# REACTIVATION BY PHOSPHOLIPIDS OF $CCl_4$ INHIBITED MICROSOMAL GLUCOSE-6-PHOSPHATASE

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Received 28 April 1969

## 1. Introduction

In 1961, Recknagel and Lombardi [1] described the destruction of the microsomal membrane enzyme glucose-6-phosphatase by treatment of microsomes with $CCl_4$ *in vitro*. This inhibitory effect of the haloalkane may be mediated by its lipid solubility. On the other hand glucose-6-phosphatase and many other membrane enzymes are known to require phospholipids for their activity [2, 3]. We therefore studied the possibility of reversing the $CCl_4$ inhibition of glucose-6-phosphatase by treatment with liver phosphatides.

## 2. Methods

We used male Wistar rats, 250–300 g (Hartelust-Thorsen Co., Uden, Holland). The animals were kept on rat chow and water ad libitum. The animals were anaesthetized with i.p. injection of ethylurethane, 1.2 g/kg. The livers were freed from blood by perfusion with cold 0.25 M sucrose solution *in situ*. Microsomes were prepared according to DeDuve [4] and resuspended in 0.25 M sucrose. The working suspensions contained about 40 mg protein per ml.

Activity of glucose-6-phosphatase was assayed in 0.1 M maleate-tris buffer [5] as follows: 10 $\mu$l of the microsome suspension were added to 0.2 ml maleate buffer and allowed to stand at 20°C (1st preincubation). After addition of 0.1 ml maleate buffer the mixture was kept again at 20° (2nd preincubation). The time of these preincubation steps was varied as indicated in the figures. Then we added 0.1 ml glucose-6-phosphate (0.1 M) and incubated at 37° for 10 min. The reaction was stopped by addition of 0.2 ml of 13% $CCl_3COOH$ and inorganic P determined after centrifugation. The activity values obtained in this way were taken as controls.

Within 10 min of incubation at 37° the enzyme activity was constant in control as well as in $CCl_4$ treated microsomes.

In $CCl_4$ inhibition experiments the first preincubation step was carried out in 0.2 ml $CCl_4$ saturated maleate buffer under a constant flow of nitrogen (75 ml $N_2$/min) containing $CCl_4$ (about 0.8 g $CCl_4$/l $N_2$).

For testing the effect of phospholipids we added 0.1 ml of a phospholipid suspension instead of maleate buffer during the second preincubation step. Appropriate blanks were run simultaneously with all assays.

Phospholipids: We used acetone precipitated pig liver phosphatides (27.7% sphingomyelin, 39.9% phosphatidylcholine, 17.3% phosphatidylethanolamine, 2.2% cardiolipin and 12.9% unidentified phosphatides, as determined by thin layer chromatography) and egg lecithin (puriss., "Merck"). The lipids were suspended in maleate buffer by means of a Potter-Elvehjem homogenizer at concentrations varying from 2 $\mu$g to 4 mg lipid phosphorus per ml. Higher concentrations were not suitable for handling.

## 3. Results

The correlation of $CCl_4$ inhibition to the time of

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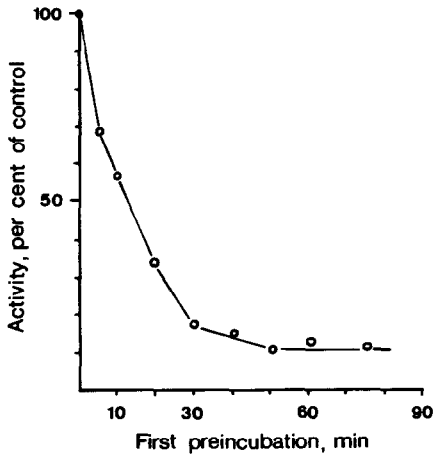


Fig. 1. Inhibition of microsomal glucose-6-phosphatase by $CCl_4$ *in vitro*. Second preincubation: 30 min.

the first preincubation is depicted in fig. 1. A constant minimal activity (about 10% of the control value) is reached within about 50 min. Possibly this phosphohydrolase activity may be due to nonspecific phosphatases not inhibited by $CCl_4$.

The reactivating effect of liver phosphatides at various degrees of inhibition is shown in fig. 2. From 0 to 50% inhibition the activity was completely restored (100 to 115% of the control). Liver phospholipids also increased the activity of untreated microsomes to about 110% of the control value. If the $CCl_4$ inhibition exceeded 50% the restorable activity concomitantly began to fall.

Fig. 3 shows the restauration of enzyme activity by different amounts of added liver phosphatides or egg lecithin, respectively. With liver phospholipids it was possible to restore fully the enzyme activity, if the residual activity was around 40% after $CCl_4$ treatment and the second preincubation time after addition of phosphatides was 30 min. A longer second preincubation time did not increase the effect of phospholipids. Egg lecithin exerted a slightly lower effect in the upper concentration range.

## 4. Discussion

$CCl_4$ is considered affecting membrane enzymes in a twofold way [6, 7]. Firstly, $CCl_4$ can attack

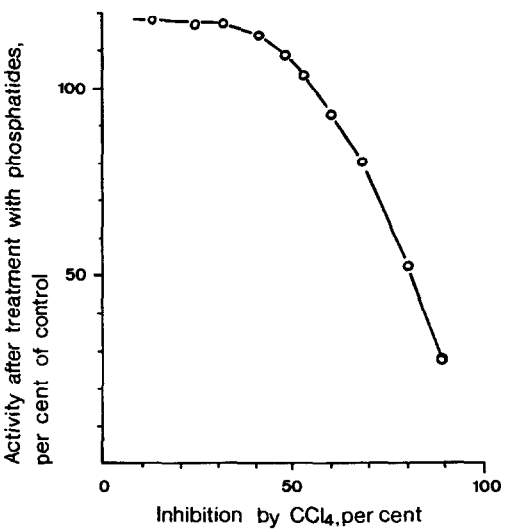


Fig. 2. Reactivation of $CCl_4$-inhibited glucose-6-phosphatase by liver phospholipids. First preincubation: 3--90 min, second preincubation: 30 min. Amount of phosphatides added: 120 μg lipid phosphorus.

membranes through a direct physicochemical action due to the solvent effect of the lipoprotein structure of cytomembranes. Secondly the hepatotoxin may

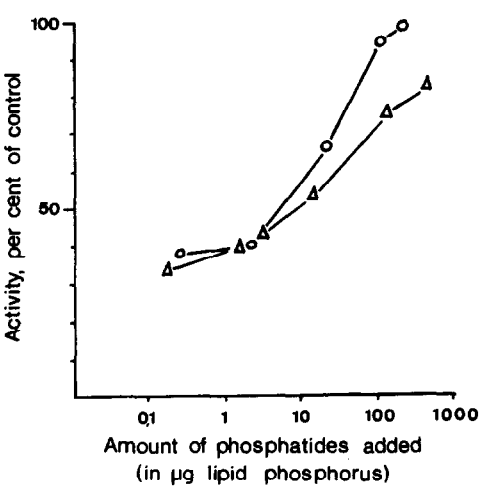


Fig. 3. Reactivation of $CCl_4$ inhibited glucose-6-phosphatase by different amounts of phosphatides added. (○) liver phosphatides, (△) egg lecithin. First preincubation: 15 min, second preincubation: 30 min.

be metabolized to an active form which can bring about further changes in membrane systems.

Our results could be explained by this double mechanism. The complete reactivation of glucose-6-phosphatase by phospholipids (fig. 2, inhibition range 0–50%) can be caused by rediffusion of $CCl_4$ from intoxicated microsomes into the added lipid micelles. This process could be responsible for the reversal of the mere physicochemical action of the haloalkane.

At inhibition values greater than 50% corresponding to longer preincubations the inhibition became partially irreversible (fig. 2). This may be contributed to effects evoked by a $CCl_4$ metabolite which cannot be removed by phosphatide micelles.

Since the described reactivation effect should be independent on the nature of the phosphatides used we checked the effectiveness of egg lecithin suspensions. As shown in fig. 3 this phospholipid was slightly less effective than suspensions of total liver phosphatides. This difference is probably due to another physical state of the lecithin suspensions which also show a greater turbidity than suspensions of phosphatide mixtures. A qualitative distinction between the two phospholipid samples was not to be noticed.

## Acknowledgement

We are indebted to Dr. H.Weiss (Fa. Etapharm, Vienna) for a gift of pig liver phospholipids.

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