prepared analogously to the chloride salt. With the quantities given in preparation No. 1, the cobalt(II) chloride solution was replaced by a solution of cobalt(II) nitrate 6-hydrate (95.5 g, 0.328 mol) in water (80 ml), and the procedure given in preparation No. 1 was followed exactly. The nitrate salt was isolated analogously by evaporation to a volume of 160 to 170 ml. The crude product was washed with 70 % v/v ethanol (three 80 ml portions) and allowed to dry in air. The yield was 82 g of red crystals of the nitrate salt (72 %). This product was not pure. The pure salt was obtained by reprecipitation from water. The crude product (20 g) was dissolved in water (40 ml) at 100° and filtered. Then 96 % ethanol (120 ml) was added quickly to the stirred solution (while hot), and the mixture was cooled for 2 h in ice. The precipitate was filtered and washed with 70 % v/v ethanol (two 20 ml portions). Drying in air yielded 16 g (80 %). (Found: Co 17.11; C 24.25; N 20.45; H 6.43. Calc. for $[Cotn_2CO_3]NO_3$: Co 16.98; C 24.21; N 20.18; H 6.39). $(\varepsilon,\lambda)_{max}$: (107.5,520); (124.9,360); $(\varepsilon,\lambda)_{min}$: (11.4,430); (50.2,327). Medium: water.

3. *Carbonatobis(trimethylenediamine)cobalt-(III) perchlorate*, $[Cotn_2CO_3]ClO_4$. Crude carbonatobis(trimethylenediamine)cobalt(III) chloride (20.0 g, 0.062 mol) was dissolved in water (50 ml) at room temperature and the solution was filtered. A saturated solution of sodium perchlorate in water (20 ml) was then added to the stirred solution with cooling in ice. The precipitation of red crystals of the perchlorate salt immediately commenced, and the cooling was continued for half an hour. The precipitate was filtered, washed with ice cold ethanol (50 % v/v, 20 ml) and 96 % ethanol (three 20 ml portions). Drying in air yielded 20.1 g (88 %). (Found: Co 16.06; C 22.74; N 15.46; H 5.52; Cl 9.65. Calc. for $[Cotn_2CO_3]ClO_4$: Co 16.07: C 22.93; N 15.28; H 5.50; Cl 9.67). $(\varepsilon,\lambda)_{max}$; (107.3,520); (124.7,360). $(\varepsilon,\lambda)_{min}$: (11.3,430); (49.2,327). Medium: water.

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# Inhibition of Duodenal Pancreatic Enzymic Activities by Polyphloretin Phosphate with Special Reference to Phospholipase $A_2$

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Polyphloretin phosphate (PPP) is a substance capable of inhibiting the effects of prostaglandins, trombin, and certain enzymes such as alkaline phosphatase and hyaluronidase. Hyaluronidase is completely inhibited in an environment containing 1 – 2 μg/ml PPP. The molecular weight of PPP is about 15 000 and it is only to a minor extent – if any – split or absorbed from the intestine.[1,2] It has been shown to be useful in cases with ulcerative colitis.[2] This beneficial effect in cases with ulcerative colitis has been ascribed its membrane tightening effect as in this disease the permeability of the colonic mucosa is increased with protein leakage as a consequence. This membrane tightening effect of PPP might be explained by its capability of inhibiting hyaluronidase. Lecithin is another compound that is one of the main components of cell membranes. In the present investigation, therefore, we have found it interesting to study the effect of PPP on the *in vitro* activity of pancreatic enzymes, especially on phospholipase $A_2$ which in the intestine splits lecithin to lysolecithin the latter of which might be highly toxic to cell membranes in patients with inflammatory intestinal diseases.

*Materials and methods.* Polyphloretin phosphate (PPP standard IV batch number Leo 101K) was a gift from AB Leo, Helsingborg, Sweden. It contained 93.5 % PPP. Contaminants were pyridine (2.7 %) sodium chloride (2.4 %), phosphoric acid (0.2 %) and water.

Phospholipase $A_2$ acitivity was estimated according to Ihse and Arnesjö,[3] lipase according to Erlansson and Borgström,[4] and trypsin by a modified version of the method of Hummel.[5] All enzyme assays were run using a pH-stat (Radiometer, Copenhagen) with a TTT2 titrator connected to an ABU11 Burette Unit with a 0.25 ml burette and a thermostatically controlled TTA31 titrator assembly.

Sodium taurodeoxycholate (NaTDC) was synthezised according to Norman [6] as modified

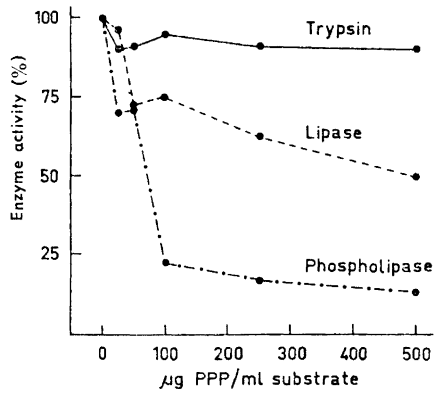


*Fig. 1.* Inhibition of trypsin, lipase, and phospholipase A by different concentrations of polyphloretin phosphate.

by Hofman.[7] Purity better than 98 % as judged by thin layer chromatography.

All experiments were run *in* vitro. The enzyme source was fresh human duodenal contents aspirated *via* a duodenal tube positioned in the ascendent part of the duodenum under fluoroscopic control and after testmeal stimulation of the pancreatic secretion. PPP in different concentrations was added to the substrate solution.

All numerical values given in results represent the average of three determinations.

*Results.* As is shown in Fig. 1 the phospholipase A activity was inhibited about 30 % with a PPP concentration of the substrate solution of 25 µg/ml, 85 % with a PPP concentration of 500 µg/ml and 100 % with 1 mg PPP/ml. Fig. 2 demonstrates the PPP inhibition with different concentrations of bile salt. The parallellity between the phospholipase activity measurements with or without PPP at different bile salt concentrations indicates that the inhibitory effect is independent of the bile salt concentrations. Duodenal phospholipase A activity is dependent on the presence of calcium.[3] As is shown in Fig. 2 the PPP inhibition of phospholipase A is the same irrespective of the calcium concentrations. The pH-optimum for the phospholipase A activity of duodenal contents is 7.5 pH.[3] This optimum was not shifted in the presence of

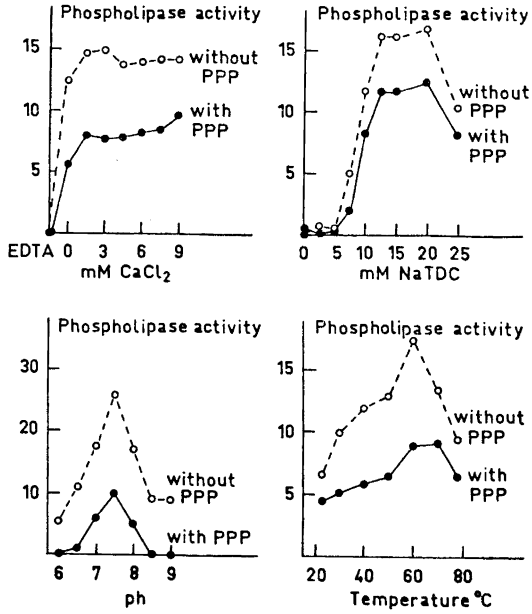


*Fig. 2.* The influence of calcium, bile salt, pH, and temperature on polyphloretin phosphate inhibition of duodenal phospholipase A.

PPP (Fig. 2). The temperature optimum was also unaltered in the presence of PPP (Fig.2).

Fig. 1 shows that the lipase activity of duodenal contents was found to be incompletely inhibited by relatively high concentrations of PPP. The inhibitory effect with a concentration of 100 μg PPP/ml substrate solution was 25 % while it was 50 % with 500 μg PPP/ml.

A maximal inhibition of the trypsin activity of duodenal contents of about 10 % was found even with high concentrations of PPP.

*Discussion.* The results of the present study indicate that PPP is a potent inhibitor of pancreatic phospholipase A in intestinal contents. This inhibitions seems to be independent of the bile salt concentrations, the calcium concentration, the pH, and the temperature of the incubation medium. Also duodenal pancreatic lipase was inhibited to a certain extent, whereas only slight inhibition, if any, of trypsin was found. PPP has been shown to exert a beneficial effect upon the protein losses *via* the colonic mucosa in patients with ulcerative colitis.[2] A subdivided dose of 2 g PPP/day was given. Under such circumstances a total inhibition of the intestinal phospholipase $A_2$ activity and, in addition, an moderate inhibition of the lipase activity should be expected. This latter possibility might explain that in cases with non-tropical sprue a beneficial effect on the protein leakage but no effect on the feacal fat excretion was obtained after peroral PPP administration.[2]

The membrane tightening effect of PPP has been ascribed its capability of inhibiting hyaluronidase.[1] Considering the fact that lecithin is a main component of cell membranes and that it is split by phospholipase A the beneficial effect of PPP in cases of ulcerative colitis may also be ascribed an inhibition of this latter enzyme by PPP.

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# An Electron-Diffraction Investigation of the Molecular Structure of 1,2,4,5-Hexatetraene (Biallenyl) in the Vapour Phase

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The molecular structure of 1,2,4,5-hexatetraene (in the following called biallenyl) has been studied by the gas electron diffraction method. The compound used in the present study was synthesized from 3-bromo-1-propyne and magnesium metal with tetrahydrofuran as solvent.[1]

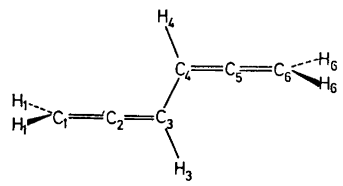


*Fig. 1.* Biallenyl. Molecular model which shows the numbering of the atoms.

A complete spectroscopic study of the compound was recently carried out by Powell *et al.*,[2] based on a sample originating from the same source. They concluded from examination of the infrared and Raman spectra that the molecule has a center of symmetry corresponding to $C_{2h}$ symmetry in which the hydrogen atoms of the $CH_2$ groups are twisted out of the molecular plane by 90°.

The electron-diffraction intensity data were obtained with the Oslo diffraction camera.[3] Diffraction photographs were taken at two camera lengths (approximately 48 cm and 20 cm) at room temperature. For each camera length four plates were selected for the structure analyses. The data were treated in the usual way [4] and yielded an experimental molecular intensity ($sM(s)$) function in the region from $s = 1.25$ Å$^{-1}$ to about 44 Å$^{-1}$.