TABLE I

THE EFFECT OF DISINTEGRATION PERIOD ON THE DISTRIBUTION OF FUMARASE AND ACONITASE IN CELL-FREE YEAST EXTRACTS

Activities measured at 240 mμ in a Unicam SP. 500 spectrophotometer: Enzyme, phosphate buffer pH 7.0 (10^{-2} *M* final) and L-malate or citrate (10^{-2} *M* final) in quartz cells, final vol. 3.0 ml. Measurements at about 20°. Blanks do not contain substrate. Specific activity = change of optical density/mg dry wt./min × 1000.

Disintegration period (sec)	*Enzyme*	*Specific activities of fractionated extracts*				
		Whole extract	*Heavy granules*	*Light granules*	*Washings of heavy granules*	*Washings of light granules*
10	Fumarase	110	130	160	70	85
30	Fumarase	120	36	54	88	121
90	Fumarase	129	5	4	86	120
10	Aconitase	40	17	25	16	15
30	Aconitase	42	9	14	34	40
90	Aconitase	54	2	1	22	21

granules, which some authors have called mitochondria[5,6,7,8]. This would be in accordance with the well-known distribution in animal cells of enzymes concerned with respiration.

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MECHANISM OF HYDROGEN TRANSFER IN MODEL DEHYDROGENASE SYSTEMS

by

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It is well known that in spite of the low redox potential of coenzyme I[1], the reduced coenzyme shows very little tendency to autoxidise in the absence of intermediaries. Although the ΔF of the reaction is very favourable there appears to be an equally important factor preventing the interaction

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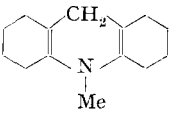
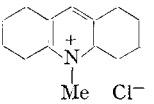
of the two systems and this must be sought in the mechanism of oxidation of dihydro-coenzyme I. This mechanism apparently permits the reduction of a flavin coenzyme, but not of oxygen itself.

In order to throw some light upon the mechanism of hydrogen transfer, the redox properties of two model substances have been studied. Nicotinamide methochloride has been electrolytically reduced and the properties of the product shown to resemble those of dihydro-coenzyme I in its slow reactivity to oxygen. The reduced model was found capable of reducing most other systems (dyes, I_2, Fe^{3+}, and $K_3Fe(CN)_6$) with a higher E_0'. The E_0' of the nicotinamide methochloride was found by potentiometric titration to be -0.36 ± 0.02 V against N.H.E at pH 9.1 and 30° C. In spite of the very different group attached to the heterocyclic N atom, this value is close to that for coenzyme I (—0.32 V at pH 7).

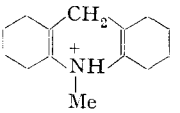
The second model examined was N-methylacridan [I] which was produced by reduction of N-methylacidinium chloride [II]



[I]



[II]



[III]

Unlike the nicotinamide model, where both *o*- and *p*-dihydro compounds can be produced, there is only one possible reduction product in this case and its E_0' was found to lie in roughly the same low range. The kinetics of oxidation of N-methylacridan by 2:6-dichlorophenolindophenol have been followed and found to proceed by way of the conjugate acid [III]. The first step in the oxidation of [III] is unlikely to involve the removal of an electron since the energy requirements to produce a double positive charge on the molecule would be prohibitive. A hydrogen atom transfer step is therefore more probable and this may well account for the very slow reaction of N-methylacridan (and its structural analogues dihydro-methylnicotinamide and dihydro-coenzyme I) with molecular oxygen. Since on the one hand oxygen reduced preferentially by an electron transfer mechanism[2] and on the other the reduced coenzyme may require the removal of a neutral hydrogen atom, there is no common mechanism by which the two systems may interact.

On this interpretation, the function of the flavin-type coenzymes would be to act as mediators, accepting the hydrogen atom from the dihydro-coenzyme and passing on an electron to cytochrome *c* in which the $Fe^{3+} \rightleftharpoons Fe^{2+}$ transformation may be effected by electron transfer. The components of the respiratory chain would then be graded with respect to chemical mechanism as well as E_0'. The concept of a hydrogen atom transfer between coenzyme I and substrate is supported by the results of WESTHEIMER, FISHER, CONN AND VENNESLAND[3] who have presented conclusive evidence of a "direct stereochemically specific transfer" of deuterium atoms. MACKINNON AND WATERS[4] have postulated a cyclic 3-step mechanism for the enzymic reduction of substrates such as ethanol, suggesting that the reduction is initiated by a radical such as R–S. Although the second stage in the reduction of the coenzyme involves a hydrogen atom transfer, the first step involves electron transfer and the scheme does not therefore appear to be compatible with ours in its present form.

It is hoped to publish a more detailed account of this work in the Australian Journal of Chemistry.

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