phosphates and that the presence of non labeled UMP diminished the incorporation of isotope from C^{14}-orotic acid into the pyrophosphates. Uridine was not an intermediate in the transformation of orotic acid into UMP.

Enzyme fractionation with $(NH_4)_2SO_4$ and methanol are being carried out to investigate the intermediate steps in these reactions.

1. Hurlbert, R. B. and Potter, V. R. *J. Biol. Chem. In press.*
2. Reichard, P. *J. Biol. Chem.* **197** (1952) 391.

Quantitative Determination of Phenylthiohydantoins from Amino Acids

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Phenylthiohydantoins are separated on a chromatographic column on a microscale and the optical density of the effluent is continuously recorded. Details of the method and the apparatus will be described.

1. Edman, P. *Acta Chem. Scand.* **4** (1950) 283.
2. Sjöquist, J. *Acta Chem. Scand.* **7** (1953) 447.

The Mode of Action of Mn^{++} on Oxidative Phosphorylation

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In a recent communication [1] it has been shown that Mn^{++} forms a prosthetic group of the hydrogen transferring catalysts involved in the generation of energy rich phosphate ($\sim$ P) bonds. The role of Mn^{++} is to link these enzymes with ATP which serves as a cofactor in coupled phosphorylation. Non-phosphorylative (as it, *e. g.*, occurs in the Keilin-Hartree heart-muscle preparation) and phosphorylative oxidation may thus be described by equations 1 and 2 respectively:

$$DPNH_2^* + 1/2\ O_2 \xrightarrow{E-CoE} DPN + H_2O \quad (1)$$

$$DPNH_2 + 1/2\ O_2 + n\cdot P \xrightarrow{(E-CoE)-Mn-ATP} DPN + H_2O + n\cdot\sim P \quad (2)$$

* DPN = diphosphopyridine nucleotide
ATP = adenosinetriphosphate

(where CoE stands for the hydrogen bearing coenzymes involved), the two enzyme complexes being correlated according to the equation:

$$(E-CoE)-Mn-ATP \rightleftharpoons (E-CoE) + Mn^{++} + ATP.$$

The question was left open whether the ATP-containing enzyme complex which catalyzes reaction 2 is responsible for the ultimate synthesis of ATP, or whether its function is merely restricted to the oxidative formation of the primary $\sim$ P bond. It has now been shown that the latter is the case.

2,4-dinitrophenol (DNP), which is known to inhibit ATP synthesis connected with hydrogen transfer between $DPNH_2$ and oxygen, stimulates respiration in mitochondrial systems (see Table 1). The latter action is, however, dependent on the concentration of ATP prevailing in the medium. Thus the stimulation can be turned into an inhibition when the concentration of ATP is kept low by adding hexokinase. Moreover, Mn^{++} ($0.5—1.0 \times 10^{-3}$ *M*) is able to prevent this inhibition. These facts indicate that $\sim$ P is formed in this system according to equation 2, while the action of DNP is confined to the blockage of the subsequent transfer of the formed $\sim$ P to the adenylic acid system.

Table 1. Effect of Mn^{++} on mitochondrial respiration inhibited by DNP. Each Warburg vessel contained: mitochondria (prepared in 0.25 M sucrose containing 0.01 M versene), 1/12 rat liver; glycylglycine, 200 µmoles; KCl, 150 µmoles; glucose, 180 µmoles; D,L-*glutamate, 60 µmoles; orthophosphate, 90 µmoles; AMP, 3 µmoles; Mg^{++}, 10 µmoles. Additions (where indicated): yeast hexokinase (prepared as described previously* [1]*), 0.1 ml; DNP, 0.4 µmoles; Mn^{++} 1.2 µmoles.—Final volume, 2.5 ml. pH, 7.8. Temp. 30° C. Gas phase, air. Time of incubation, 45 min.*

Additions	Respiration µatoms O	Phosphorylation µmoles P
None	15.2	9.6
DNP	44.0	4.9
Hexokinase	36.5	85.5
DNP + hexokinase	8.4	9.7
DNP + hexokinase + Mn^{++}	45.5	12.2

1. Lindberg, O. and Ernster, L. *Nature* **173** (1954) 1038.