# THE EFFECT OF PHOSPHATE ON GLUTATHIONE INDUCED LIPID PEROXIDATION AND SWELLING IN RAT LIVER MITOCHONDRIA

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Both phosphate (Hunter et al., 1959) and glutathione (Lehninger and Schneider, 1959) induce swelling of fresh rat liver mitochondria in dilute suspension as judged by the turbidity or density ($D_{520}$) decrease. The glutathione swelling occurs only if both GSSG and GSH are present (Neubert and Lehninger, 1962) and is intimately associated with lipid peroxide formation (Hoffsten et al., 1962) as judged by the thiobarbituric acid method. Both phosphate and glutathione induced swelling occur in KCl and in sucrose media, but KCl media must be used when the thiobarbituric acid method is to be applied because of interference by sucrose.

When both phosphate and glutathione are present the glutathione induced changes are prevented but the phosphate induced swelling occurs in a normal fashion (Fig. 1). It may be seen that relatively low concentrations of phosphate, such as 0.2 mM, largely prevent glutathione induced lipid peroxide formation and correspondingly shift the $D_{520}$ or swelling curve toward that typical for phosphate alone. One mM phosphate completely prevents the action of glutathione. Phosphate induced swelling appears to be typical in the presence of glutathione with one characteristic change. Any lag period seen with phosphate alone

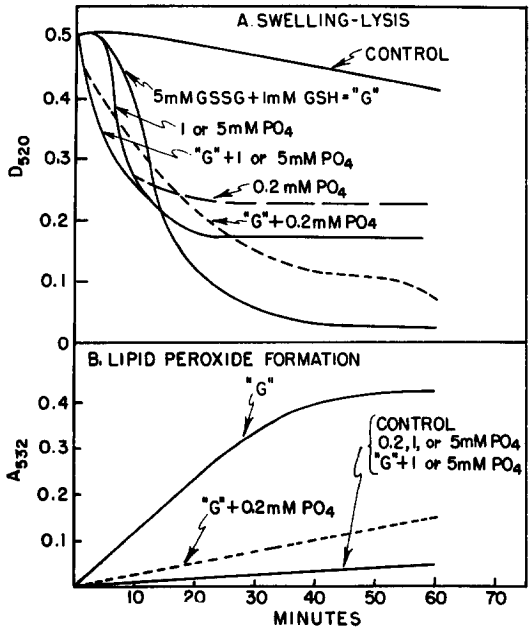


Fig. 1. The effect of various concentrations of phosphate on glutathione induced lipid peroxidation and swelling-lysis. Fresh mitochondria. 0.175 M KCl + 0.025 M Tris buffer pH 7.4 medium, 24°C.

is usually eliminated so that in the presence of glutathione there is a very rapid initiation of swelling by phosphate.

Fig. 2 presents the effect of phosphate added late, during and after the short lag period seen with 5 mM GSSG + 1 mM GSH. The glutathione initiates lipid peroxidation, but as soon as phosphate is added the appearance of lipid peroxide virtually stops. Not only can the reactions leading to lipid peroxide be prevented by phosphate, they are also interrupted by phosphate and no significant lipid peroxide chain reaction continues. When phosphate is added during glutathione induced swelling there is a rapid additional swelling due to the phosphate and some decrease in the extent of glutathione induced swelling-lysis if the phosphate is added early enough.

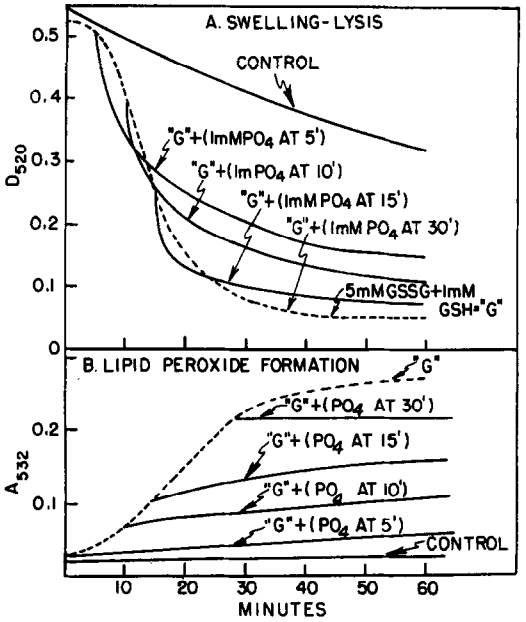


Fig. 2. The effect of 1 mM phosphate added at various times after GSSG + GSH on lipid peroxidation and the course of the swelling curve. Fresh mitochondria, KCl-Tris medium, 24°C. When added, the glutathione mixture was always present from zero time.

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Fig. 3 illustrates an experiment indicating that the protective effect of phosphate is not due to catalyzing the oxidation of GSH to GSSG during the lag period. When GSH is added after GSSG and $PO_4$, there is no swelling of the glutathione type.

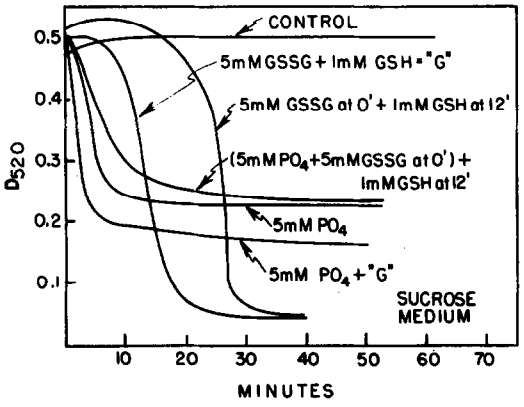


Fig. 3. The effect of adding GSH after the mitochondria have been exposed to GSSG and phosphate for a period of time. Fresh mitochondria. Medium = 0.33 M sucrose + 0.025 M Tris buffer pH 7.4, 24°C.

Arsenate produces effects like phosphate in almost identical concentrations. Sulfate has no effect. Thyroxine at 1 μM partially inhibits and 10 μM completely eliminates the peroxide formation and glutathione induced swelling. Uncoupling agents like DNP do not alter the lipid peroxide formation or glutathione induced phase of the swelling. However, they eliminate any phosphate induced swelling in the presence of glutathione just as they do in its absence.

These effects of relatively low concentrations of phosphate and arsenate raise the question whether the action of glutathione with thiol or disulfide groups at phosphorylation sites in the mitochondrial membrane leads to intermediates which initiate lipid peroxidation under certain conditions, but which are diverted to other pathways when phosphate or arsenate are present. In further investigations on this point serious attention must also be given to the possibility that phosphate and arsenate have a direct antioxidant action themselves or potentiate other antioxidant material in the mitochondria. Added known antioxidants prevent glutathione swelling but do not cause the simultaneous and independent electron transport supported swelling seen with phosphate, arsenate, and thyroxine.

Oxygen consumption measurements with the Beckman micro oxygen electrode indicate that the entire oxygen consumption associated with lipid peroxidation is eliminated by phosphate. This means that phosphate is interrupting the process in its initial stages and not just inhibiting the final steps which lead to formation of malonaldehyde, the substance actually measured by the thiobarbituric acid method.

Investigators dealing with oxidative phosphorylation mechanisms and other enzyme systems must be alert to effects such as described here. In many cases lipids or lipoproteins are in-

volved and glutathione may be present. Depending on the particular situation involved either protective effects or inhibition by phosphate might be seen. If alteration in lipids caused inactivation, then phosphate might preserve activity. If alteration in lipids caused release from a latent or inhibited state, then phosphate might prevent "activation". Only further work can determine whether the reactions which initiate lipid peroxidation or the lipid peroxides *per se* have any physiological role. It is of great significance, however, that Neubert, Wojtczak, and Lehninger (1962) have identified C-factor, which is essential for mitochondrial contraction and improves phosphorylation in submitochondrial particles, as glutathione peroxidase. A distinct selectivity of phosphate relative to glutathione induced changes is seen in the fact that 1 mM phosphate has no effect on ascorbate induced swelling-lysis in sucrose medium, and produces only slight inhibition of ascorbate induced swelling and lipid peroxidation in KCl.

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