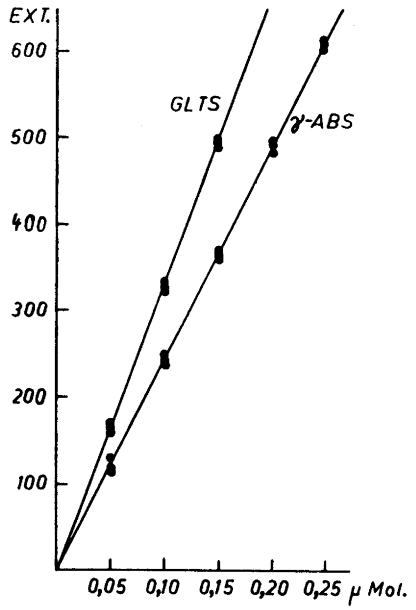

Abb. 2.

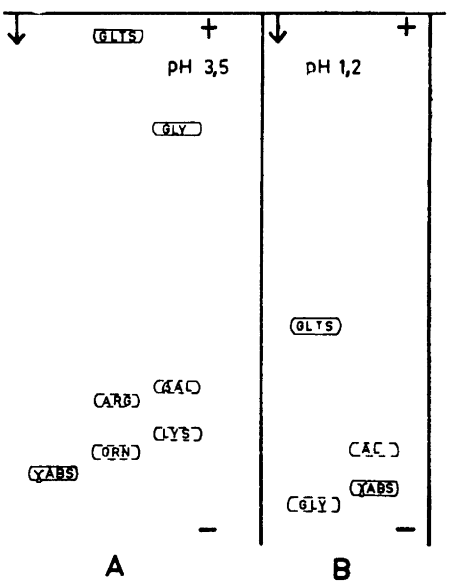

Abb. 3, A und B.

sungen erhaltenen Extinktionen (nach Korrektion mit dem Leerwert) werden Eichkurven angefertigt und so die Konzentration an γ-ABS und GLTS in der zu untersuchenden Lösung rechnerisch ermittelt. Beispiel siehe Bild 2. — Sollten in dem zu untersuchenden Material (z.B. Serumextrakt) grössere Mengen Glycin auftreten, so kann es vorkommen, dass in Folge eines "Overloading"-Effektes die γ-ABS nicht klar vom Glycin zu trennen ist. Letzteres hat eine etwas grössere Mobilität als γ-ABS. (Siehe Bild 3 A.) Hier kann man sich weiter helfen in dem man in einem Puffermilieu von pH 3,5 arbeitet. (Puffer: Eisessig:Pyridin:Wasser, 15:1:89, v/v.) Bei gleichen Versuchsbedingungen wie oben erhält man ein Trennungsbild wie im Bild 3 B; Auftragsstelle 12 cm von der Anode. γABS wandert 15 und Glycin nur 5 cm zur Kathode. Auch GLTS ist in diesem Milieu gut zu bestimmen und wird bei ihrer kurzen Wanderungsstrecke (1 cm) nicht von anderen Aminosäuren überlagert.

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On Light-Induced Phosphorylation in *Rhodospirillum rubrum*

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Light-induced phosphorylation (LIP) was described in 1954 by Arnon, Allen and Whatley [1] for plant and by Frenkel [2] for bacterial systems. Among the concepts about its mechanism are: (1) the phosphorylation reactions are coupled to an electron carrier chain, which recombines the photochemical oxidant (OH) and the

photochemical reductant (H) from the Hill reaction, or its equivalent in bacteria, to H_2O, and (2) P + ADP = ATP * is the net chemical reaction in LIP. Molecular oxygen is not involved in the process. Arnon, Whatley and Allen [3] have suggested that FMN, vitamin K and ascorbate are three of the components in the postulated carrier chain in isolated spinach chloroplasts. Recently, however, they have included TPN and excluded ascorbate and postulated that vitamin K and FMN participate in "alternative pathways for cyclic photophosphorylation" [4], in agreement with the hypothesis of Wessels. [5] Smith and Baltscheffsky [6] have shown that a substance with an absorption spectrum peak at 434 mμ appears to be involved in LIP in extracts of *Rhodospirillum rubrum*. It very probably is identical with the hemoprotein isolated by Vernon and Kamen [7] and recently described by Bartsch and Kamen [8]. Phenazine methosulfate stimulates the phosphorylation in *R. rubrum* [9] and in isolated chloroplasts [10]. The mechanism of the phosphorylation reactions is unknown.

This preliminary communication about LIP in cell-free extracts of *R. rubrum* deals with inhibitor effects, the action of phenazine methosulfate, ATP-ase and ^{32}P-ATP-exchange reactions. The bacteria were grown anaerobically at 30°C in light from incandescent lamps, in essentially the medium of Gest, Kamen and Bregoff [11]. After one washing of the harvested bacteria with distilled water and one with 0.02 M glycylglycine, pH 7.4, the extracts were prepared by grinding with alumina [6] and securing the supernatant from a 10 min centrifugation at 10 000 × *g*. The phosphorylation experiments were performed at saturating light intensities and 30°C. Phosphate was measured according to Lindberg and Ernster [12].

Table 1 shows the effect of some inhibitors on our system. In agreement with earlier data by Smith and Baltscheffsky [13] the inhibitors of mitochondrial respiration, KCN and NaN_3 did not influence LIP, whereas the uncoupler of oxidative phosphorylation dicoumarol had a strong effect. The observations of Smith and Baltscheffsky [6] that HOQNO and of Geller and Gregory [9] that antimycin A inhibit LIP are confirmed. Both inhibit electron transport between cytochrome b and cytochrome c_1 in mitochondria [14], thus it would seem logical to assume that the point of action is the same in LIP as in mitochondria. The absence of an effect of KCN and NaN_3 on LIP is in line with the fact that the hemoprotein, which Bartsch and Kamen [8] described and which very probably is the compound suggested by Smith and Baltscheffsky [6] to take part in LIP, does not form addition compounds with KCN and NaN_3. We consider the possibility that this hemoprotein, assumed by Chance and Smith [15] and by Bartsch and Kamen [8] to be a terminal oxidase for *R. rubrum*, reacts in LIP with oxidizing equivalent produced by the bacterial equivalent of the Hill reaction.

Table 1. Effect of different inhibitors on LIP. Medium: 2 ml 0.2 M glycylglycine pH 7.4, 20 μmoles K_2HPO_4, 10 μmoles ADP, 35 μmoles $MgCl_2$, 30 μmoles NaF, diluted to a final volume of 3.0 ml.

Inhibitor	Final concentration of inhibitor M	% Inhibition
KCN	10^{-3}	0
NaN_3	10^{-3}	0
Dicoumarol	10^{-4}	> 90
HOQNO	2×10^{-6}	> 95
Antimycin A	3×10^{-6}	> 95
Atebrin	7×10^{-3}	about 25

Table 2 shows a typical stimulation of the LIP obtained by 0.02 % phenazine

Table 2. Effect of HOQNO and phenazine methosulfate on LIP. Medium: as in Table 1. Where added: 2×10^{-6} M HOQNO and 0.01 % phenazine methosulfate, final concentrations.

Addition	μmoles P/hour/"OD_{800}" *
—	22
HOQNO	0
Phenazine methosulfate	180
HOQNO + phenazine methosulfate	130

* Abbreviations: P, orthophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; ATP-ase, adenosine triphosphatase; TPN, triphosphopyridine nucleotide; FMN, flavin mononucleotide; HOQNO, 2-*n*-heptyl-4-hydroxyquinoline-N-oxide; M, moles per liter.

* "OD_{800}" stands for the optical density of the absorption spectrum peak near 800 mμ of the bound chlorophyll (compare with Frenkel [17]).

methosulfate and the effect of HOQNO in the absence and the presence of phenazine methosulfate. Similar effects as with HOQNO are obtained with antimycin A and dicoumarol. Geller [16] assumes that phenazine methosulfate serves as a fast "by-pass" around the site which is rate limiting in the system. Our data indicate that a site which is inhibited by antimycin A and HOQNO is "by-passed" by phenazine methosulfate. The point at which dicoumarol acts, which is also "by-passed" may or may not be the same as is acted upon by antimycin A and HOQNO.

Frenkel [17] has reported that an active ATP-ase is present in his "crude" preparation of *R. rubrum*. In our system both a Mg++-stimulated ATP-ase and a ^{32}P-ATP-exchange reaction are present. The ATP-ase activity was inhibited to more than 90 % and the ^{32}P-ATP-exchange activity to about 80 % by 7 mM atebrin, which Löw [18] has shown to inhibit respiration, ATP-ase and ^{32}P-ATP-exchange reactions in liver mitochondria. The possible relation between these reactions and LIP is under investigation.

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Chromatography of Tropolones on Paper Impregnated with Ethylenediaminetetraacetic Acid and Dimethyl Sulphoxide

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Tropolones have recently been found in a number of conifer heartwoods and their identification by paper chromatography is therefore a matter of considerable interest. Conventional methods for the identification of phenols have been tried but give bad results due to tailing of the spots. This disadvantage was successfully eliminated by Zavarin and Anderson [1] by using paper impregnated with phosphoric acid and toluene — *iso*octane as mobile phase. In a search for a chemically less agressive stationary phase, which would also be suitable for preparative work, the use of dimethyl sulphoxide was investigated. Although highly polar, this is a good solvent for most lipophilic compounds and has been used with advantage in paper chromatography of sugar acetates and related compounds [2].

Preliminary experiments using dimethyl sulphoxide impregnated paper with light petroleum as mobile phase indicated favourable R_F-values although the spots were still very elongated, extending from the starting line. The length of the spots was dependent on the amount of substance applied but the distance travelled by the lower edge of the spot reached a limit when the amount of tropolone was increased. These results point to an irreversible adsorption of the tropolones.

Tropolones are known to form stable chelates with a number of multivalent cations and an obvious explanation of their