EFFECT OF GLUTATHIONE MIXTURES AND OF PHOSPHATE AND ARSENATE ON PEROXIDATION OF UNSATURATED FATTY ACIDS

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The swelling and lysis of rat liver mitochondria induced by mixtures of GSSG and GSH is intimately associated with lipid peroxide formation (Hoffsten et al., 1962). In trying to determine whether lipid peroxide formation is the cause of this type of swelling it is important to know whether the lipid peroxidation is the result of a selective attack through membrane thiol and disulfide groups or a non-specific and general direct attack on membrane lipids. Therefore, we have investigated the effect of glutathione on pure unsaturated fatty acids under the same experimental conditions used in the mitochondrial studies. Lipid peroxides were determined by the thiobarbituric acid method as previously described (Hunter et al., 1963a). Ethyl linolenate and methyl arachidonate were used since the thiobarbituric acid method measures principally malonaldehyde derived from fatty acids with three or more double bonds (Dahle et al., 1962).

As may be seen in Fig. 1, a small amount of lipid peroxide (TBA color material) is formed when a linolenate suspension is exposed to 5 mM GSSG + 1 mM GSH at 25° in a 0.175 M KCl - 0.025 M Tris pH 7.4 medium. Much more lipid peroxidation occurs with arachidonate, so studies with this acid will be discussed in greater detail. The distinction between the fatty acids may be of real significance, as mitochondrial membranes have essentially

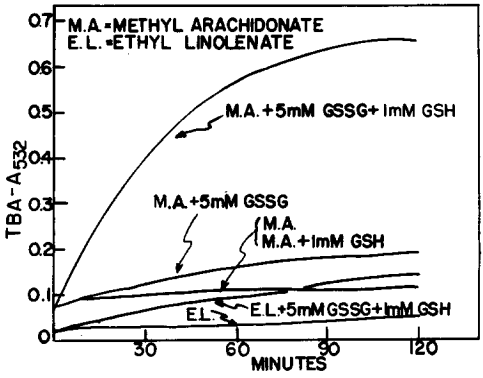

Fig. 1. Lipid peroxidation induced by GSSG + GSH compared with that due to GSSG or GSH alone. 0.3 mM methyl arachidonate or ethyl linolenate suspension, 0.175 M KCl + 0.025 M Tris buffer, pH 7.4 medium, 25°C.

no linolenic acid but do have fairly large amounts of arachidonic and similar acids (Getz et al., 1962, and Richardson et al., 1961).

Neither GSH nor GSSG alone gives much lipid peroxidation over the very small amount observed with arachidonate alone, but the combination of the two produces fairly rapid lipid peroxidation. Thus, peroxidation of pure lipids in the presence of glutathione seems to require the addition of both the oxidized and the reduced forms, just as is the case for swelling and lipid peroxidation in mitochondria.

Since phosphate and arsenate in low concentrations prevent GSSG + GSH induced swelling and lipid peroxidation in rat liver mitochondria (Hunter et al., 1963b), we investigated the effect of these substances in the pure lipid non-mitochondrial system to determine whether non-specific antioxidant actions of phosphate and arsenate were great enough to possibly account for their action in mitochondria. In Fig. 2 it may be seen that 5 mM phosphate completely prevents lipid peroxidation induced by GSSG + GSH. Lower concentrations, such as 1 mM, are almost as effective. In general terms the concentration of phosphate required for inhibition of glutathione induced peroxidation with

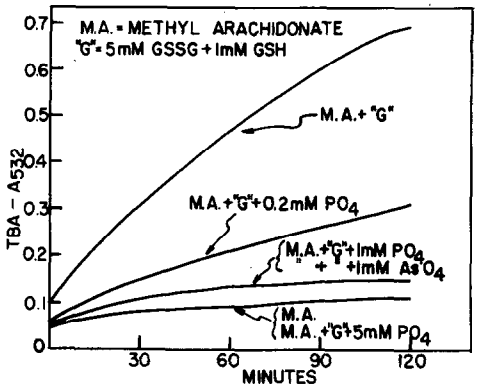

Fig. 2. Inhibition of glutathione induced lipid peroxidation by 0.2 to 5 mM phosphate and by 1 mM arsenate. 0.3 mM methyl arachidonate + suspension. KCl-Tris medium, 25°C.

methyl arachidonate is the same as with mitochondria. Arsenate has effects very similar to phosphate in the same concentrations.

Other observations suggest that there may be some degree of specificity of phosphate inhibition with respect to glutathione initiated lipid peroxidation. "Spontaneous" peroxidation of either linolenate or arachidonate at 60° is delayed but not prevented by PO_4. Low concentrations of ascorbate, which produce a mitochondrial swelling-lysis somewhat like that with glutathione, also induce lipid peroxidation with methyl arachidonate but not with ethyl linolenate under these conditions. Phosphate has little effect on ascorbate induced peroxidation under conditions where it completely prevents GSSG + GSH induced peroxidation. Phosphate slightly inhibits cytochrome C catalyzed peroxidation of linolenate but has little effect in the case of arachidonate. In this regard it is of some interest that a number of studies in the literature on peroxidation in tissue and food materials were actually carried out in 20 to 100 mM phosphate buffer, circumstances under which glutathione induced peroxidation would not be seen. Fe^{++} ion, as expected on the basis of known infor-

mation, initiates peroxidation of arachidonate. This also is inhibited by phosphate, but the complexing of Fe by phosphate may be entirely responsible for this effect.

The mechanism by which a combination of GSSG and GSH act to cause lipid peroxidation is not known. In pure solutions the addition of GSSG considerably increases the rate of oxidation of GSH, so there is the possibility that an intermediate oxidized form or complex is the agent which initiates lipid peroxidation. The fact that phosphate has relatively little effect on the oxidation of GSH in the presence of GSSG suggests that phosphate may react with such an intermediate but does not prevent its formation. However, phosphate may react with the initial lipid peroxide intermediates rather than with glutathione intermediates. Such a reaction might have physiological implications. Another possibility is that these reactions are determined by the presence of Fe in the solutions, such as the GSSG. EDTA at 0.1 mM has little effect on oxygen consumption with GSSG + GSH, but 1 mM partially inhibits. If phosphate were acting solely by complexing Fe, it is hard to understand why ascorbate induced lipid peroxidation is not also affected.

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