steepness of the curve. Figure 2b shows another current-voltage curve taken from another, less vigorous, axon showing a point in the negative conductance region. These curves are very similar in all essential features to those observed in the squid giant axon (*3*) and *Homarus* axons (*11*).

In three cases, membrane currents were also recorded in ASW with a low sodium concentration. In each case, the action potential was abolished. Figure 3 shows the current-voltage relation of an axon in ASW, in ASW with low sodium concentration and back again to ASW. In this axon, recovery was incomplete. The action potential before sodium replacement was 82 mv, and in ASW again after exposure to the ASW with a low sodium concentration it was 57 mv. In this and in every other case, there was no inward current for any values of depolarizing pulses in a low sodium concentration. There was very little effect on steady-state currents. The transient current therefore may be identified at least tentatively as being carried by sodium ions. Sodium, then, would seem to carry the current responsible for the depolarization phase of the action potential.

The membranes of *Myxicola* giant axons, then, seem to be very similar in essential features to those of the squid giant axons.

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12. We thank Dr. D. L. Gilbert for generous contributions of his time during early stages of this project. We also thank Drs. K. S. Cole, R. E. Taylor, and E. Rojas for critical reading of the manuscript. Supported in part by grant NB-06436 from the US. Public Health Service.

4 October 1967

Cis-Trans Isomerism in Naphthoquinones: Interconversion and Participation in Oxidative Phosphorylation

Abstract. *Synthetic phylloquinone was resolved into* cis *and* trans *isomers by thin-layer chromatography. The two isomers had identical ultraviolet spectra characteristic of vitamin K_1 and were differentiated by nuclear-magnetic-resonance spectroscopy on the basis of the displacement of the peak corresponding to the olefinic methyl group in the naphthoquinone side chain. Studies on the restoration of electron transport coupled to phosphorylation in irradiated preparations of* Mycobacterium phlei *showed that only the* trans *isomer was active with substrates linked to nicotinamide-adenine dinucleotide. The purified* trans *phylloquinone was enzymatically converted to the* cis *isomer. Under similar conditions,* cis *vitamin K_1 gave rise to the* trans*-naphthoquinone. The natural naphthoquinone of* M. phlei *vitamin MK_9(II-H) was similarly resolved into* cis *and* trans *isomers.*

Restoration of oxidative phosphorylation in a quinone-depleted system from *Mycobacterium phlei* requires the addition of the natural naphthoquinone [MK_9(II-H)], vitamin K_1, or its homologues (*1*). Examination of the specificity for restoration of both oxidation and phosphorylation revealed a requirement for the 1,4-naphthoquinone nucleus with a methyl group in the carbon 2-position and at least one isoprenoid unit in the carbon-3-position (*2*). A number of compounds, such as dihydrophytyl vitamin K_1 and lapachol, which have been shown to restore oxidation by the main respiratory pathway, fail to restore phosphorylation (*2*, *3*). The requirements for restoration of phosphorylation by quinones appears to be more specific than that required for restoration of oxidation. Synthetic vitamin K_1 contains a mixture of *cis* and *trans* isomeric forms (*4*, *5*). The resolution of the synthetic quinone into its geometric isomers has permitted a further definition of the stereospecific requirements for restoration of coupled phosphorylation. Of particular interest was the finding of an enzymatic interconversion of the isomers of vitamin K_1. The ability of the *trans* isomer to restore oxidative phosphorylation and a consideration of the energetic processes involved in *cis-trans* isomerization may provide knowledge of the bioenergetic process and of the role of quinones in oxidative phosphorylation.

Chromatography of synthetic vitamin K_1 (*6*) was performed on a thin layer of silica gel G containing 0.1 percent rhodamine 6G with 10 percent butyl ether in hexane as solvent. The rhodamine plates fluoresced in ultraviolet light while ultraviolet-absorbing material quenched the fluorescence and gave rise to a dark band (*7*). Synthetic vitamin K_1 could be resolved into two bands, with R_F being 0.44 and 0.51, respectively (Fig. 1). Both of these compounds were identified as phylloquinones by ultraviolet spectroscopy, reverse-phase thin-layer chromatography, and infrared spectroscopy. With the molecular extinction coefficient reported for the absorption maximum at 269 mμ of phylloquinone in isooctane (*8*), the ratio of the amount of the slow- to the fast-moving band was about 10 : 1. A further identification of both bands as isomers of the same synthetic compound was obtained by nuclear-magnetic-resonance (NMR) spectroscopy (*9*). Both compounds yielded identical NMR spectra except for a difference in chemical shift in the region of 8.2 to 8.4 (Fig. 2). The peak in this area

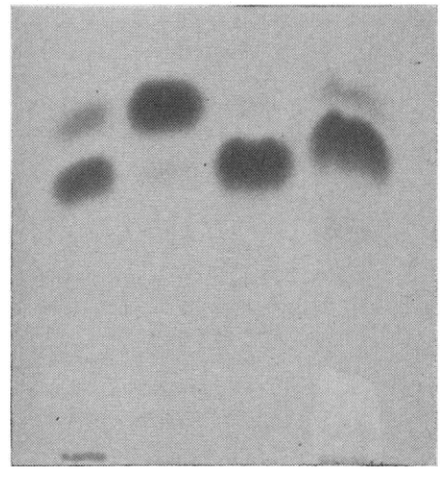

Fig. 1. Thin-layer chromatographic separation of geometrical isomers of phylloquinone and vitamin K_9(II-H). Chromatography was done on a thin layer (250 μ) of silica gel G impregnated with rhodamine 6G and developed in butyl ether: hexane (1 : 9 by volume). Spots visualized as dark bands under ultraviolet light (left to right): a sample of synthetic vitamin K_1; *cis* K_1, and *trans* K_1, the repurified bands from the synthetic K_1 (R_F *cis* K_1, 0.51, R_F *trans* K_1, 0.44); K_9(II-H), the natural naphthoquinone prepared from whole cells of *Mycobacterium phlei*. The quinone was purified by column chromatography prior to thin-layer chromatography according to the procedure of Dunphy *et al.* (*13*).

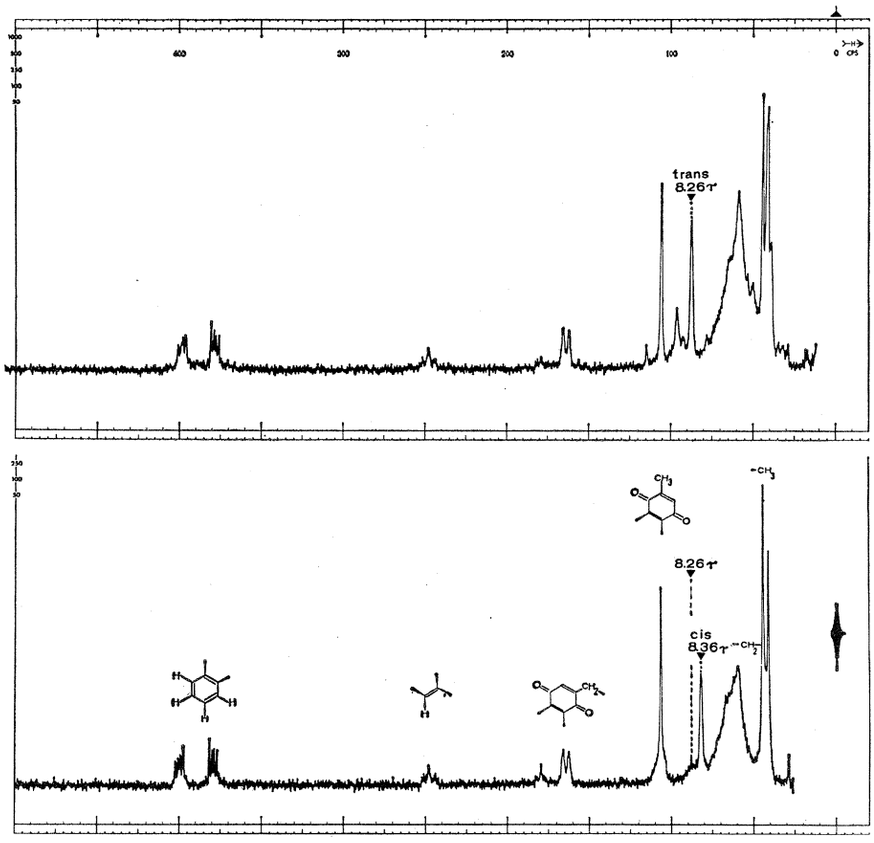

Fig. 2. The NMR spectroscopy of *cis* and *trans* vitamin K_1. Upper spectrum, *trans* K_1; lower spectrum, *cis* K_1. Spectra were recorded on a Varian Model HA 100 NMR spectrometer at a frequency of 100 mc/sec in carbon tetrachloride incorporating 1 percent tetramethylsilane as internal standard (*10*).

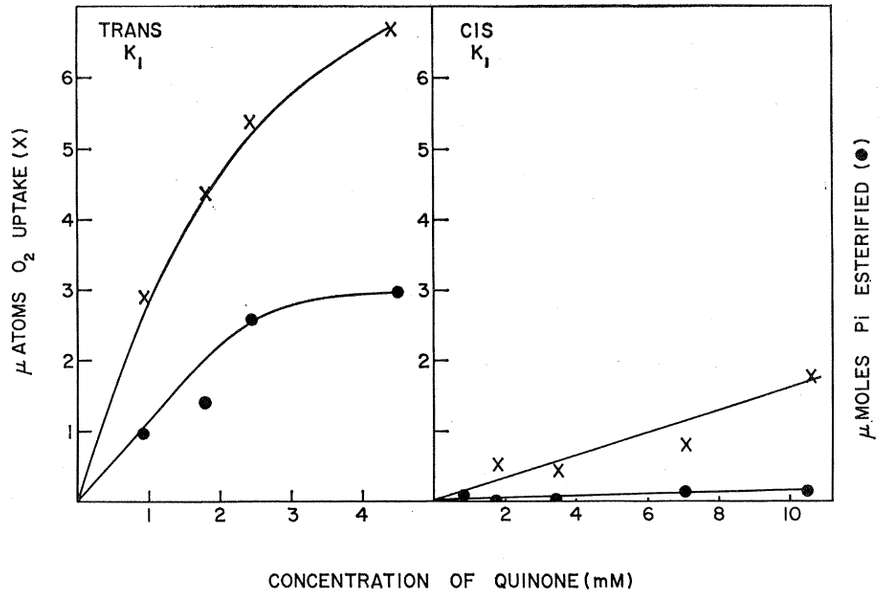

Fig. 3. Restoration of coupled oxidation and phosphorylation by isomers of vitamin K_1. The restoration of oxidation and phosphorylation was measured according to the procedure of Brodie and Ballantine (*1*). The system consisted of 0.5 ml of irradiated particles (45 mg of protein per milliliter), 0.6 ml of supernatant (fractionated with 30 to 60 percent ammonium sulfate; 15.5 mg of protein per milliliter), 10 μmole of adenosine monophosphate, 25 μmole of potassium fluoride, 15 μmole of nicotinamide-adenine dinucleotide, 15 μmole of $MgCl_2$, 25 μmole of glucose, 3 mg of hexokinase, 10 μmole of inorganic phosphate, 100 μmole of HEPES buffer *p*H 7.5 (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid), *cis* or *trans* vitamin K_1, and water to a final volume of 2.5 ml. The reaction was allowed to proceed for 8 minutes after addition of the substrate.

corresponds to the olefinic methyl group in the isoprenoid unit of the naphthoquinone side chain. In the *trans* isomer (slow-moving band, Fig. 1; upper spectrum, Fig. 2), this appears at 8.26τ, whereas in the *cis* isomer (lower spectrum, Fig. 2) the olefinic methyl signal appears at 8.36τ. A similar displacement of chemical shift in the region between 8 and 9τ was reported by Isler and Montavon (*5*) and by Bates *et al.* (*10*). A small band at 8.08τ was also present in the spectrum of the *trans* but absent from that of the *cis* isomer.

The two isomers of vitamin K_1 were purified by preparative thin-layer chromatography, and the ability of each to restore oxidative phosphorylation to an irradiated particulate preparation of *M. phlei* was tested according to the procedure previously described (*1*) (Fig. 3). With exogenously added nicotinamide-adenine dinucleotide which bypasses the amytal-sensitive site and reenters the respiratory chain at or before the quinone level (*11*) and a supernatant fraction which lacked the coupling factors for the third site of phosphorylation [cytochrome *c* → oxygen (*12*)], it was possible to measure the phosphorylation occurring at the second site (quinone → cytochrome *b*). The effect of increasing concentrations of the *cis* and *trans* isomers on the restoration of coupled activity is shown in Fig. 3. The *cis* isomer partially restored oxidation but failed to restore phosphorylation, whereas the *trans* isomer of vitamin K_1 was capable of restoring both activities with a quinone-depleted system. The phosphorus-oxygen ratio observed with increasing concentrations of *trans* vitamin K_1 ranged from 0.35 to 0.50. Although both the synthesis of adenosine triphosphate and coupled electron transport increased and were dependent on the concentration with increasing amounts of *trans* K_1, there appeared to be some quantitative variation from one experiment to the next. This variation was primarily due to the difficulty of suspending the highly lipophilic naphthoquinone. The actual proportion of the quinone emulsion (prepared by sonic oscillation of a mixture of phylloquinone and dialyzed irradiated supernatant) that participated in the coupled electron transport could not be quantitatively evaluated. Nevertheless, the *trans* vitamin K_1 was always as effective as the unresolved synthetic naphthoquinone in restoring coupled phosphorylation.

Although the precise relation be-

Table 1. Interconversion of *cis-trans* isomers of vitamin K_1.

Quinone	Amount of quinone added (μmole)		Added quinone recovered (μmole)		Newly formed quinone recovered* (μmole)	
	Cis	*Trans*	*Cis*	*Trans*	*Cis*	*Trans*
Synthetic† K_1	4.0‡	36.7‡	4.8	22.6		
Trans K_1	0	11.0		8.7	0.31	
Cis K_1	11.0	0	5.8			0.3

* These values represent the quantity of the purified isomer former from either *cis* or *trans* K_1 which was not present in the initial starting material. † Four identical flasks, each containing 5 mg of synthetic K_1, were incubated separately, and the acetone extracts were combined prior to purification. ‡ The amount of each of the geometric isomers present in the synthetic naphthoquinone was obtained spectrophotometrically after chromatographic resolution. The synthetic vitamin K_1 was incubated without prior separation into isomers.

tween the two isomers is not completely understood in terms of their possible participation in oxidative phosphorylation, the fact that incubation of the pure *trans* isomer resulted in the production of the *cis* isomer provided a clue (Table 1). The system consisted of 0.5 ml of washed irradiated particles (30 to 40 mg of protein per milliliter), 5 mg of *cis*, *trans*, or synthetic vitamin K_1 (10 ml/ml suspended by sonic oscillation in the presence of dialyzed irradiated supernatant), 0.2 ml of untreated supernatant (15 to 10 mg of protein per milliliter), 1.5 μmole of nicotinamide-adenine dinucleotide, 40 μmole of β-hydroxybutyrate, 20 μmole of inorganic phosphate, 10 μmole of adenosine diphosphate, and 30 μmole of $MgCl_2$. The reaction was terminated by the addition of 10 ml of cold acetone. The precipitated protein was separated from the organic phase and reextracted with a further 10 ml of acetone. The acetone extracts were combined and mixed with 20 ml of diethyl ether and then with 30 ml of water. The ether layer containing the quinone was washed three times with water, dried over sodium sulfate, and evaporated to dryness under nitrogen. The lipid extract was then fractionated by thin-layer chromatography as previously described and eluted from the silica gel. The amount of quinone was measured spectrophotometrically. In addition, it was observed that the ratio of *trans* to *cis* decreased after incubation of the unresolved synthetic mixture and that the amount of *cis* K_1 recovered in this experiment was actually higher than in the starting material, an indication of the possible formation of the *cis* isomer from the *trans* quinone. Nevertheless, a precise quantitative estimation of this conversion could not be made due to the variation in the recovery of quinone which was observed with different preparations. The enzymatic interconversion of the *cis* and *trans* isomers of vitamin K_1 was also observed with the resolved isomers as starting material. Even though the *cis* isomer was relatively inactive in terms of its ability to restore oxidative phosphorylation (Fig. 3), *trans* vitamin K_1 was produced from the *cis* isomer when the latter was added to an irradiated particulate preparation. The interconversion of the isomers was not observed with zero time controls.

Although a structural and stereospecific requirement for restoration of oxidative phosphorylation in quinone-depleted *M. phlei* preparations was demonstrated with vitamin K_1, this synthetic quinone differs from the natural quinone in this microorganism. Nevertheless, the ability of vitamin K_1 to restore oxidative phosphorylation and the interconversion of the isomers suggest that the natural quinone in *M. phlei* may also be present as *cis* and *trans* isomers. Dunphy *et al.* (*13*) demonstrated the existence of two isomers of the natural naphthoquinone vitamin K_9 (II-H) in *M. phlei* (Fig. 1). The two isomers were shown to be of identical molecular weight by mass spectroscopy, and the identification of the two isomers as *cis* and *trans* was confirmed by NMR analysis. The requirements for restoration of oxidative phosphorylation have been shown to be stereospecific and dependent on *trans* vitamin K_1. Nevertheless, *cis* vitamin K_1 is enzymatically formed from the *trans* isomer. Preliminary studies have shown that the conversion of *trans* vitamin K_1 to the *cis* isomer requires conditions of oxidative phosphorylation, whereas the conversion of the *cis* isomer to *trans* may be independent of this process.

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14. We thank Miss Patricia Brodle for technical assistance. Supported by grant AT (11-1) 113 from AEC, by grant AI-05637 from NIH and by the Hastings Foundation of the University of Southern California School of Medicine. This communication is the 32nd in a series entitled "Oxidative Phosphorylation in Fractionated Bacterial Systems."

22 June 1967; revised 30 October 1967

Defensive Use of a "Fecal Shield" by a Beetle Larva

Abstract. *The larva of* Cassida rubiginosa *carries a tight packet of cast skins and feces on a fork held over its back. The packet is a maneuverable shield used by the larva to protect itself against attack. It is highly effective in blocking the bite of ants.*

The larva of *Cassida rubiginosa*, a beetle of the family Chrysomelidae widely distributed through the Northern Hemisphere, has long been known to naturalists for its odd habit—shared with other members of the genus—of carrying a trash packet above its back (*1*) (Fig. 1A).

The packet, which is held by a two-pronged fork that projects forward from the tip of the abdomen (see Fig. 1C), consists of the molted skins previously shed by the larva, together with the dried remains of its accumulated fecal wastes. The mechanism whereby the cast skins are retained on the fork, and the procedure by which the fecal droplets are discharged onto the packet by the protrusible anal turret (Fig. 1B), have been described (*2*). The function of this "stercoraceous parasol" (*3*)—or "fecal shield" as we shall call it—has remained a matter of dispute. It has been said to serve for camou-