreased glycerol ester hydrolase activity (GEH); PUPC hydrosol decreased GEH and cholesterol ester hydrolase (CEH) activities; GEH was inhibited more than CEH; PUPC- sodium deoxycholate solution was more effective than the hydrosol; and phosphatide acyl hydrolase (PAH) was not affected at all. The results are shown in Table 2. They are compatible with those obtained in long-term experiments, except for the relative (8) and absolute increase in CEH activity (2) (Table 1), and with the *in vitro* lower activity of PAH in the presence of soya polyunsaturated phosphatidylcholine (8,10) and higher inhibition of GEH than CEH by sodium deoxycholate (9). Thus species differences, the kind and duration of the diets, the components, and dosage of the drug are important for the enzyme activity patterns obtained. However, a regulating effect of the drug(s) including two types of action (viz., a stimulation and stabilization) may be considered in relation to enzyme-metabolite interactions in the metabolism and accumulation of lipids in the arterial wall.

MATERIAL AND METHODS

Ratios of enzyme-specific *(mU/mg),* relative (%), and metabolic activities (enzyme-specific or relative activity/substrate metabolite concentration in molar units), and correlations were calculated in order to define normal and atherosclerotic patterns and effects of the drug(s) in the arterial wall (2- 4,7,8,14).

RESULTS

Regulating Effect of the Drug on Enzyme Activity Ratios in the Aorta
The ratio of GEH/CEH specific activities amounted to 1.3 to 1.5, 1.7, and 2.3 in the aorta in normal rabbits, baboons (Table 3), and rats (7), respectively. It was elevated in

four out of five experimental series depending on the kind and duration of diets, and was reduced or normalized by PUPC solution in all the experiments (Table 3).
The ratio of ACAT/CEH relative activity values varied depending on the conditions of experimental diets and was elevated by PUPC solution in control but decreased in experimental animals (Table 4). High and significant negative correlations of the GEH/CEH and ACAT/CEH activity ratios produced by the diets and appropriate differences between these values and those produced by the diets and PUPC solutions were calculated (Tables 3 and 4).
STIMULATION and STABILIZING EFFECTS of the DRUG on ENZYME METABOLIC ACITIVITIES of the AORTA
The enzyme metabolic activities related to synthesis and hydrolysis of cholesterol esters, ACAT/C and CEH/CE, respectively, were considerably changed in experimental atherosclerosis, and thus the ratios of both (by analogy with ACAT/CEH) were markedly elevated (Table 5). The metabolic activity of ACAT and CEH were stimulated and stabilized by PUPC solution of a different degree but so that the ratio ACAT/C: CEH/CE was normalized or remained unchanged, respectively, in approximately 100 % (Table 5).

DISCUSSION

The negative correlation between the initial values of GEH/CEH and ACAT/CEH and the differences depending on PUPC solution, along with a decrease (normalization) of appropriate variances which may be calculated (Tables 3 and 4), indicates a regulating effect (5,6) of the drug. It means that low ratios of the enzyme activities can be enhanced, unchanged, or depressed within a relatively narrow range of fluctuations, whereas markedly elevated ratios are reduced to the normal fluctuation range. This effect may be explained by higher inhibition of GEH than CEH by sodium deoxycholate, as shown by the *in vivo* (Table 2) and *in vitro* experiments (9).
Normalization and stabilization of the metabolic activities ACAT/C:CEH/CE can hardly be attributed to a direct influence of sodium deoxycholate on ACAT, as this enzyme seems to be much less sensitive than CEH to the inhibitory effect of the bile salt, as indicated by a comparison of results obtained *in vitro(9,13).* Therefore other enzyme mediated effects of the drug must be taken into account.
There is a close interrelationship of enzyme catalyzed hydrolysis of triglycerides and cholesterol esters and the
ATP-dependent synthesis of the latter in the arterial wall (7,8,12). The inhibition of GEH by PUPC solution (Tables 2 and 3) with a concomitant decrease in the activity of acid:CoA ligase (AMP) (14) indicates a lower turnover of free fatty acids. Thus a deficit of fatty acids will lead to decreased accumulation of cholesterol esters by the ACAT-catalyzed synthesis and reduced substrate and/or product inhibition of CEH. This is what is reflected by the normalized or unchanged ratios of both metabolic and specific activities of the enzymes.
It may be concluded that the polyunsaturated phosphatidylcholine-sodium deoxycholate solution regulates hydrolysis of triglycerides and the energy-dependent synthesis of cholesterol esters in the arterial wall, the two components being involved as a substrate (11) and effector, respectively, in stabilizing and normalizing effects of the drug.

1 ACA T = acyl-CoA: cholesterol acyltransferase (EC 2.3.1.26). GEH = glycerol ester hydrolase (EC 3.1.1.3). PAH = phosphatide acyl hydrolase (EC 3.1.1.4). CEH=sterol ester hydrolase (EC 3.1.1.13). AL=acid: CoA ligase (AMP) (EC 6.2.1.3).

4.3 Plasma lipid response to the i.v. administration of CDP-Choline, SAMe and citiolone in hyperlipemic subjects:*Galeone F, Salvadorini F, Int Conf on Atherosclerosis Milan, Sept 09-12, 1977*

The effect of some drugs stimulating a number of liver enzymatic activities (CDP-choline, S-adenosyl-methionine and acetyl-homocysteinathiolactone) was studied in hyperlipemic patients, to explore the role of liver function in hyperlipidemia. Recently a close connection between liver impairment and hyperlipemia has been reported. 28,13 and 10 hyperliepmic subjects received i.v. respectively CDP-choline 300mg, SAMe 45 mg and citiolone 300 mg in 100 ml saline solution, during a 30 minute period. Basally and at the end of the injection, plasma lipid fractions were evaluated. CDP-choline caused a significant decrease in plasma total lipids ($p > 0.001$), cholesterol ($p > 0.01$) and triglycerides ($p > 0.01$) as well as a slight increase in beta-LP ($p > 0.05$).
SAMe injection was followed by a significant fall in plasma total lipids ($p > 0.05$), cholesterol ($p > 0.001$), and beta-LP + pre-beta LP/ alpha-LP ratio ($p > 0.05$), while alpha-LP increased significantly ($p > 0.05$).
Citiolone determined only a decrease in plasma cholesterol values ($p > 0.001$). The AA. suggest that the hypolipidemic effect of the three compounds tried can be due to a common mode of action, i.e. the stimulation of hepatic phospholipids biosynthesis, though in a different way.

4.4 Incorporation of polyenephosphatidylcholine into serum lipoproteins after oral or intravenous administration: *Zierenberg O, Odenthal J, Betzing H, Atherosclerosis. 1979 Nov; 32(3):259-76*

SUMMARY: Radioactive polyenephosphatidylcholine (PPC) was injected i. v. in dogs, rats and rabbits. In addition, PPC was administered orally to dogs and rats. The incorporation of the PPC applied into serum lipoproteins was determined. After i.v. injection in dogs approximately 80% of the radioactive dose was transported by HDL. Only around 50% of the dose was found, however, in the HDL fraction of rats and rabbits.
Radiochemical analyses provided a quantitative determination of the time course of PPC incorporation into HDL as well as of the metabolites of PPC. After in vitro incubation with rat and human serum PPC was incorporated into HDL by approximately 50% of the applied dose and by 5-14% into LDL, by approximately 15% into VLDL and by 20-30% into other serum constituents. In vitro incubation of PPC with human HDL yielded the complete incorporation into this lipoprotein fraction .

4.5 Inhibition of endocytosis in the arterial smooth muscle cells by means of highly unsaturated phosphatidylcholine: *Bowyer DE et al, Med Welt 1979 Sep 28;30(39):1447-8. German*

The incorporation of lipids into the smooth muscle cells of the arterial wall is a significant part of the pathogenesis of atherosclerosis. Pharmaceuticals that can reduce this lipid depositing can be used therapeutically.
…….
The lab developed a method based on the technique of Williams by which the endocytosis rate of the epithelial and smooth muscle cells can be measured. This

method allows the examination of substances that reduce the endocytosis rate and thus reduce the intracellular lipid collection. As the endocytosis is dependent on the constitution of the plasma membranes, it was obvious to examine polyunsaturated phosphatidylcholine and its effect on the endocytosis rate. The following results show that polyunsaturated phosphatidylcholine significantly reduce the rate of endocytosis in smooth muscle cells. …..
DISCUSSION: Our experiments show that it is possible to measure the endocytosis rate of cells in a culture. They further show that the endocytosis rate is significantly reduced by polyunsaturated phosphatidylcholine. This effect can be produced in real time as well as by prior incubation and is probably caused by a change in the cell membrane. The results lead to the conclusion that polyunsaturated phosphatidylcholine can inhibit the atherosclerotic process by reducing the endocytosis of plasma constituents, if the cells of arterial walls are changed the same way in vivo as our cells in vitro.
The results of the second experiment show that this inhibition declines fast when polyunsaturated phosphatidylcholine no longer is in the medium. This would underline the necessity to maintain a high enough plasma concentration of polyunsaturated phosphatidylcholine by repeated application.

4.6 The effect of essential phospholipids (EPL) on the activity of plasma-lecithin-cholesterol-acyl-tranferase (LCAT) in vivo and in vitro in the rabbit. *Horsch AK et al, Verhandlg d Deutsch Gesellsch für innere Med 81 (1975) 1457-1459. German*

The major part of the serum cholesterol is in the form of esterized cholesterol, mostly as linolic acid ester. Responsible for the esterification of the cholesterol bound to the high density lipoprotein HDL is the lecithin-cholesterol-acyl-transferase (LCAT), which transfers a fatty acid from the beta-position of lecithin to the C3-atom of cholesterol and changes lecithin into lysolecithin. This enzyme thus plays an important role in the metabolism of lipoproteins and lipids. Its significance for the metabolism of the arterial wall, particularly in atherosclerosis, has not yet been understood completely.
The atherosclerotic lesion is characterized through the accumulation of intra-and extracellular cholesterol esters and other lipids, that partly ingress from the plasma and partly are synthesized locally. The esterization of cholesterol can increase 20-fold. Cholesterol ester from saturated fatty acids are highly sclerogenic, whereas linolic acid esters show no such effect and can be mobilized out of the tissue more easily. Essential phospholipids (EPL), named because of their high content of linolic acid, are lecithins, with which the extent of the atherosclerotic changes in experimental atherosclerosis could be significantly reduced. This effect was attributed to the influence on the LCAT enzyme by EPL: on one hand linolic acid would be freed by forming non-sclerogenic cholesterol ester, on the other hand a faster turn over of cholesterol in the serum could be achieved. To test the hypothesis the LCAT activity in the serum in experimental atherosclerosis was measured with EPL present.
……
RESULTS: No significant influence of EPL on the activity of LCAT could be seen in vivo [in rabbits]. The initially mentioned anti-atherogenic effect of EPL, that was found by us and told of by others, can therefore not be caused by increased LCAT activity. The effect is probably caused by releasing unsaturated fatty acids for the intramural esterization of cholesterol and thus the formation of little sclerogenic cholesterol esters.

4.7 Effect of polyenoic phospholipid therapy on lecithin cholesterol acyltransferase activity in the human serum: *Dobiasova M et al, Physiologia Bohemoslovaca. 37(2):165-72, 188*

Abstract
The effect of polyenoic phospholipids on the concentration of serum lipids and the activity of lecithin cholesterol acyltransferase (LCAT, E.C. 2.3.1.43) was investigated in 18 patients with chronic glomerulonephritis accompanied by hyperlipaemia and reduced rate of cholesterol esterification in the plasma. The effects of therapy were evaluated immediately after a 2-month period of treatment and again after a 3-month drug free interval following termination of the therapy. An immediate effect of the treatment was reflected in a significant increase in the fractional esterification rate (FER% .h-1) and a marked reduction of the concentration of triglycerides (TG). Discontinuation of the drug resulted in the return of TG and FER values to the initial levels and in a rise of total (TCH) and unesterified cholesterol (UCH), HDL-cholesterol (HDL-TCH) and the molar esterification rate (MER
mumol.1-1.h-1) .The activity of LCAT estimated by radioassay in common and endogenous substrates varied in parallel.

4.8 Incorporation of polyenephosphatidylcholine into serum lipoproteins after oral or intravenous administration: *Zierenberg O et al, Atherosclerosis. 32(3):259-76, 1979 Nov.*

Abstract
Radioactive polyenephosphatidycholine (PPC) was injected i.v. in dogs, rats and rabbits. In addition, PPC was administered orally to dogs and rats. The incorporation of the PPC applied into serum lipoproteins was determined. After i.v. injection in dogs approximately 80% of the radioactive dose was transported by HDL. Only around 50% of the dose was found, however, in the HDL fraction of rats and rabbits. Radiochemical analyses provided a quantitative determination of the time course of PPC incorporation into HDL as well as of the metabolites of PPC. After in vitro incubation with rat and human serum PPC was incorporated into HDL by approximately 50% of the applied dose and by 5--14% into LDL, by approximate1y 15% into VLDL and by 20--30% into other serum constituents. In vitro incubation of PPC with human HDL yielded the complete incorporation into this lipoprotein fraction.

4.9 Effect of polyenephosphatidylcholine on cholesterol uptake by human high density lipoprotein: *Zierenberg O et al, Atherosclerosis. 39(4):527-42, 1981 Jul.*

Abstract
The lipid and protein composition of human HDL was changed by incorporation of polyenephosphatidylcholine (PPC) into HDL in vitro. HDL with incorporated PPC (HDL-PPC) had a higher molar PC/apoprotein ratio than native HDL. PPC accounted for up to 50% of the PC fraction of HDL. The fluidity of HDL-PPC was higher than that of native HDL but lower than that of PPC liposomes. Zonal ultracentrifugation separated HDL-PPC into a major and a minor component. The AI/AII ratio of the major fraction was reduced compared with native HDL. The storage capacity of HDL-PPC and native HDL for cholesterol was studied by incubation of these fractions with and 2 over black square];. [1 and 2 over black square]4] cholesterol-LDL. Significantly more cholesterol (55%) was taken up by HDL-PPC from LDL than by native HDL. The transfer of cholesterol from LDL to HDL in human serum was studied by an in vitro and 2 over black square]; .[1 and 2 over black square]4C] cholesterol distribution test. In this test

the lipoproteins of serum were labeled with and 2 over black square];. [1 and 2 over black square]4C] cholesterol. An analytical procedure was developed to quantify the transfer of cholesterol from LDL to HDL after addition of PC. The transfer depended on the fluidity and the dose of the PC fraction used as well as on the initial LDL + VLDL/HDL ratio and was independent of LCAT activity.

5. Studies of EPL in hepatology

5.1 The "essential" phospholipids in hepatology – 50 years of experimental and clinical experiences: *Kuntz E, Z Gastroenterol (Suppl 2) 1991: 29:7-13*

SUMMARY: Phospholipids are the most important structural and functional components of all biological membranes –thus also of the liver cell and its organelles – and of sinusoidal endothelia. Membrane damage always means cell damage – liver damage always involves membrane damage. The energy quantity of 5600 cal/Mol required for the cellular biosynthesis of phosphatidylcholine cannot be supplied by the damaged liver cell. Therefore, the incorporation of exogenous high-energy phosphatidylcholines is decisive for the restoration of the morphology and function of biological membranes. The cytoprotective effect of "essential" phospholipids (EPL) has been evidenced in 5 in-vitro studies with EPL in 18 different models of liver damage and with 5 animal species. The hepato-protective effect of EPL has clearly been confirmed. Also curative and regenerative effects have been seen in the experiments. The hepatoprotective action of EPL seems to be based on the inhibition of lipid peroxidation as the earliest measurable event on the molecular level. The fundamental knowledge on the pharmacokinetics of EPL (intestinal absorption, distribution in plasma, metabolism, enterohepatic circulation) is largely supported by over 15 experimental studies. Toxicity of EPL can certainly be excluded on the basis of 20 experimental in-vitro and in-vivo studies. This is also true when excessive doses are given both in short term and long term use. There is no fetal toxicity or any mutagenic potential. A carcinogenic potential can be ruled out.
EPL entered the German market in 1952 and, in the meantime, it has been registered in 53 countries. Within these 38 years, 126 clinical studies with a total of 8'334 patients including a phase-IV multi center study with 2'862 patients have been carried out. The critical assessment of clinical, biochemical and histological or electron-microscopic findings shows the effect of EPL: 1) accelerated improvement or normalisation of the subjective complaints, of the clinical findings and the numerous biochemical parameters, 2) better histological or electron-microscopic findings as compared with control groups (more important regression of inflammatory reactions, clear reduction of liver cell necrosis, less fatty infiltration, less fibrosis, improved liver cell regeneration), 3) shortened time of hospitalisation, and 4) less post-hepatic residues. The tolerance of EPL proved to be excellent. In the course of the hitherto 38-year application in 53 countries no severe side-effects have been reported, nor have been observed any adverse reactions in the 126 clinical studies. There are no interactions with other drugs either. On the basis of the astonishingly good therapeutic results, EPL is of value for the following indications:
- protective/curative supporting therapy in toxic liver damage and fatty liver
- flanking supporting therapy in chronic hepatitis and liver cirrhosis

NOTE: This study also mentions the pharmacokinetics of EPL:

Within 24 hours > 90 % of orally administered EPL is absorbed and transported to the liver. The maximum absorption is reached after 6 – 8 hours. After this time the plasma level is 5 – 10 %. The half time of elimination from the plasma is 30 hours.

NOTE: Thus to increase the percentage of EPL in the vascular system, intravenous application is important, as >90% of oral administration enters the enterohepatic pathway.

5.2 Protective effect of essential phospholipids on liver injury due to total parenteral nutrition: *Lata J et al, Vnitrni Lekarstvi. 47(9):599-603, 2001 Sep*

Abstract
OBJECTIVE: It is known that total parenteral nutrition (TPN) causes liver damage by various mechanisms and leads to an increase of transaminases and obstructional enzymes. From this aspect TPN can be considered an external factor which causes liver damage. In our investigation we wanted to find out whether parenteral administration of essential phospholipids (EPL) can have a protective effect on this damage.
PATIENTS AND METHODS: Our investigation comprised 20 patients where TPN was indicated, usually on account of severe acute exacerbation of a non-specific inflammation of the gut. The patients were divided into two groups. Ten patients were treated by intravenous administration of essential phospholipids (EPL,), 50 mg every 6 hours for a period of two weeks. The control group comprised ten patients without hepatoprotection. The bilirubin, ALT, AST, GMT, ALP values were assessed before the initiation of the study, on the seventh and fourteenth day. The results were statistically processed by the paired and non-paired t-test.
RESULTS: The baseline results of the mentioned tests did not differ significantly between groups. Bilirubin and AST did not change significantly during the investigation. In the control group we found, as compared with baseline values, a significantly increase of ALT on the seventh and fourteenth day, a significantly increase of GMT on the seventh and fourteenth day and a slight non-significant rise of ALP on the fourteenth day. In the EPL treated group, as compared with baseline values, a significant rise of ALT occurred on the fourteenth day. We did not observe a significant rise of GMT and ALP.
Between the EPL treated and control group a significant increase of GMT and ALP occurred in the control group, the other values did not differ between groups.
CONCLUSION: Parenteral EPL administration can have a favourable effect on liver damage caused by TPN, associated with cholestasis and biliary sludge. This conclusion can be hypothetically explained by improved bile fluidity and protection of the bile pole of the hepatocyte by essential phospholipids. Therefore their administration during TPN can be recommended.

5.3 Essential phospholipids in the treatment of hepatic encephalopathy: *Bruha R et al, Vnitrni Lekarstvi. 46(4):199-204, 2000 Apr*

Abstract
THE AIM: To evaluate the effect of treatment with intravenous EPL phospholipids in patients with liver cirrhosis and hepatic encephalopathy grade III-IV.

PATIENTS AND METHODS: l2 patients with hepatic encephalopathy grade III-IV due to decompensated liver cirrhosis, age 35-67 years, were randomized to two groups: EPL 2.0 g i.v**.** daily was given to 6 patients in combination with standard therapy for 2 weeks; 6 patients had only standard therapy. The patients were followed up for 90 days. The diagnosis of hepatic encephalopathy was based on clinical examination, psychometrical tests and neurophysiological examination-EEG and evoked potentials.
RESULTS: Mean survival in the group treated with EPL i.v. was 50.3 days in comparison to 34.7 days in control group. P300 latencies improved significantly in the EPL group in comparison to control group (427.5 ms before vs 366.3 ms after treatment period in the treated group; 346.6 ms before vs 347.5 ms after treatment period in controls) .Ammonia level decreased from 95 mumol/l to 49.7 mumol/l in the treated group, while in controls remained unchanged (46.5 mumol/l before vs 53.5 mumol/l after treatment period). No adverse reactions were observed during the treatment with EPL i.v.
CONCLUSION: Administration of EPL i.v. is a safe treatment of advanced liver disease. In the group of patients treated with EPL i.v. was observed prolonged survival, as an improvement of hepatic encephalopathy in comparison to control group.

5.4 Prevention and treatment of liver fibrosis based on pathogenesis: *Lieber CS, Alcohol Research and Treatment Center, Bronx Veterans Affairs Medical Center and Mount Sinai School of Medicine, New York 10468, USA. Clinical & Experimental Research. 23(5):944-9, 1999 May*

Abstract
Multiple agents have been proposed for the prevention and treatment of fibrosis. S-adenosylmethionine was reported to oppose CC14-induced fibrosis in the rat, to attenuate the consequences of the ethanol-induced oxidative stress, and to decrease mortality in cirrhotics. Anti-inflammatory medications and agents that interfere with collagen synthesis, such as inhibitors of prolyl-4-hydroxylase and antioxidants, are also being tested. In nonhuman primates, polyenylphosphatidylcholine (PPC), extracted from soybeans, protected against alcohol-induced fibrosis and cirrhosis and prevented the associated hepatic phosphatidylcholine (PC) depletion by increasing 18:2 containing PC species; it also attenuated the transformation of stellate cells into collagen-producing transitional cells. Furthermore, it increased collagen breakdown, as shown in cultured stellate cells enriched with PPC or pure dilinoleoyl PC, the main PC species present in the extract. Because PPC and dilinoleoyl PC promote the breakdown of collagen, there is reasonable hope that this treatment may be useful for the management of fibrosis of alcoholic, as well as nonalcoholic, etiologies and that it may affect not only the
progression of the disease, but may also reverse pre-existing fibrosis, as demonstrated for CC14-induced cirrhosis in the rat and as presently tested in an ongoing clinical trial.

5.5 EPL in the treatment of viral hepatitis B in subjects who abuse alcohol: *Tsyrkunov VM, Klinicheskaia Meditsina. 70(1):75-8, 1992 Jan*

Abstract
A trial was conducted of clinical efficacy of EPL. Twenty-three EPL-treated patients with hepatitis B abusing alcohol were compared to a control group receiving standard treatment. EPL has advantages as it shortens jaundice, cholestasis, enhances recovery of lipid metabolism in erythrocytic membranes and antioxidant properties of blood. EPL holds promise in multimodality treatment of combined hepatic lesions.

5.5 Polyenylphosphatidylcholine attenuates alcohol-induced fatty liver and hyperlipemia in rats: *Navder KP et al, Journal of Nutrition. 127(9):1800-6, 1997 Sep*

Abstract
Chronic administration of a soybean-derived polyenylphosphatidylcholine (PPC) extract prevents the development of cirrhosis in alcohol-fed baboons. To assess whether this phospholipid also affects earlier changes induced by alcohol consumption (such as fatty liver and hyperlipemia), 28 male rat littermates were pair-fed liquid diets containing 36% of energy either as ethanol or as additional carbohydrate for 21 d, and killed 90 min after intragastric administration of the corresponding diets. Half of the rats were given PPC (3 g/l), whereas the other half received the same amount of linoleate (as safflower oi1) and choline (as bitartrate salt) .PPC did not affect diet or alcohol consumption and 2 over black square];. [1 and 2 over black square] 5.4 +/- 0.5 G/(kg.d)], but the ethanol-induced hepatomegaly and the hepatic accumulation of lipids (principally triglycerides and cholesterol esters) and proteins were about half those in rats not given PPC. The ethanol-induced postprandial hyperlipemia was lower with PPC than without, despite an enhanced fat absorption and no difference in the level of plasma free fatty acids. The attenuation of fatty liver and hyperlipemia was associated with correction of the ethanol-induced inhibition of mitochondrial oxidation of palmitoyl-1-carnitine and the depression of cytochrome oxidase activity, as well as the increases in activity of serum glutamate dehydrogenase and aminotransferases. Thus, PPC attenuates early manifestations of alcohol toxicity, at least in part, by improving mitochondrial injury. These beneficial effects of PPC at the initial stages of alcoholic liver injury may prevent or delay the progression to more advanced forms of alcoholic liver disease.

5.6 Polyenylphosphatidylcholine prevents carbon tetrachloride-induced lipid peroxidation while it attenuates liver fibrosis: *Aleynik SI et al, Alcohol Research Center, Bronx, NY, Journal of Hepatology. 27(3):554-61, 1997 Sep.*

Abstract
BACKGROUND/AIMS: Polyenylphosphatidylcholine protects against alcoholic cirrhosis in the baboon and carbon tetrachloride-induced cirrhosis in rats. This study addresses the possible mechanism of the protective effect of polyenylphosphatidylcholine. METHODS: For 8 weeks, rats were injected with either carbon tetrachloride in peanut oil or peanut oil alone (control), and pair-fed nutritionally adequate liquid diets with equivalent amounts of linoleic acid either as polyenylphosphatidylcholine or as safflower oil. Other rats were injected for 9 weeks with heterologous albumin and fed the same liquid diets. Lipid peroxidation was measured by F2-isoprostanes and 4-hydroxynonenal. RESULTS: Carbon tetrachloride-induced lipid peroxidation was strikingly attenuated with polyenylphosphatidylcholine supplementation. Levels of hepatic F2-isoprostanes and 4-hydroxynonenal paralleled liver fibrotic scores and collagen accumulation. Polyenylphosphatidylcholine also attenuated the fibrosis induced in rats with human albumin, but in this case, levels of hepatic 4-hydroxynonenal did not change, nor were they significantly affected by polyenylphos-phatidylcholine. Neither carbon tetrachloride injection nor polyenylphosphatidylcholine treatment changed the arachidonic acid content (a major precursor of F2-isoprostanes and 4-hydroxynonenal) in liver phospholipids, and hepatic vitamin E was not significantly altered. CONCLUSIONS: The hepatic protection of polyenylphosphatidylcholine against carbon tetrachloride appears to be due, at least in part, to an antioxidanACt effect, whereas the protection against heterologous albumin-

induced fibrosis suggests that an additional mechanism, such as stimulation of collagenase activity, may also be responsible.

6. Other studies & reports with reference to EFA and phospholipids

6.1 Advances in nutrition and dietetics: *HM Sinclair, Practitioner 181, 468 (1958)*

……. The rapid increase in notified deaths from pulmonary embolism and infarction, which is occurring in this country at the same rate as deaths from ischaemic heart disease, could be similarly attributed to a relative deficiency of EFA. **This deficiency causes a structural defect in cell membranes and connective tissue, and the body in consequence becomes increasingly susceptible to injurious agents. Among these are infections, ultra-violet light, x~rays, chemical carcinogens and hydrochloric acid in the duodenum.** It is possible therefore that a relative deficiency of EFA could play a part in the etiology of coryza and poliomyelitis by allowing the virus to traverse the mucous membranes of nose and gut, of leukaemia, of lung cancer and of' duodenal ulcers. In lower animals deficient in EFA the matrix of bone is defective causing easy fractures, and this could be related to senile osteoporosis, another disease that is increasing at the same rate is ischaemic heart disease. The requirement of EFA in lower animals is about seven times greater in males than in females during the reproductive period. This fits the incidence of the diseases mentioned.
PRACTICAL IMPLICATIONS

KinseII (Essential Fatty Acids, 1958) has reported therapeutic success in the treatment of intermittent claudication with maize oil as a source of EFA. Work on lower animals strongly suggests that the deposition of cholesterol in tissues can be removed by administration of EFA and that maize oil (which is rich in EFA) will prevent coronary thrombosis even if saturated (non-EFA) fat is included in the diet. In the present state of knowledge it seems sensible to treat hypercholesterolremla, severe atheroma and myocardial infarction with diets high in EFA-fat and low in non-EFA fat; obviously I am only referring to dietetic treatment in general; various factors that increase the requirement of EFA (e.g. hypothyroidism, diabetes mellitus) may be present. A fat-free diet has the effect of increasing EFA-deficiency since the fat available to the body is then the fat the body can form from carbohydrate, and this is relatively saturated (non-EFA) fat. Conversely, a high-fat diet is also in general harmful since the ordinary fats included in our diet are relatively deficient in EFA (butter, lard, dripping, meat-fat, margarine- though this last can be an important source of EFA depending upon the method of manufacture). What is required is to decrease the non-EFA fat in the diet and increase the EFA. There are pharmaceutical preparations available for this and there is a large use of vegetable seed oils (maize or corn oil, sunflower seed oil) in the United States. There is no great difficulty in taking 3 ounces (85 g,) of maize oil daily) which provides about 50 g of linoleic acid and also vitamin E. Since pyridoxine (vitamin B6) appears to be becoming deficient in our diets (Sinclair 1958b) this must also be supplied (10 to 50 mg daily). It may cause surprise that as much as 50 g of linoleic should be administered; 3 ounces (85 g) of corn oil provides about 750 calories and might lead to obesity unless other fat and carbohydrate are reduced as is strongly desirable. **There is evidence that if linoleic acid is ingested as neutral fat (triglyceride), which is the form in maize oil, it is rapidly oxidized in the body and only part is available for EFA-functions; this is true even in an animal that is**

suffering from deficiency of EFA. But if linoleic acid is given in the form of a lecithin (a phospholipid) then it may be absorbed as such and protected from rapid oxidation for energy purposes; it is probably as part of phospholipids that EFA play their most important functions. In this way quite small amounts of EFA may be sufficient to treat these diseases. A pharmaceutical preparation of lecithin rich in EFA is marketed in Germany; until more research is done -and research is urgently needed in the whole field of EFA- we cannot say what foodstuffs are good sources of lecithins containing EFA ; eggs, for instance, vary according to the diet of the hen. So for the present we must rely mainly on vegetable seeds (nuts, whole-wheat bread) and their oils such as maize oil) and certain fish, such as herring…….

6.2 An estimation of arteriosclerosis by the measurement of pulse wave velocity and an analysis of the clinical effect of therapeutic agents on arteriosclerosis: *Yoshimura S et al, Cor Vasa, 1968;10(3):173-82*

SUMMARY: The pulse wave velocity of the isolated human aortae was studied with special reference to their sclerotic changes examined pathologically. The pulse wave velocity was mainly determined by the distensibility of the arterial wall and internal pressure. The effect of other factors on the pulse wave velocity was almost negligible. This means that the internal pressure must be taken into consideration by measuring the pulse wave velocity. The pulse wave velocity increased with the elevation *of* diastolic pressure. The pulse wave velocity correlated weIl with the grade of the pathological changes. The difference between the pulse wave velocities measured ante - and postmortem was not significant. Thus the pathological conditions of the aorta of living subjects can be estimated by measuring PWV. The PWV in the clinical cases increased with the elevation *of* diastolic pressure and age. High blood pressure may be one of the factors which accelerate sclerosis of the aorta and metabolic disorders would be an essential cause of the arteriosclerosis. The effect of therapeutic agents on arteriosclerosis can be proved quantitatively at a subclinical stage by the PWV method.

6.3 Use of isradipine and EPL for protection of the kidney during extracorporal lithotripsy: *Nei'mark AI, Zhukov VN et al, Urologiia I Nefrologiia. (6):19-21, 1998 Nove-Dec*

Abstract
The authors analyse the effects of ESWL on renal function in 180 patients with nephrolithiasis. Renal performance was judged by the level of enzymes. Pharmacological defense of the kidney was made with isradipine and EPL given for 12 weeks before lithotripsy and 4 weeks after it. Isradipine proved a good corrector of renal function after lithotripsy as it decreased enzymuria, promoted normalization of the activity of alkaline phosphatase, gamma-glutamyl transferase, alpha-glucosidase and lactate dehydrogenase to the end of the first postoperative month. This indicates quicker recovery of renal parenchyma after ESWL. EPL also improved enzymic indices. Its moderate protective action on renal parenchyma normalized levels of some enzymes one month after ESWL.

6.4 Effect of intravenous polyunsaturated phosphatidylcholine infusions on insulin receptor processing and lipid composition of erythrocytes in patients with lver cirrhosis: *Cantafora A et al, European Journal of Clinical Investigation. 22(12):777-82, 1992 Dec.*

Abstract
The aim of this study was to determine whether insulin receptor processing capabilities of human erythrocytes could be improved by changing the cell membrane lipid composition using an intravenous infusion of polyunsaturated phosphatidylcholine. Thirteen cirrhotics were submitted to the i.v. infusion of phosphatidylcholine (2 g day-1 for 3 days) .Both erythrocyte lipid composition and insulin receptor processing ability were examined at the beginning of the study and at 0**,** 3 and 11 days after the end of the treatment. This treatment decreased the erythrocyte cholesterol to phospholipid molar ratio and increased the proportion of polyunsaturated fatty acids (mainly linoleic acid) immediately after the end of the treatment. The proportion of arachidonic acid increased immediately in the phosphatidylserine class and, a few days later, also in phosphatidylethanolamine. The phospholipid class distribution did not show any relevant modification in the course of the study. Surface insulin receptors, which generally were up-regulated in the untreated subject (-7.1 +/- 20.4%), showed an improvement in down regulation capabilities that appeared to be well correlated with the changes in lipid composition of cell membranes induced by i.v. infusion of polyunsaturated phosphatidylcholine. The confirmation of these findings also in target cells for insulin may open new perspectives in the treatment of diabetes mellitus.

6.5 Use of preparation essential and hemosorption in the complex treatment of chronic alcoholism: *Khodzhaeva NI, Zhurnal Nevropatologii I Psikhiatrii Imeni S-S-Korsakova. 90(2):59-62, 1990.*

The results of the treatment of three groups of patients with chronic alcoholism are compared. Group I (n=26) received essential combined with hemoperfusion, group II (n=18) placebo combined with hemoperfusion and group III (n=20) was on teturam. It has been established that as compared with other treatment measures, essential combined with hemoperfusion contributed to the occurrence of long remissions and disappearance of somatic complications of alcoholism to a greater degree.