# Effect of Formaldehyde on Certain Oxidative Enzymes. (18413)

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Although formaldehyde is a highly toxic substance its effect on enzyme systems has apparently not been studied. In order to determine whether it was equally toxic to all enzymes, the oxidation of various compounds by a standard rat liver or kidney homogenate was studied in the presence of small amounts of formaldehyde. The results showed that the succinic acid, choline, sarcosine, and L-proline oxidases were inhibited by formaldehyde in concentrations which were without effect on the cytochrome, amine, and D-amino acid oxidases.

*Experimental.* Liver or kidney homogenates were made by grinding the tissue in a mortar with 0.05 M Na-K-phosphate buffer pH 7.8, 1.0 ml per 0.5 g tissue, and straining through muslin. 0.5 ml of the resultant suspension was placed in each Warburg flask which had a final volume of 2.0 ml made up of buffer and substrate. The latter was added either before or after the formaldehyde in order to see whether it protected the enzyme. Saturation concentrations of each substrate were used. All the enzymes tested have pH optima at or near pH 7.8.

Formaldehyde acts rapidly. Immediately after the addition of 6.2 x $10^{-3}$ M to the liver suspension the rate of oxygen uptake is reduced 40-60%. Even though the homogenate was made in a standard way variations in the percentage inhibition occurred. As a basis for the following comparison the inhibition of the succinoxidase was used. In the presence of the above concentration of formaldehyde added before the substrate the succinoxidase of liver was, in this instance, inhibited 42%, the choline oxidase 75%, L-proline oxidase 74%, sarcosine oxidase 30% and tyramine and cytochrome oxidases 0%. In the kidney when the succinoxidase was inhibited 80% there was no inhibition of the oxidation of D-methionine or D-alanine. When tyramine, D-alanine or p-phenylene diamine were added to the succinic acid, the inhibition of the succinoxidase still occurred which indicated that these compounds were not preventing the formaldehyde from reacting with the respective enzymes.

TABLE I. Effect of 6.2, 9.3, and 12.4 × $10^{-3}$ M Formaldehyde Added 5 Minutes Before and After 4.2 × $10^{-2}$ M Sodium Succinate on the Oxidation of the Latter by Rat Liver Homogenate at pH 7.8, 37°. The succinoxidase activity under these conditions was approximately 100 $\mu$l $O_2$ per 10 min.

| | % inhibition | | | | | |
|---|---|---|---|---|---|---|
| | 6.2 × $10^{-3}$ M formaldehyde | | 9.3 × $10^{-3}$ M formaldehyde | | 12.4 × $10^{-3}$ M formaldehyde | |
| Time, min. | Before succinate | After succinate | Before succinate | After succinate | Before succinate | After succinate |
| 0-5 | 16 | 0 | 34 | 0 | 57 | 0 |
| 10-15 | 14 | 0 | 30 | 10 | 48 | 18 |
| 20-25 | 18 | 6 | 31 | 20 | 47 | 29 |
| 50-60 | 21 | 21 | 35 | 34 | 49 | 42 |

Table I shows the effect of different concentrations of formaldehyde on the succinoxidase of liver and the effect of adding the succinic acid before and after the formaldehyde. If added before, the substrate protects the enzyme for a short time from the formaldehyde. The affinity of the latter for the enzyme is, however, so great that it displaces the succinic acid regardless of the concentration of the latter. Fumaric acid offers no protection. Similar results were obtained with the other enzymes.

In the rat liver, the order of the oxidation rates of the various added compounds was p-phenylene diamine 1, succinic acid 2, choline 3, L-proline 4, tyramine 5, sarcosine 6. In the kidney the succinoxidase was more than twice as active as the D-amino acid oxidase. It is obvious therefore the observed effects of formaldehyde are not correlated with the relative amounts of the enzymes present in the homogenates. Either the amino group of various enzymes have different affinities for formaldehyde or these

groups are not important for the action of the three enzymes which are not affected by the reagent. Since doubling the formaldehyde concentration still does not inhibit these three, it is probable that the latter explanation is correct. Finally it may be mentioned that the formaldehyde inhibition of the succinoxidase is the same at pH 6.0 as it is at pH 8.0.

*Summary.* 1) Formaldehyde inhibits the succinic acid, choline, L-proline and sarcosine oxidases in liver homogenates. In equal concentrations, it has no effect on the amine and cytochrome oxidase of liver or the D-amino acid oxidase of kidney. 2) The prior addition of substrate protects the enzymes from the formaldehyde for a short period of time.

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## Can Experimental Shock be Induced by Coronary Occlusion?* (18414)

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*Objectives of the Experimental Approach.* Of the several hazards of coronary occlusion, the clinical development of a shocklike state has been discussed a good deal in recent years. The clinical inference that this is due to supervention of peripheral circulatory failure has not, however, received valid support. Experimental work appears to favor the conclusion that circulatory failure not attributable to severe irregularity of the heart beat is due successively to defection of useful contractions in the ischemic area, loss of total contractile energy through expansion of the affected area, and failure of the still viable fractions to compensate adequately(1-4). However, such conclusions are based on experiments extending only an hour or so beyond the time of coronary occlusion, after which the animals usually die due to ventricular fibrillation. Thus the most complete dynamic study of changes in intraventricular and aortic pressure pulses, that of Orias(2), stressed chiefly (a) that occlusion of the ramus descendens anterior is followed immediately by hypodynamic beats with shortened systoles, but (b) that these effects are usually compensated within 4 to 7 minutes through increase in initial tension in the left ventricle, which restores aortic and left ventricular pressures to normal levels. This compensation failed to occur in only three of 23 experiments and in these rapid dilation and failure of the heart supervened. The subject has also been studied on survival animals of which the report by Gross and his associates(5) may serve as an illustration. These investigators found, in dogs that survived for a day or more, a decrease in cardiac output, some irregular decline of arterial pressure, and evidence of pulmonary congestion based on a prolonged cyanide time. Since they were unable to establish any reduction in blood volume or fall in peripheral venous pressures they concluded that evidence for peripheral circulatory failure was lacking.

Despite the negative character of previous experimental work it seemed important that another attempt be made to determine whether hemodynamic signs of peripheral circulatory failure develop in animals which have survived occlusion of the ramus descendens anterior for 5 hours or more and in which serious arrhythmia has been prevented or mini-

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