soluble cytoplasmic components have to be included in the system [1]. It has been shown previously that microsomes, isolated from regenerating rat liver, work more effectively in incorporation systems of this kind than normal microsomes [2,3]. The present report is concerned with the effects of regenerative growth induction on the soluble components of the incorporation system. As is well known, these components are required for the initial activation of the labeled amino acids at the expense of high-energy phosphates [4]. The complete soluble system used in these experiments contained the following essential components: (1) a mechanism for ATP-generation (in the present experiments composed of phosphoenolpyruvate, pyruvate kinase and adenylate kinase), (2) guanosine triphosphate [5], (3) amino acid activating enzymes, associated in an hitherto unknown way with soluble RNA.

With standard amounts of liver microsomes as acceptor for activated amino acids, cell fluid from regenerating livers effected, per mg protein, a more rapid incorporation of ^{14}C-L-leucine into protein than cell fluid from normal liver. The increase was preceded by a lag period of 12—14 h and culminated about 30 h after hepatectomy. As a rule, the activity then was 60—80 % higher in the case of regenerating livers.

In order to elucidate which of the components of the activation chain were affected by the regeneration, some of these components were studied separately. The higher efficiency of the cell fluid from regenerating liver was not due to increased contents of phosphoenolpyruvate, ATP or guanosine triphosphate, since these compounds were added separately to the incorporation system in adequate amounts. The concentrations of pyruvate kinase and adenylate kinase were not significantly higher in the preparations from regenerating liver. In several experiments, moreover, these enzymes (prepared from rabbit muscle) were added in excess to the incubation samples without influencing the regeneration effects. The amounts of amino acid activating enzymes were determined in dialyzed cell fluid from normal and regenerating livers by measuring the rate of ^{32}P-exchange between ^{32}P-pyrophosphate and ATP in the presence of L-leucine or an amino acid mixture. An increase of nearly 40 % was observed in the regenerating livers. The RNA-content showed a slight increase at the same time.

It is concluded, therefore, that the higher efficiency of the amino acid activating system, observed in regenerating liver, was primarily due to a higher activity of the amino acid activating enzymes proper.

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The Initial Reactions Involved in Respiratory Chain Phosphorylations

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Recent studies have indicated that the mitochondrial PO_4-ATP ** exchange and DNP-activated ATPase reactions involve a reversible electron transport through the diaphorase-FAD of the respiratory chain [1,2]. On the basis of these conclusions and of certain quantum-chemical considerations [3,4] pointing to a possible participation of phosphorylated quinoid structures as intermediates in respiratory chain phosphorylations the following reaction sequence appears to be a satisfactory formulation of the mechanism of the first of the three phosphorylations occurring along the mitochondrial oxidation of DPNH by oxygen:

1) DPNH is oxidized by the diaphorase-FAD with the simultaneous cleavage of one of the P—O bonds of a molecule of inorganic phosphate and the attachment of the phosphoryl radical to the 2-keto group of the *iso*-alloxazine ring:

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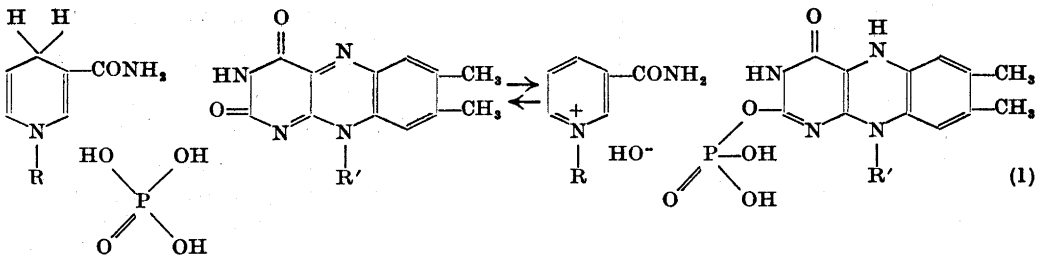
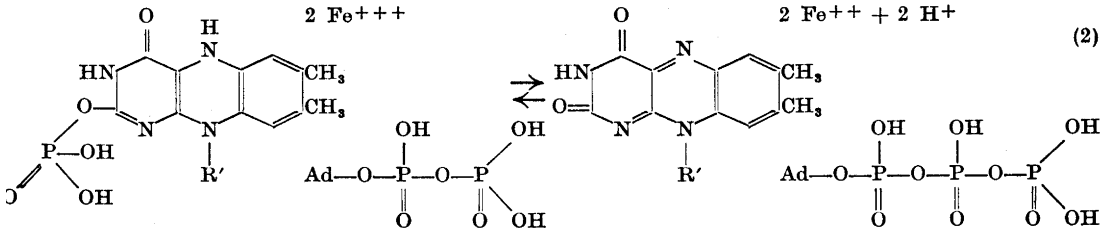
** Abbreviations: PO_4, inorganic phosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; DPNH, reduced diphosphopyridine nucleotide; FAD, flavin adenine dinucleotide; DNP, 2,4-dinitrophenol.



(1)

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)

Reaction (1) is dependent on the spatial arrangement of the reactants. It requires that the pyridine ring of DPNH and the *iso*alloxazine 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 amytal *, which blocks both electron trans fer 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 known [5] discrepancy between the rates of H_2O—$P^{18}O_4$ and $^{32}PO_4$—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.

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* 5-Ethyl-5-*iso*amylbarbituric acid.

Toxic Liver Injury: Some Biochemical Changes in the Liver caused by Dimethylnitrosamine

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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 weight [1]. 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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