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On the Effect of Organic Mercury Compounds and Copper Salts on Catalase Cyanide

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In connection with studies of different antidotes in cyanide poisoning it became of interest to investigate the action of certain heavy metal compounds on cyanide inhibited heme enzymes. The toxicity of cyanide is attributed to its inhibitory action on cytochrome oxidase, but another heme enzyme, catalase, was used as a model enzyme in these studies, as it in contrast to cytochrome oxidase can be obtained in a pure state. It is of importance in this connection that catalase qualitatively behaves as cytochrome oxidase in its reaction with cyanide, as both the association and dissociation velocity constants are high¹. It has previously been demonstrated by Chance¹ that silver ions generate free catalase from the catalase cyanide complex, due to the fact that silver ions combine with the free cyanide in equilibrium with catalase cyanide. The high toxicity of silver salts, however, apparently prevents their use as antidotes in cyanide poisoning. Incidentally we observed that organic mercury compounds had a high affinity for cyanide and could completely reactivate cyanide inhibited catalase. This suggested that these compounds may have an antidotal effect in

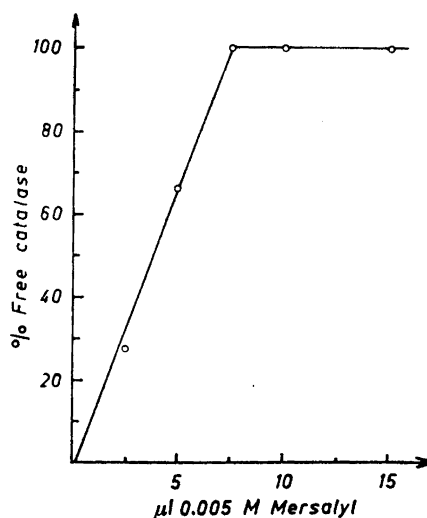


Fig. 1. Effect of Mersalyl on catalase cyanide. Horse blood catalase 0.8×10^{-6} M, cyanide 1×10^{-5} M, phosphate buffer 0.025 M of pH 7.4, final volume 3.0 ml. Indicated amounts of the mercurial added and the free catalase formed was determined spectrophotometrically from the decrease in absorbancy at 425 m μ .

cyanide poisoning. Of special interest were those organic mercury compounds which are used as diuretics, as they might be easily available for use as antidotes. The complete reactivation of cyanide inhibited catalase by one of these diuretics, Mersalyl (Salyrgan), is shown in Fig. 1. Experiments on rabbits² demonstrated, however, that Mersalyl had no antidotal effect in cyanide poisoning. Presumably the mercury compound was bound to sulfhydryl groups in the body before it could react with cyanide. (The mercurials have a very high affinity for sulfhydryl groups and their diuretic action is in fact attributed to an inhibition of sulfhydryl enzymes in the kidney.)

Agner³ reported that cupric salts could be used as antidotes in cyanide poisoning. This was disputed by Walther and Meyer⁴ but Agner's results have been confirmed in connection with the present work⁵. However, when the effect of cupric chloride on catalase cyanide was studied, it was found that even a considerable excess of cupric

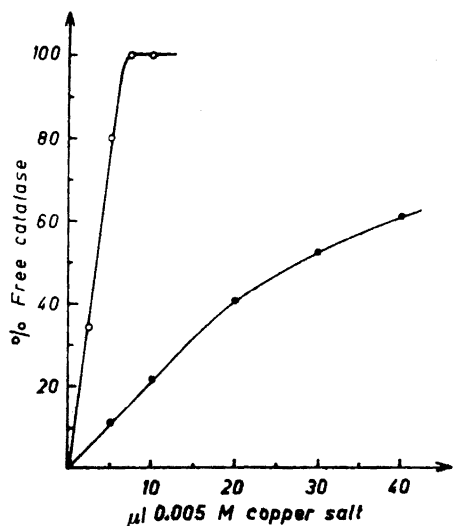


Fig. 2. Effect of cupric and cuprous ions on catalase cyanide. Conditions as in Fig. 1. ○ ○ ○ CuCl₂, ● ● ● CuCl. The latter prepared immediately before use by dissolving the salt (a colorless preparation from Schering-Kahlbaum) in 5% KCl under nitrogen.

ions could not completely reactivate cyanide inhibited catalase, Fig. 2. Cupric chloride was thus inferior to Mersalyl in this respect, although it was effective as an antidote, whereas Mersalyl was not. This discrepancy could be explained when further experiments demonstrated that cuprous ions in contrast to the cupric ions very effectively liberated catalase from catalase cyanide; see Fig. 2. (The antidotal effect of cuprous chloride was not determined, as this compound is only sparingly

soluble and is rapidly oxidized to cupric chloride in the presence of air). If cupric ions could be reduced to the cuprous state in the vicinity of the cyanide inhibited cytochrome oxidase, an efficient reactivation of the latter could be expected. Cupric ions are in fact reduced by ferrocytochrome c⁵ and as cytochrome c is mainly present in the reduced form in cyanide poisoned cells, good possibilities exist for an intracellular reduction of the cupric ions. It is relevant that cupric ions have much less affinity for sulfhydryl groups than cuprous ions or organic mercury compounds^{6,7}. (The reaction between mercaptans and cupric ions involves a reduction of the latter to the cuprous state.) This suggests that cupric ions are not so rapidly bound to sulfhydryl groups in the body as the mercurials and could then better reach the cyanide inhibited cytochrome oxidase in the central nervous system.

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