6. GENERAL SUMMARY AND CONCLUSION
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The effect of cadmium on levels of hepatic riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) as well as the activities of flavokinase and FMN-phosphatase were studied following injection of cadmium sulfate solution subcutaneously to male Sprague-Dawley rats at a dose of 0.44 mg/kg body weight, every alternate day for 15 days. The body weights and wet weights of liver were found to increase significantly following cadmium treatment. The levels of hepatic free riboflavin and FAD increased, while the FMN level remained unaltered. A significant decrease in the activities of hepatic flavokinase and FMN-phosphatase were observed both in in vivo and in vitro experiments, although the degree of inhibition was found to be more pronounced in case of flavokinase than FMN-phosphatase.

In order to elucidate how Cd\(^{2+}\) affects flavokinase, the crucial enzyme for flavin coenzyme biosynthesis, further studies were undertaken with a partially purified (dialyzed preparation) as well as a nearly purified preparation of the enzyme. Our investigations reveal that Cd\(^{2+}\), Cu\(^{2+}\) and Hg\(^{2+}\) exhibit a non-linear inhibition of the enzyme with the dialyzed preparation. However, this non-linearity was not observed with the purified preparation.

Similarly, a non-linear activation of flavokinase by ATP was also observed. Although Cd\(^{2+}\) inhibits the reaction,
the pattern of the reaction remain unaffected. Again this non-linearity in kinetic pattern was not observed with the purified preparation.

Zinc (Zn$^{2+}$) has been found to be an activator of flavokinase and the inhibition brought about by Cd$^{2+}$ was reversed by Zn$^{2+}$. Our studies further show that Cd$^{2+}$ competitively inhibits flavokinase. This competition was found to be with both Zn$^{2+}$ and riboflavin. In addition, the enzyme appears to contain essential, accessible and functional thiol group for activity and that Cd$^{2+}$ probably interacts with the thiol group at or near the active centre to decrease the enzyme activity. However, thiol protectors like glutathione and dithiothreitol reversed the inhibition brought about by the metal.

Thus, from the present study it can be concluded that

(1) the subcutaneous administration of Cd$^{2+}$ in the present dose interferes with hepatic riboflavin metabolism as is evident from altered hepatic flavin levels,

(2) this alteration appears to result from the inhibitory effect of the metal on two of the enzymes studied concerning riboflavin metabolism viz. flavokinase and FMN-phosphatase,

(3) the results of in vitro experiments strengthen the in vivo observations regarding the effects of Cd$^{2+}$,

(4) Zinc (Zn$^{2+}$) is an activator of flavokinase,
(5) Cadmium (Cd\(^{2+}\)) appears to compete with Zn\(^{2+}\) and riboflavin for binding with the same position on the active site of the enzyme molecule,

(6) Zinc (Zn\(^{2+}\)) reverses the inhibition of the enzyme brought about by Cd\(^{2+}\),

(7) sulfhydryl groups appear to be essential for flavokinase activity, and

(8) Cadmium and other divalent cations such as Cu\(^{2+}\) and Hg\(^{2+}\) inhibit flavokinase activity probably by interacting with the thiol group at or near the active site of the enzyme.