2) The reduced phosphoryl-FAD is reoxidized by the subsequent carrier along the respiratory chain, with the simultaneous transfer of the phosphoryl group to ADP:

2. Löw, H., Siekevitz, P., Ernster, L. and Lindberg, O. *Biochim. et Biophys. Acta. In press.*
3. Grabe, B. *Exptl. Cell Research* **13** (1957) 588.

Reaction (1) is dependent on the spatial arrangement of the reactants. It requires that the pyridine ring of DPNH and the isoalloxazine ring of FAD be oriented in two parallel planes close enough to be bridged over by a phosphate ion, the latter being an obligatory component of the reaction; one of the OH-groups of the phosphate may be attracted by the amide group of DPNH.

Reaction (1) is thought to be sensitive to amyltal*, which blocks both electron transfer and the concomitant attachment of phosphate, and to DNP, which interferes with the attachment of phosphate in the left-to-right reaction, thus rendering the electron transfer uncoupled from, and independent of, the activation of inorganic phosphate.

By assuming that Reaction (1) is more rapid than Reaction (2) in the intact mitochondria the proposed mechanism can be visualized to account for the known⁵ discrepancy between the rates of $\text{H}_2\text{O}-\text{P}^{18}\text{O}_4$ and $^{32}\text{PO}_4\text{-ATP}$ exchange during oxidative phosphorylation.

The possibility to generalize the present type of mechanism to be valid also for the two other instances of respiratory chain phosphorylation will be discussed.

1. Siekevitz, P., Löw, H., Ernster, L. and Lindberg, O. *Biochim. et Biophys. Acta. In press.*

* 5-Ethyl-5-isoamylbarbituric acid.

4. Grabe, B. *Exptl. Cell Research. In press.*
5. Boyer, P. D., Falcone, A. B. and Harrison, W. H. *Nature* **174** (1954) 401.

Toxic Liver Injury: Some Biochemical Changes in the Liver caused by Dimethyl- nitrosamine

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Dimethylnitrosamine (N-nitrosodimethylamine) is used industrially as a solvent and as an intermediate in synthetic processes. It has been shown to cause acute haemorrhagic centrilobular necrosis of the liver in the rat and other laboratory animals when administered in doses of the order of 25 mg pr kg body weight¹. The acute liver lesions may be accompanied by bleeding into the gut and haemorrhagic peritoneal effusion, but there is little evidence of damage to other tissues and organs.

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When dimethylnitrosamine was added to the normal diet of rats at a level of 50 parts per million a high incidence of malignant tumours of the liver was found between the 26th and 40th weeks². Dimethylnitrosamine is a useful agent for the experimental study of toxic liver injury because its acute effects are regular and reproducible and also it can be readily estimated in tissues and body fluids by the polarographic method of Heath and Jarvis³. The compound is rapidly metabolised *in vivo*, mainly, and perhaps exclusively by the liver^{4,5}. It is distributed almost uniformly in the different tissues and organs with no preferential accumulation in the liver, and there is evidence that it becomes uniformly distributed in the body water soon after administration⁵. Metabolism occurs *in vitro* by liver slices and broken-cell preparations, but not to a significant extent with other tissues with the possible exception of kidney. There is a strong requirement for oxygen and complete inhibition by anaerobiosis. Metabolic activity of broken-cell preparations can be removed by dialysis and partially restored by the addition of TPN and to a less extent DPN. All the activity occurs in the microsome + cell sap fraction of the liver with negligible metabolism by the nuclear + mitochondrial fraction⁶.

Incorporation of labelled amino acids into rat liver protein *in vivo* is markedly reduced 3 h after the animal has received a necrotising dose of dimethylnitrosamine but incorporation into kidney and spleen protein is not altered significantly nor is the labelling of the free amino acids in the liver. Incorporation of ³²P into acid-soluble, lipid and residual phosphorus fractions of the liver is not significantly changed 6 h after the same dose of dimethylnitrosamine, but incorporation into a partially purified RNA fraction is reduced at this time⁷.

Some histochemical and biochemical changes found in the liver during the development of the necrosis will be described, also some recent work on the metabolism of ¹⁴C-dimethylnitrosamine and on the effect of the compound on rat liver slices *in vitro*.

1. Barnes, J. M. and Magee, P. N. *Brit. J. Ind. Med.* **11** (1954) 167.
2. Magee, P. N. and Barnes, J. M. *Brit. J. Cancer* **10** (1956) 114.
3. Heath, D. F. and Jarvis, J. A. E. *Analyst.* **80** (1955) 613.
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5. Magee, P. N. *Biochem. J.* **64** (1956) 676.
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Structural Factors Involved in the Diaphorase and Cytochrome c-Reductase Activities of Mitochondria and Microsomes

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As indicated by previous studies^{1,2}, the low activity at which TPNH* is oxidized by way of the cytochrome system may constitute a limiting factor for the function of TPN-dependent oxidations in liver tissue. The great discrepancy in activity, known to prevail between the liver TPNH- and DPNH-cytochrome c reductase systems³, and recent evidence reported in the literature^{4,5} that the former may be selectively regulated by metabolic control mechanisms, prompted a comparative study of the two systems with respect to their catalytic components. Interest was in the first place focused on the diaphorase components, since these are believed to constitute the first catalytic links of the cytochrome c-reductase systems with the reduced pyridine nucleotides.

While the TPNH- and DPNH-cytochrome c reductases are located exclusively in the particulate elements of the cytoplasm^{3,6,7}, recent studies⁸ revealed the occurrence of a considerable diaphorase activity in the soluble cytoplasm. This activity was found to be equally high with DPNH and TPNH, in contrast to those present in mitochondria and microsomes, which are 3—7 times higher with DPNH than with TPNH. Since there are indications that the TPNH- and DPNH-diaphorase activities of the soluble cytoplasm are catalyzed by one common enzyme⁸ it appeared to be of interest to investigate whether the same could be valid for the particulate DPNH- and TPNH diaphorases, their discrepancy in activity being due to secondary factors, connected with the organized nature of the structures in which they are located. The data collected in Tables 1 and 2 seem to support this concept.

As emerges from Table 1, precipitation of a suspension of mitochondria or microsomes with acetone resulted in a selective stimulation of the TPNH-diaphorase activity, with a shift of the TPNH/DPNH activity ratio towards unity. A similar effect ensued if mitochondria or microsomes were exposed to a hypotonic

* The symbols, DPNH and TPNH, stand for the reduced forms of di- and triphosphopyridine nucleotide, respectively.