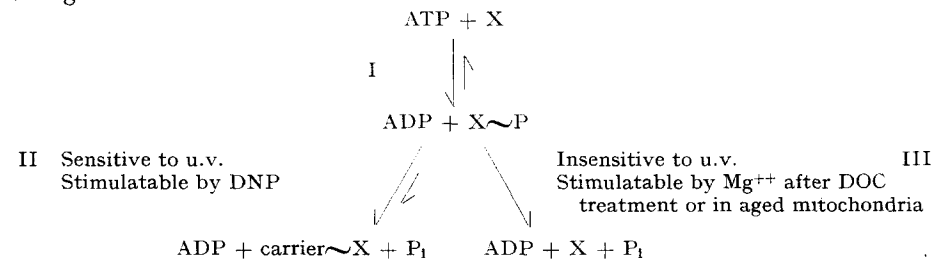
Evidence in support of two adenosine triphosphatase pathways in rat-liver mitochondria

The question as to the number of ATPases which are active, or may be activated, in rat-liver mitochondria is as yet an open question (*cf.* ref.[1,2]). We have recently obtained evidence[3] which may be interpreted to indicate the existence of two distinct pathways in the sequence:

$$ATP \longrightarrow ADP + P_i$$

Fresh mitochondrial suspensions were prepared and irradiated* as previously described[4] in either 0.25 *M* sucrose or 0.25 *M* sucrose–0.01 *M* Tris–0.001 *M* EDTA, pH 7.4. Control mitochondrial suspensions in pyrex tubes were removed from the irradiator with the last irradiation sample. Incubations were performed in 25-ml Erlenmeyer flasks at 30° for 20 min. Each flask contained 50 mequiv. rat-liver mitochondria, 0.05 *M* Tris, 1 m*M* ATP, 0.25 *M* sucrose; when added, 0.1 % DOC, 4 m*M* Mg^{++}, and 0.1 m*M* DNP. The final volume was 2 ml. Release of P_i from ATP was measured according to LINDBERG AND ERNSTER[5].

Fig. 1 shows the effect of varying times of irradiation with far-u.v. light on DNP-stimulatable ATPase and Mg^{++}-stimulatable ATPase, the latter following DOC treatment. DNP-stimulatable ATPase is readily inactivated by far-u.v. light, while, after DOC treatment, Mg^{++}-stimulatable ATPase is resistant to such inactivation. Identical results have been obtained whether mitochondria are prepared in 0.25 *M* sucrose or in sucrose containing Tris and EDTA (a Mg^{++} chelator), indicating that Mg^{++} is not a limiting factor in the DNP-stimulatable ATPase. Modifying WADKINS AND LEHNINGER'S[6] scheme for the transphosphorylative chain, we may make the following notations:



In this scheme fresh mitochondria, capable of catalyzing electron-transport phosphorylation of ADP, would carry out reactions I and II. Aged or DOC-treated mitochondria would have a lesion at reaction II and would transfer phosphate from X~P to water via reaction III. Reaction II, which would be coupled to electron transport in a phosphorylating system, would be blocked by far-u.v. light. The u.v.-sensitive site may possibly be a quinone acting in an energy-conservation role[7,8]. These data would support the view that two pathways for the transfer of phosphate from ATP to water exist, one presumably involved in oxidative phosphorylation, and

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; P_i, inorganic orthophosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate; DOC, deoxycholate; DNP, 2,4-dinitrophenol; mequiv., mitochondria derived from 1 mg of fresh rat liver.

* An error appeared in ref. [4], p. 433, concerning the value of lamp emission. The correct value is 62 ergs/cm²/sec.

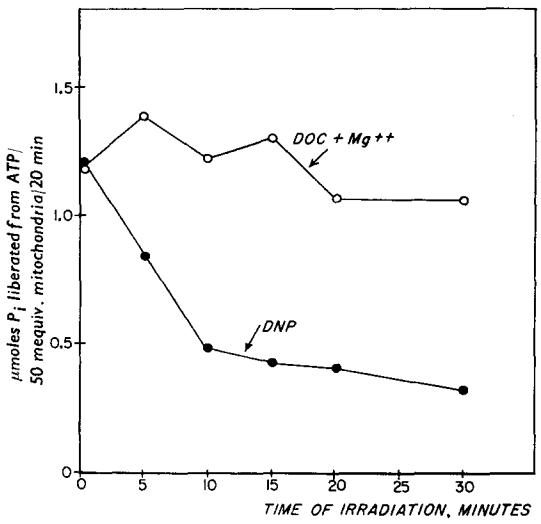


Fig. 1.

the other brought about by dislocation of components due to aging or DOC treatment. The latter pathway, involving reaction III, would presumably be irreversible.

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Photooxidation of cytochrome c by illuminated chromatophores of *Rhodospirillum rubrum* under anaerobic conditions

The photosynthetic process in bacteria is thought to involve the simultaneous production of an oxidizing and reducing power in the bacterial chromatophore under the the influence of light. This oxidizing and reducing power would result from the shifting of electrons in the organized pigment system of the chromatophore, and would be expressed in the bacterial cell as cellular oxidation–reduction reactions. It has been shown by the experiments of VERNON[1] and more clearly by the work of FRENKEL[2] that