# INHIBITION OF MALIC DEHYDROGENASE BY CYCLIC DISULFIDES

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Lipoic acid, a cofactor required for certain α-keto acid dehydrogenases, profoundly affects normal processes in several systems undergoing developmental changes (Runnström, 1956; Machlis, 1957; Ham and Eakin, 1958; Henderson and Eakin, 1959). That this effect is unrelated to any coenzymatic or vitamin-like functions of the compound was demonstrated when it was found that similar cyclic disulfides having little or no nutritional or catalytic activity are nevertheless just as potent inhibitors of regeneration in hydra and planaria as is lipoic acid itself (Henderson and Eakin, 1959; Spangenberg, 1960). In investigating the mechanisms of inhibition of regeneration, it was found (a) that the presence of oxalacetate (but not aspartate or α-ketoglutarate) during the exposure of regenerating systems to cyclic disulfides prevented the latter from arresting regeneration and (b) that these cyclic disulfides would inhibit certain enzymatic activities of planaria homogenates and preparations from mammalian tissues. Further investigation on enzymes related to oxalacetate metabolism showed the DPN-dependent malic dehydrogenase to be unusually sensitive to the cyclic disulfide but not to the reduced (dithiol) derivatives. Hence a detailed

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study has been made of the effect of such compounds on this and other DPN- and TPN-dependent systems using refined preparations in standardized procedures measuring spectral changes resulting from the oxidation or reduction of the coenzymes (Colowick and Kaplan, 1955).

The enzymes can be classified in three groups according to their reaction to added cyclic disulfides:

(A) Relatively insensitive (inhibited less than 25% by $10^{-4}$M disulfide): alcohol dehydrogenase, glutamic dehydrogenase.

(B) Moderately sensitive (50% inhibited by a disulfide concentration between $10^{-4}$ and $10^{-5}$M): glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitric dehydrogenase, lactic dehydrogenase.

(C) Very sensitive (Table I): malic dehydrogenase.

Because malic dehydrogenase is sensitive to a concentration of disulfide only one-hundredth that needed to inhibit the next most sensitive enzyme (glyceraldehyde-3-phosphate dehydrogenase) and on the basis of other observations to be reported in detail (effects of added metallic ions, sulfhydryl compounds, etc. on the inhibitions), we believe the mechanism of action of these disulfides upon the malic dehydrogenase to be unique.

The $C_7$, $C_8$ (lipoic acid) and $C_9$ homologs cause comparable inhibition in refined porcine preparations, but when planaria extracts are used, there is approximately a four-fold increase in the inhibitor activity for each increase of one carbon atom in the side chain. This difference parallels the relative activities of the three homologs in arresting regeneration of planaria, a system in which the effective concentrations are: $C_7$, $8.0 \times 10^{-5}$M; $C_8$, $2.5 \times 10^{-5}$M; and $C_9$, $0.90 \times 10^{-5}$M. Because of this parallelism between the enzymatic and develop-

Table I

Inhibition of Malic Dehydrogenase Activity

by Cyclic Disulfides

| Enzyme Source | Cyclic Disulfide* | Molar Concentration Inhibiting Enzyme 50 Per Cent** |
|---|---|---|
| Refined preparation*** | DL-1,2-dithiolane-3-butyric acid ($C_7$) | $1.5 \times 10^{-7}$ |
| | DL-1,2-dithiolane-3-valeric acid ($C_8$) | $1.5 \times 10^{-7}$ |
| | (+)-1,2-dithiolane-3-valeric acid ($C_8$)**** | $1.5 \times 10^{-7}$ |
| | (-)-1,2-dithiolane-3-valeric acid ($C_8$) | $1.5 \times 10^{-7}$ |
| | DL-1,2-dithiolane-3-caproic acid ($C_9$) | $2.5 \times 10^{-7}$ |
| Acetone powder of planaria homogenate | DL-1,2-dithiolane-3-butyric acid | $10.0 \times 10^{-5}$ |
| | DL-1,2-dithiolane-3-valeric acid | $2.5 \times 10^{-5}$ |
| | DL-1,2-dithiolane-3-caproic acid | $0.7 \times 10^{-5}$ |
| Mouse liver extracts | DL-1,2-dithiolane-3-valeric acid | $4.0 \times 10^{-6}$ |

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**Final concentrations: oxalacetate, $2.5 \times 10^{-4}$ M; DPNH, $5 \times 10^{-5}$ M; and malic dehydrogenase 10 units ($\Delta A_{340\mu m}$/min/unit = 0.010) per 3 ml. cuvette.

Buffer: $\frac{M}{50}$ potassium phosphates, pH 7.4. 25°C.

***A refined preparation from pig heart obtained from the Worthington Biochemical Corporation.

****The biologically active antipode of lipoic acid.

mental systems and because of the counteracting effects of oxalacetate mentioned above, we believe that the basic factor involved in the cyclic disulfides' deleterious *in vivo* effects is the inhibition of malic dehydrogenase.

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