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 $\rightarrow$ 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-

phorylation sites are involved, one of which is affected, whereas the other one remains unaffected. When the rate of light-induced phosphorylation is stimulated several fold by phenazine methosulfate, only a small part of the phosphorylation, which is about equivalent to the sensitive part of the unstimulated system, is inhibited by valinomycin. This supports the conclusion that one of two sites, the "valinomycin-insensitive" one, is activated by addition of phenazine methosulfate. "Cyclic" light-induced phosphorylation in spinach chloroplasts remains unaffected by valinomycin, which may indicate that only a site corresponding to the "valinomycin-insensitive" site in bacterial chromatophores is participating in light-induced phosphorylation of the isolated spinach chloroplasts.

(3) When a preparation of bacterial chromatophores has been aged at 55—57°C, the initially very low stimulation obtained by menadione increases with the aging time, up to 15—25 minutes of preaging, after which a very rapid decrease occurs. In contrast to the stimulation obtained by phenazine methosulfate, that obtained by menadione is very sensitive to low concentrations of the inhibitors antimycin A and 2-*n*-heptyl-4-hydroxyquinoline-N-oxide. Thus a difference has been found between the modes of action of the two stimulatory agents on light-induced phosphorylation.

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Glycolysis in Human Erythrocytes Studied with ^{14}C-labelled Glucose

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The energy required for the maintenance of viability of the erythrocytes is obtained through breakdown of glucose mainly to lactate. Some hemolytic anemias and the aging of erythrocytes *in vitro* or *in vivo* have been shown to be accompanied by disorders in the glycolytic system [1-4].

In order to study these disorders more closely a test system has been worked out. The erythrocytes are washed at 4°C and finally suspended in an inorganic buffered salt solution with the following composition: NaCl 108 mM; KCl 3.9 mM; $MgCl_2$ 5 mM; Na-phosphate (pH 7.4) 20 mM. 2 ml of the suspension (holding 15 % hemoglobin) are incubated at 37° in the two media described in Table 1. After 60 min the glycolysis is stopped by adding perchloric acid. Different perchloric acid soluble substances are then separated using stepwise elution through a Dowex 1 column according to Bartlett [4]. Methylene blue stimulates breakdown along the pentose phosphate shunt [5] and the CO_2 formed during incubation is absorbed to NaOH moistened filter paper strips in the center well of the Erlenmeyer

Table 1. Composition of media.

	− methylene blue ml	+ methylene blue ml
Erythrocyte suspension	2.0	2.0
Buffered salt solution	0.6	0.6
0.05 % methylene blue	—	0.2
Distilled water	0.2	—
0.15 M ^{14}C-glucose (uniformly labelled, containing $1.3 \cdot 10^6$ cpm).	0.2	0.2
Total volume	3.0	3.0

Table 2.

	− methylene blue	+ methylene blue
ATP, μmoles per incubation flask	0.38	0.45
2,3-diP-glycerate, μmoles per incubation flask	2.8	2.9
% radioactivity in CO_2	—	3.5
% » » 2,3-diP-glycerate	2.7	4.8
% » » lactate	1.9	2.9