moles/l for K_i (s = 0.12), in both cases the correlation coefficients of the regression lines being > 0.989.
The enzyme inhibition caused by **III** was also of the non-competitive type (figure 3). The values calculated in this case were 0.130×10^{-3} moles/l for K_m (s = 0.007) and 2.02×10^{-3} moles/l for K_i (s = 0.11), and r > 0.992. This means that **III** inhibits about 4 times as strongly as **I**.
From these results obtained in vitro it can be seen that silymarin components **I–III** clearly inhibit, depending on concentration, the peroxidation of linoleic acid catalysed by lipoxygenase. An analogous interaction with animal lipoxygenase and thus an inhibition of the peroxidation of the FA in vivo is self-evident. It therefore appears that the assumption that silymarin has a protective function for the ML also holds true experimentally.

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Silymarin, an inhibitor of prostaglandin synthetase

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Summary. Silybin (**I**), silydianin (**II**) and silychristin (**III**), the main constituents of silymarin, inhibit the formation of prostaglandins in vitro. The inhibition is log-linearly dependent on the concentration of the effectors.

The antihepatotoxic effect[3] of silymarin is due, amongst other things, to a particular 'protective effect' which the active components of this natural substance[4] have on certain membrane lipoids[5–7]. In the preceding report[7a] we show that these substances inhibit the activity of lipoxygenase in a specific manner. We have already found a similar inhibitory effect on vegetable peroxidases[8].
Lipoid peroxidation[5–7,9], which is increased by certain hepatic toxins until clinical-pathological symptoms occur, is closely linked to the formation of prostaglandins (PG). There is a high degree of correlation between the 2 processes[10]. The primary substrate for the formation of PG is formed by the polyunsaturated C20 fatty acids, especially arachidonic acid, which are released from the membranes by lipolysis[11]. We were able to show that the effect of arachidonic acid on lipoid peroxidation and PG formation in individual organs depends directly exactly on dosage[10]. On the other hand, the inflammatory processes that can occur in tissues and organs as a result of this and similar pathogenic phenomena are linked to the formation of PG[11,12]. The inhibition of prostaglandin synthetase (PG synthetase) by various corticosteroids[13] and non-steroid antiinflammatory medicaments[14,15], and the fact that inflammatory symptoms can be induced by the application of exogenic PG[12,16], are conclusive indications of the involvement of PG and its precursors in the inflammation process. Investigation into the effect of substances **I–III** on the formation of PG acquires particular significance because various liver diseases are also accompanied by inflammatory processes (hepatitis).
PG synthetase is a membrane bound enzyme complex. This complex combines the properties of a phospholipase, a

lipoxygenase, a peroxidase, a cyclooxygenase, a dehydrogenase and a reductase[11–17]. The enzyme preparation needed for the experiments was produced from the fundi of rats' stomachs using the method of Pace-Asciak and Wolfe[13]. Using chromatographic and mass spectrometric analysis of the reaction products of arachidonic acid, we found that PG of the types A, E and F was produced in the present experimental arrangement (figure 1)[13,18].

Material and method. Test substances. Stable, water-soluble, uniform TLC derivates of silybin (**I**), silydianin (**II**) and silychristin (**III**) as Na salts of dihemisuccinates[19]; solutions of the effectors were prepared ad hoc in buffer I.

Substrate. 1-^{14}C arachidonic acid in benzene, sp. act. 50 mCi/mmole (Radiochemical Centre, Amersham); 'cold' arachidonic acid, concentration > 75% (GLC of the methyl ester) (Merck-Schuchardt). 3 µl 1-^{14}C arachidonic acid, 15 µl 'cold' arachidonic acid and 2 µl acetone were used per 10 ml of experimental preparation.

Buffer[13]. 50 mM phosphate buffer pH 7.4+20 mM EDTA (buffer I). 50 mM phosphate buffer pH 7.4+20 mM EDTA+5.7 mg hydroquinone+56 mg reduced glutathione/l (buffer II).

Experimental animals. Male SPF albino rats, breed OFA (SD), from the Vienna University research institute for the breeding of experimental animals (Himberg, Austria), weight 200–250 g were used. Feeding was with standard dry feed; 12 h before commencement of the experiment, feeding of the animals was stopped. Water was given ad libitum.

Equipment. Isocap 3000 liquid scintillation counter (Nuclear, Chicago); model 7201 plate scanner (Hewlett-Packard); Ultra-Turrax (Janke und Kunkel, Staufen i.Br.); pH meter (Seibold, Vienna); Rotavapor (Büchi, Flawil, Switzerland); d.c. equipment (Desaga, Heidelberg); Varian-Matt 111 mass spectrometer (ionisation 80 eV, ion source 300 °C); HP-67 programmable electronic computer (Hewlett-Packard), STD-03 programme.

Experimental method[13]. The stomachs of 8 rats killed by a blow on the neck were rapidly prepared, carefully cleaned of any remaining fatty tissue and rinsed in ice-cold buffer I. The fundi were separated from the pyloric parts, cut into small pieces and homogenised for 1 min in 56 ml of ice-cold buffer II. In each batch 7 ml of homogenate were mixed with substrate and effector (3 ml) in the specified concentrations (figure 2) (control samples: 3 ml buffer I) and incubated for 30 min at 37 °C. A moderate flow of O_2 was introduced and the specimens shaken slightly. The reaction was then stopped by the addition of 200 ml of chloroform-methanol (2:1, v/v). A trace of BHT-antioxidant (butylated hydroxy toluene) prevented further oxydation during processing. The mixture was quickly filtered in a separating funnel, washed with 20 ml 0.03 M HCl and dried at room temperature in a stream of N_2 (Rotavapor). The residue was absorbed in 0.3 ml chloroform-methanol and chromatographed on silica gel GF_{254} finished plates (Merck) (solvent: chloroform-methanol-glacial acetic acid, 18:1:1, v/v/v); R_f values: $PGF_{2\alpha}$ 0.05–0.10; PGE_2 0.20–0.25; PGA_2 0.40–0.50; arachidonic acid 0.60. The substances were identified by mass spectra[13] (methyl ester, trimethylsilylether) of the eluted zones (figure 1). They were detected using a spray reagent[20] consisting of 1 ml anisaldehyde, 9 ml 95% ethanol and 1 ml concentrated H_2SO_4.

Evaluation. a) The d.c. plates were scanned and the activity in the individual zones automatically recorded as an activity curve. The areas underneath the peaks were measured by a planimeter. The total area was made equal to 100% and the partial areas related to this value.

b) The coloured zones were scraped off, mixed with 15 ml of scintillation liquid and the DPM counted. The total activity was made equal to 100% and the activities of the individual zones related to this value.

The total PG (ΣPG%) represents the percentage of substrate converted by PG synthetase compared to the arachidonic acid used (= 100%). The data obtained from 3–4 similar experiments were averaged ($\bar{x}$) and SD calculated. $\bar{x}$ (and in some instances s) were entered in a semilogarithmic grid against effector concentration (figure 2) and the optimally fitted curve, the regression equations and correlation coefficient (r) calculated.

Results and discussion. PG synthetase from the fundus of rats' stomachs converts about 30% of arachidonic acid to defined PG (figure 1). This formation of PG is significantly inhibited by **I**, **II** and **III**, depending on their concentration (figure 2). The effective area ranges from about 0.001 up to around 1 mM effector in the incubation medium. The maximum concentration is predetermined in each case by the solubility limit, whilst the lowest corresponds to that of the lowest detectable inhibitory effect. Concentrations below 0.001 mM show no significant effect in comparison to the control samples.

The quantities of PG formed correlate log-linearly with the actual effector concentration (figure 2). The following regression equations (correlation coefficients) were determined:

I: $y = -2.34 - 2.33 \ln x$ ($r = 0.991$)
II: $y = 5.85 - 2.49 \ln x$ ($r = 0.979$)
III: $y = 11.40 - 2.20 \ln x$ ($r = 0.972$)

Whereas **I** almost completely suppressed the formation of PG at the highest concentration (0.3 mM), **II** and **III** reduced it in the most extreme case to about one third or

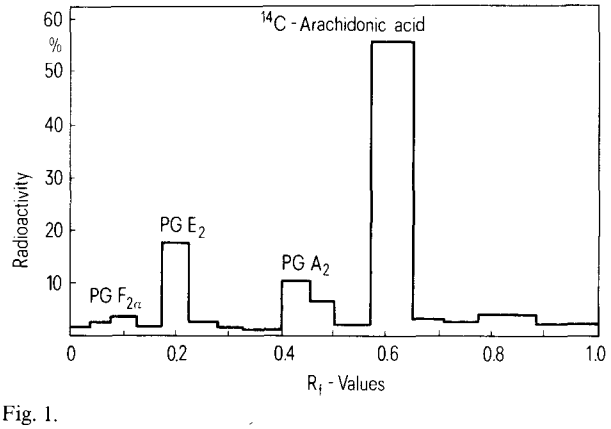

Fig. 1.

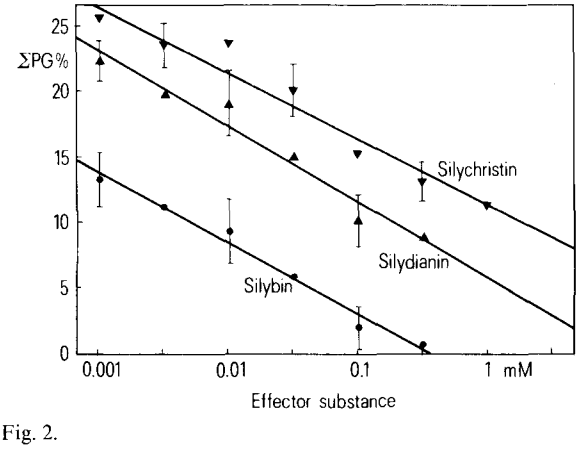

Fig. 2.

one half of the unchecked production of PG respectively. I thus proved to be the strongest inhibitor of PG synthesis in vitro.

In the case of the partial destruction of membrane structures in the liver which occurs following organic diseases or intoxications, increased quantities of C20 fatty acids are released by lipolysis, which leads, amongst other things, to the increased activity of PG synthetase. Silymarin can counteract this deleterious process. The suppression of the (pathological) decomposition of membrane lipoids together with the inhibition of PG formation could provide a plausible explanation for the way in which this hepatotropic complex functions.

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Causes of artificially high blood glucose values in experiments with diabetic rats and mice

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Summary. Unusually high blood sugar values may be caused by the slightest contamination of the blood obtained after puncture of the tail vein by diabetic urine, that has dried onto the tails of the animals. This can be avoided by collecting blood that is allowed to drip from the severed tip of the tail or by carefully cleaning the tail before puncturing the tail vein.

During the course of a long-term study with streptozotocin-diabetic rats we noticed occasional blood sugar values that were very much higher than the usual blood sugar values for such animals and which also were not in accordance with the corresponding glucose excretion in the 24-h urine. In occasional cases, the values measured were more than 1200 mg/dl and thus markedly higher than the values given in the literature for very severe streptozotocin diabetes in the rat[1-4].

The hexokinase technique which was used for the glucose determinations is regarded as the scientific reference method and cannot be considered as the cause of the high blood sugar values because of its very low variability[5-10]. The 'Capiletten' (Labora Mannheim, Mannheim) that are used in our laboratory have an accuracy of ± 1-2% and can therefore also be excluded as a possible cause. Other explanations, such as the chance coincidence of changed eating habits, stress during blood collection, and obtaining blood from different vascular areas, can be excluded because of the size of the deviations. The only remaining possibility was a volume displacement of the plasma space, and this was the subject of our 1st investigations.

Materials and method. In experiment I, a series of blood sugar values and haematocrit values were determined in the same animals (Sprague-Dawley rats siv 50 SPF, Iwanova, Kisslegg). The blood collection was carried out on the one hand by means of puncture of the tail vein with a steel cannula followed by pipetting of 10 µl from the drops of blood that developed on the tail, and on the other by the almost simultaneous cutting off of the extreme tip of the tail (0.5-1 mm). The blood sugar determination was carried out by means of the hexokinase G-6-PDH method (Glucoquant®, Boehringer) in a haemolysate (Hämolyse-Reagenz, Boehringer). The scatter of the blood sugar values was determined in each group and was expressed as the coefficient of variation (table 1).

In order to clarify the results obtained, streptozotocin-diabetic rats were randomised into 2 groups of 10 in a 2nd experiment (II). Before removing blood, the tails of the animals in group A were carefully washed in water at body