Table 1. Endogenous phosphorylation. Each flask contained: 80 μmoles of glycylglycine buffer, pH 7.4; 20 or 30 μmoles of orthophosphate, pH 7.4; 5 μmoles of ADP; 5 μmoles of DPN; 0.03 μmoles of cytochrome c; 50 μmoles of glucose; 0.5 mg of hexokinase; 10 μmoles of $MgCl_2$, and 0.5 ml of mitochondria in a final volume of 2 ml. 50 μmoles of DL-β-hydroxybutyrate were added only where indicated. Incubated in air for 20 min at 30°. Other details given in Ref.[2].

Added substrate	Oxygen uptake μatoms/mg N	Acetoacetate formed μmoles/mg N	Phosphate uptake μmoles/mg N	P/O
None	3.1	0	5.7	1.8
β-Hydroxybutyrate	8.5	5.3	22.0	2.6
Substrate minus endogenous	5.4	5.3	16.3	3.0

highest rate of endogenous phosphorylation were most stable to storage at 4°. Furthermore, certain pretreatments of liver mitochondria, such as freezing and thawing, or preincubation with low concentrations of phosphate which, among other detrimental changes (*cf.* Ref.[7]), abolished the endogenous activity, also decreased the stability of the stored preparations. The implication of these findings with regard to a possible correlation between endogenous phosphorylation and mitochondrial stability will be discussed.

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Particulate and Soluble D-Lactic Cytochrome c Reductase of Yeast

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The main fraction of the D-lactic cytochrome c reductase activity (D-LDH) of aerobic yeast was associated with respiratory particles. By means of the extraction of lipids with *n*-butanol, acetone, and ether, 40 % of the enzyme was solubilized. The solubilized enzyme has been purified one thousand fold. The difference spectrum of oxidized/reduced enzyme had absorption maximum at 450 mμ. At pH 3, flavin fluorescence appeared. The flavin concentration, calculated from the spectral difference, agreed well with that obtained from fluorescence intensity. In contrast to the flavohaemoprotein L-lactic cytochrome c reductase (L-LDH), there was no haem associated with the D-enzyme, and the D-enzyme did not reduce the haem of L-LDH. The turnover number of D-LDH was 20 000 min^{-1} $mole^{-1}$, compared with 15 000 for L-LDH.

Particulate as well as solubilized D-LDH were absolutely specific for D-lactate. A preparation of D-LDH, which was obtained directly in a soluble form in the cell homogenate[1], has erroneously been determined to have relative stereospecificity [2]. The apparent Michaelis constant of D-lactate for D-LDH was under certain conditions one thousand fold lower than that of L-lactate for L-LDH.

The oxidation of D-lactate to pyruvate went to completion with excess of cytochrome c. At a relatively high buffer concentration (μ 0.08), the initial rate of reaction has been determined for a series of cytochrome c concentrations, each with a series of D-lactate concentrations. The results can be summarized as follows: The apparent Michaelis constant of D-lactate was proportional to the cytochrome c concentration, and the rate, obtained with excess of lactate, was proportional to the cytochrome c concentration. These criteria, which were the same for soluble and particulate enzyme, are fullfilled in the following reaction scheme (where E = enzyme, SH_2 = lactate, and A = cytochrome c):

$$SH_2 + E \rightleftharpoons SH_2 \cdot E \xrightarrow{k_3} S + EH_2$$

$$EH_2 + A \xrightarrow{k_4} E + AH_2$$

In this scheme, $k_3 \gg k_4 \times A$

The fresh preparations of soluble D-LDH reduced ferricyanide at a rate which was 5 % of that with cytochrome c. On storage, the ferricyanide reduction was lost more rapidly than the cytochrome c reductase activity. This could either be ascribed to a modification of D-LDH, or to the presence of another enzyme. The aerobic "D-lactic ferricyanide reductase" had reaction properties strikingly different from those of the corresponding anaerobic enzyme.

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Purification of Yeast Hexokinase by Cellulose Ion Exchanger Chromatography

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In an earlier communication from this laboratory, it was reported that radioactive phosphorylserine could be isolated from a hydrolysate of a crude yeast hexokinase preparation which had been incubated with its radioactive substrate, AT^{32}P or glucose-6-^{32}P[1]. It seemed necessary to perform further studies with a more purified enzyme. Yeast hexokinase was isolated in a crystalline form several years ago [2,3]. By using cellulose ion exchanger chromatography, it now appears possible to obtain a more active preparation than the crystalline enzyme in an easy way.

As starting material, a commercial preparation (Sigma hexokinase, Type III) was used having a specific activity of 100–150 units per mg of preparation (estimated according to Kunitz *et al.*[3]). The activity was raised by two consecutive chromatographic separations using carboxymethyl cellulose columns (Serva Entwicklungslab., Germany) to between 1 200 and 1 900 units/mg protein, respectively. The elution was performed at pH 5 with a gradient increase in the concentration of the acetate buffer (0.02 N → 0.30 N with regard to sodium acetate). The enzyme was then chromatographed on a column of diethylaminoethyl cellulose (Eastman Org. Chem., U.S.A.). It was eluted with a potassium phosphate buffer of pH 7.0, increasing gradually from 0.05 M to 0.30 M. The enzyme was eluted as a main peak with nearly constant specific activity from fraction to fraction. No inactive protein could be separated from the enzyme activity by similar rechromatography of the main part of the enzyme peak. The specific activity was about 3 000 units/mg protein (as compared to about 1 400 units/mg protein found by Kunitz *et al.*[3]). The average yield in the three step purification was about 10 %.

When a sedimentation analysis was carried out in a Spinco ultracentrifuge, Model E, only one boundary was obtained. A single determination of the molecular weight was performed according to Archibald [4], and it was found to be about 50 000.

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On Electron Transport and Phosphorylation in Plant and Bacterial Light-Induced Phosphorylation

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The following findings from experiments on light-induced phosphorylation (aerobic conditions, saturating light-intensities) will be discussed:

(1) Coenzyme Q_0 stimulates light-induced phosphorylation in isolated spinach chloroplasts. It is, however, less efficient as stimulatory agent than menadione. At 10^{-4} M concentration, for example, coenzyme Q_0 gives only 70 % of the stimulation obtained with the same concentration of menadione.

(2) Valinomycin, which completely uncouples oxidative phosphorylation in animal mitochondria at low concentrations [1], inhibits light-induced phosphorylation in isolated chromatophores of the photosynthetic bacterium *Rhodospirillum rubrum* to approximately 50 %. This may indicate that two phos-