Oxalacetate Decarboxylation by Iron and Copper

THE catalysis of oxalacetate decarboxylation by metallic ions constitutes a simple 'model' system, the properties of which may throw some light on the biological decarboxylation mechanisms. Kornberg, Ochoa and Mehler[1] have described the formation of an unstable complex between oxalacetate and aluminium ions, which possesses a characteristic ultra-violet absorption spectrum. Recent studies from this laboratory[2] have shown that iron and copper differ in many respects as regards their ability to accelerate the decomposition of oxalacetate.

The effects of iron and copper were therefore examined in the light of their ability to form complexes such as that with aluminium. A Beckman Model *DU* quartz spectrophotometer was used, measurements being made at room temperature in 1-cm. cells. The 100 per cent transmission blanks contained all the constituents of the experimental cells except oxalacetate.

The ultra-violet spectrum of oxalacetate depends intimately on *p*H. The light-absorbing constituent (that is, either the enol tautomer or possibly the oxalacetate ion) increases with increasing *p*H. It might be expected, therefore, that formation of complexes involving this form (as suggested to occur for aluminium ions by Ochoa's team) would be maximal at high *p*H values. This is found to be the case with cupric ions, *but not with ferric ions.*

In unbuffered solutions, the absorption spectra of the ferric and cupric complexes resemble each other closely, both possessing very marked bands with maxima at 270 mμ. The ferric complex, however, shows an additional faint band extending from 355 to 375 mμ. The copper complex decomposes much more readily than that of iron when the same metal concentration is used. Whereas the copper complex forms almost spontaneously (that is, in less than 30 sec.) at acid *p*H, the iron complex formation reaches a maximum only after 1–5 minutes (depending on the oxalacetate to ferric iron ratio).

Ferrous iron did *not* cause formation of a complex resembling the above two. This is unexpected, as manometrically ferrous iron has been shown to be an active oxalacetate-decomposing agent[3]. However, since that work was carried out at 38° C. in acetate buffer, and a strong oxygen uptake was observed, it may be assumed that conversion to ferric iron preceded the decomposition of oxalacetate. Results of the spectrophotometric examination suggest that ferrous iron as such may not be a catalyst for the decarboxylation of oxalacetate.

The effect of *p*H on the rates of formation and decomposition of the two metal-oxalacetate complexes shows that, in the case of iron, formation of the complex is maximal at the lowest *p*H. The decomposition-rate does not vary greatly from *p*H 1·1 to *p*H 3·2, but is much slower at *p*H 4·5. A steady rise in optical density at and above *p*H 6 was so unusual that the spectrum produced under those conditions was re-examined. It was found that the absorption peak at 270 mμ *was no longer formed*, and that the whole spectrum resembled that of oxalacetate alone. However, in the presence of ferric iron, the whole spectrum was of higher density. Thus, the effect of ferric iron at and above *p*H 6 seems to be to increase the amount of the light-absorbing form of oxalacetate without forming a complex.

In the case of copper, complex formation increases with increasing *p*H, and thus appears to be dependent on the amount of enol-oxalacetate or oxalacetate ions present. The decomposition-rate appeared to be maximal between *p*H 3·5 and *p*H 4·5, decreasing sharply on either side of that range.

These results confirm the differences between cuprous and ferric ions observed in the manometric work, and show once again the intimate connexion between catalytic effectiveness and *p*H. The investigation is being extended to the effect of buffers, and to the blood catalysis. Full details of this work will appear in the *Australian Journal of Experimental Biology and Medical Science.*

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This work was carried out as part of the programme towards elucidating the mechanism of oxalacetate decarboxylation by blood[4,3,5].

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Effect of Acridine Compounds on the Respiration of the Brain of the Six-Day Chick Embryo

IN relating the effect of certain acridines on mitosis to that on respiration, the inhibition of the oxygen consumption of the isolated brain of the six-day chick embryo by these compounds has been measured. It is thought that a note on these results would be of interest as a comparison with the results of Albert and Marshall[1] on chick red cells infected with *Plasmodium gallinaceum.*

Compound	Percentage inhibition of respiration of isolated chick brain at concentration of :			Concentration producing pycnosis of dividing cells
	1 : 12,000	1 : 6,000	1 : 3,000	
5-Aminoacridine hydrochloride	14	31	45	1 : 125,000
5-Amino-1-phenyl-10-methylacridinium bromide	nil	nil	nil	1 : 55,000
5-Amino-10-methylacridinium bromide	nil	nil	nil	1 : 140,000
5-Amino-2-trifluoromethylacridine hydrochloride	68	100	100	1 : 110,000

These results were obtained by a modification of the Cartesian diver micromanometer, using buffered Tyrode solution as a medium, and are interpolations from curves relating inhibition of oxygen consumption to concentration.

Exposure of the isolated brain tissue to the acridine compounds resulted, within two hours, in swelling and fusion of the chromosomes in dividing cells (pycnosis). The concentrations producing this change are recorded in the table; at such concentrations there was no measurable inhibition of respiration.

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