An Hypothesis for Oxidative Phosphorylation [1,2]

The myriad of obscure chemical processes whereby Pi eventually combines with ADP to yield ATP concomitant with the oxidation of reduced respiratory components such as diphosphopyridine nucleotide, flavoprotein and cytochrome have been encompassed by the term *oxidative phosphorylation*[3]. The observed P/O ratios have been idealized as 3, 2, and 1 and are related to the positions in the respiratory chain occupied by the starting reduced coenzyme and the final oxidizing agent[4]. The experimental data has frequently been summarized in a symbolic fashion as the intermediates are as yet unknown. In this paper a working hypothesis based on chemical principles will be proposed.

The 1, 4 addition of Pi (acting as a nucleophile) to an unsaturated carbonyl system, followed by dehydrogenation would yield the moiety $H_2O_3P\text{–}\vec{O}\text{–}\vec{C}\!=\!\vec{C}\text{–}C\!=\!O$ in which phosphorus is susceptible to nucleophilic attack. Such susceptibility is a requisite for a phosphorylating agent[5]. This sequence is reminiscent of the 1, 2 addition of Pi to the carbonyl group followed by dehydrogenation which occurs in the transformation of 3-phosphoglyceraldehyde to the effective phosphorylating agent 1, 3-diphosphoglyceric acid[6].

The past[7] and current[8] emphasis on the participation of quinones in cellular respiration suggested the selection of the vinyl carbonyl system of a quinone to be part of the mechanism of oxidative phosphorylation. The mechanism (Fig.) has been orientated to start with DPNH, as is

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[2] The abbreviations used are DPN diphosphopyridine nucleotide, DPNH reduced diphosphopyridine nucleotide, ATP Adenosine triphosphate, ADP Adenosine diphosphate, Pi orthophosphate, P/O ratio of atoms of phosphorous and oxygen coupled through oxidative phosphorylation, DNP 2,4-dinitrophenol, E enzyme.

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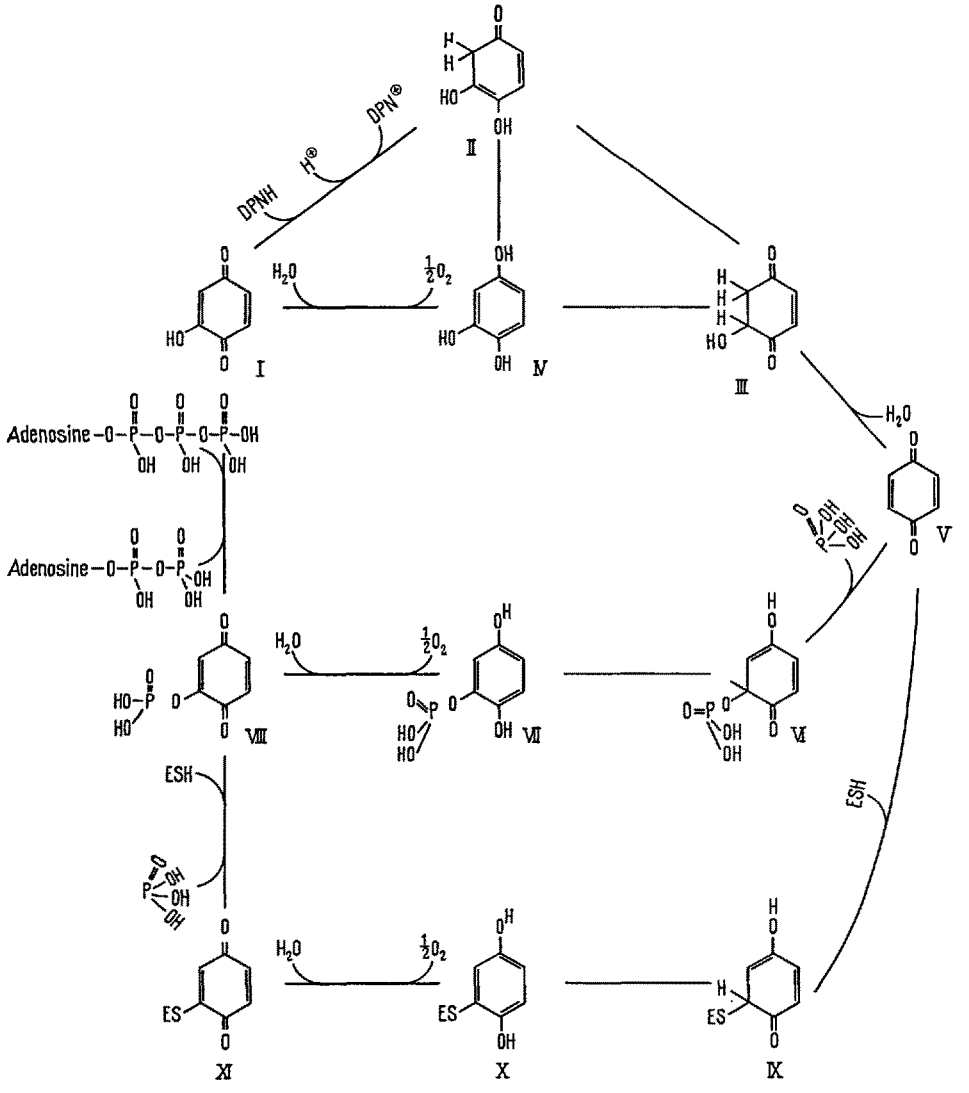
[6] Ref. [3], p. 325.

[7] L. F. FIESER and M. FIESER, *Organic Chemistry*, 3rd ed. (Reinhold Publishing Corporation, New York 1956), p. 722.

[8] For many of the references in this area see: A. F. BRODIE and J. BALLANTINE, J. biol. Chem. *235*, 232 (1960). – D. E. GREEN and R. L. LESTER, Fed. Proc. *18*, 987 (1959). – C. MARTIUS, *Ciba Foundation Symposium on the Regulation of Cell Metabolism* (J. O. Churchill, London), p. 194.



DPNH and ½ O_2 represent appropriate reduced and oxidized portions of the respiratory chain.

customary in such discussions. The rationalization will begin, however, with Pi acting as a nucleophile.

The transformation of V to VI is the recognized 1, 4 addition of an acid to a quinone[9]. The hydroquinone VII, a tautomer of VI, is then oxidized to the quinone VIII. In VIII phosphorus is susceptible to nucleophilic attack. This structure is analogous to known metabolic phosphorylating agents since VIII may be considered as either an enol phosphate or a mixed anhydride of phosphoric acid with a vinylogue of a carboxylic acid. The structure I is obtained as a consequence of the phosphorylation of ADP. As I converted to V (presumably through tautomers such as II and III) the hydroxyl group is eliminated by a sequence of reduction and dehydration. This type of reaction and tautomerism has recently been described and discussed[10-12]. Structures II and III are reasonable intermediates, since DPNH may be considered as the source of the hydride anion, which behaves as a nucleophile and participates in 1, 4 addition to I[13,14].

The hydroxyhydroquinone IV, which is tautomeric with II and III, is an alternative reduction product of I. Reoxidation of IV would yield I. The sequence I, II, III, V, VI, VII, VIII, I is a cyclic mechanism which accounts for oxidative phosphorylation with a P/O ratio of 1[15]. The sequence I, II or III, IV, I involves oxygen uptake without concomitant uptake of Pi. The enhanced formation of the tautomer IV from II or III could account for the uncoupling of oxidative phosphorylation by an agent such as DNP[16]. In view of the participation of enzyme sulphhydryl groups during the conversion of 3-phosphoglyceraldehyde to 1, 3-diphosphoglyceric acid[17] the transformation of V to VIII may be mediated through IX, X, and XI rather than through VI and VII.

The nature and degree of substituents and number of aromatic rings markedly influence: (a) the oxidation-reduction potential of a quinone[18], (b) the keto-enol tautomerism of a hydroxyhydroquinone[12], (c) the ease of the reductive elimination of a hydroxyl group from a hydroxyquinone[12]. The existence of semiquinones[19] permit the various oxidation-reduction steps outlined in the Figure to take place either through a single two electron transfer or through a sequence of two transfers, each of one electron. Thus appropriate quinones may mesh with various oxidation-reduction processes in the respiratory chain including those involving free radicles[20,21].

Some striking agreement with the hypothesis may be found in the experimental literature. COHEN[22] and BOYER[23] demonstrated that an oxygen atom from Pi ended up in water during oxidative phosphorylation. In the proposed scheme ADP is a nucleophile acting on VIII, hence one of the oxygen atoms derived from Pi remains with 1 and ends up in water. BOYER's symbolism[23] which summarizes much data, may be related to the above structures as follows: Y is V, $YOPO_3^=$ is VII, $Y'O \sim PO_3^=$ is VIII, Y'OH is I. BOYER also reasoned 'if DNP acts by preventing the initial phosphate uptake then its action is in some manner to allow dissipation of the energy of oxidation prior to formation of a compound or state that otherwise would react with Pi'. The proposal that DNP facilitates the conversion of II or III to IV conforms with this view. The formation of V is consistent with the data which PINCHOT[24] obtained with a bacterial particulate system. Subsequent to the action of DPNH PINCHOT found a soluble acceptor of Pi whose acceptor activity was not diminished by DNP. The inclusion of DNP during the treatment with DPNH yielded variable amounts of the phosphate acceptor. The inhibition of electron transport by the lack of ADP and Pi is in agreement with the hypothesis[25]. The isolation from natural sources of the 1, 4-diketo tautomer of 1, 4, 5-trihydroxy-7-methylnaphthalene[26-28] supports the significance ascribed to tautomerism in the hypothetical scheme.

The mechanism, suggests the search for quinones and vinyl carbonyl compounds unsubstituted in a distal vinyl position. In view of the lability of substituents on a quinone nucleus, especially under oxidizing conditions[29,30], this mechanism, with suitable modification, may be used to rationalize the mode of action of substituted quinones e.g. coenzyme Q_{10} and compounds such as vitamins K and E and dicoumarol. The isolation of hydroxyquinones from organisms with the capacity for rapid metabolism e.g., parasitic fungi, seeds and eggs[31] and also as a metabolite from a carcinogenic polynuclear hydrocarbon[32] is of cogent interest.

Résumé. Présentation d'une hypothèse pour la phosphorylation oxidative qui comprend des substances vinyl carbonyl, suivie d'une esquisse d'un système cyclique utilisant des quinones, hydroxyquinones, hydroxy-hydroquinones et leurs dérivés phosphorilatés.

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