Reaction (1) is presumably catalyzed by a flavoprotein, since diaphorase activity, and the transfer reaction[4] (which involves a transfer of electrons from DPNH to the 3-acetyl pyridine analog of DPN) can be demonstrated in the ammonium sulfate fractionated and dialyzed extracts. At present it is not known whether reaction (2) is enzymic or non-enzymic in nature. Although, in soluble preparations, it is probably non-enzymic, the recent demonstration by COLPA-BOONSTRA AND SLATER[5] of a reduced menadione oxidase offers an alternative possibility. If reaction (2) proves to be non-enzymic, then the menadione reductase (reaction (1)) like Straub's diaphorase, catalyzes the reduction of menadione, which in turn reduces cytochrome *c*[6].

The possible role of these soluble enzymes in coupled oxidative phosphorylation in this organism will be discussed in a more detailed paper to follow.

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The role of vitamin K_1 in coupled oxidative phosphorylation*

Roles for vitamin K analogues in electron transport[1,2,3] and in oxidative phosphorylation[4,5,6] have been suggested. Evidence will be presented in this communication that vitamin K_1 is an essential coenzyme necessary for coupled oxidative phosphorylation in extracts of *Mycobacterium phlei*. The bacterial system requires active subcellular particles[7,8] and the soluble enzymes of the supernatant which include menadione reductase[5].

Evidence that vitamin K_1 plays an essential role as a physiological electron carrier, and is a key compound in oxidative phosphorylation, now has been obtained in several ways: (1) restoration of oxidative phosphorylation in light-inactivated preparations by the addition of vitamin K_1; (2) the reconstitution of coupled phosphorylation by adding K_1 after extensive fractionation of the supernatant; and (3) the specific requirement for certain configurations which occur in vitamin K_1, but not in related compounds.

Exposure of isolated particles and active supernatant to light emitted at 3600 A (Gates Raymaster Lamp, tube B) results in a complete loss of oxidation and phosphorylation. Addition of vitamin K_1 suspended in the inactive lipids obtained from extracts after centrifugation[5] completely restores both oxidation and coupled phosphorylation (Table I).

Exposure of the lipid-K_1 suspension to light at 3600 A results in complete inactivation. Lipid-extractable material obtained from untreated (active) supernatants has an absorption spectrum similar to that of the K-like compounds. Furthermore, this exposure to light alters in a similar manner the characteristic absorption spectrum of the lipid-extractable material from supernatant and of vitamin K_1.

This requirement for vitamin K_1 can also be demonstrated in the presence of enzymes obtained by ammonium sulfate fractionation and in which restoration of activity previously required the addition of heated crude supernatant. Both oxidation and phosphate esterification are restored by the addition of vitamin K_1.

After exposure of both particle and supernatant to light, the bacterial system exhibits a specific dependence on vitamin K_1 for coupled oxidative phosphorylation. Lapachol methyl ester and vitamin K_1 diacetate, compounds which closely resemble vitamin K_1 in the R_2 and R_3 position of the naphthoquinone, show some activity. Vitamin K_2, menadione, menadione diphosphate, dimethyl naphthoquinone, phthicol, lapachol, quinone and α-tocopherol are inactive. The system reactivated with vitamin K_1 is uncoupled by dinitrophenol and dicumarol; it is not affected by antimycin A. Addition of riboflavin monophosphate (FMN) to the light-treated particles and supernatant restores oxidation but not the ability to esterify inorganic phosphate.

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TABLE I

THE EFFECT OF VITAMIN K_1 ON LIGHT-EXPOSED PARTICLES AND SUPERNATANT (3600 A)

System	Additions	Oxygen μatoms	Δ Pi μmoles	P:O
P + S	none	2.32	1.8	0.77
P + S	vit. K_1	3.46	2.7	0.81
TP + TS	none	0.62	0.3	
TP + TS	vit. K_1	2.92	3.0	1.02
TP + TS	crude heated supernatant	2.20	1.6	0.71
TP + TS	treated vit. K_1	0.78	0.2	

The system contained 0.3 ml (7.0 mg protein) of the particulate fraction (TP) treated for 1.5 h with a Gates Raymaster lamp (3600 A), 0.4 ml (17 mg protein) of supernatant (TS) treated in a similar manner, 4.0 μmoles enzymically reduced DPN, 3.9 μmoles vitamin K_1 (oil) suspended in inactive lipid, 15 μmoles $MgCl_2$, 25 μmoles KF and 7.8 μmoles inorganic phosphate. The acceptor system consisted of 2.5 μmoles ADP, 20 μmoles glucose, and 1 mg yeast hexokinase. P and S are untreated particles and supernatant, respectively. The oxygen uptake was measured at 30° for 10 min after the addition of DPNH. The reaction was stopped with 10% TCA and the mixture analyzed for phosphate disappearance.

The FMN type system appears to represent the non-phosphorylative pathway in extracts from this organism[7,9].

Vitamin K_1 is involved as a coenzyme in both electron transport and coupled oxidative phosphorylation. Preliminary evidence indicates that ~ P is incorporated in vitamin K_1. This monophosphate ester has also been postulated by WESSELS[10] to be the activated intermediate.

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Purification of a serum protein required by a mammalian cell in tissue culture

The studies of EAGLE and his co-workers on the nutrition of tissue culture cells have defined their amino acid and vitamin requirements[1]. The nature of the serum components necessary for their growth, however, has not been elucidated. A protein which is required for the growth of a tissue culture cell, human appendix – A 1*, has now been purified about 15-fold from calf serum by conventional protein fractionation procedures.

The assay procedure used to follow the purification of the protein depends upon two marked effects produced by it on washed tissue culture cells inoculated into a synthetic medium[4]. Under the conditions of the assay, in the absence of the protein factor, few cells attach to a glass surface

* Appendix – A 1 is a single clone isolate obtained in Dr. PUCK's laboratory[2] from a strain isolated by Dr. CHANG[3].