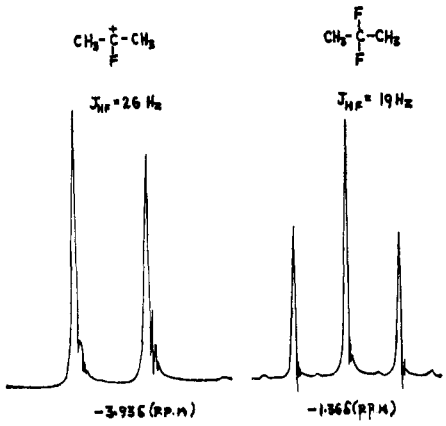

Figure 1.

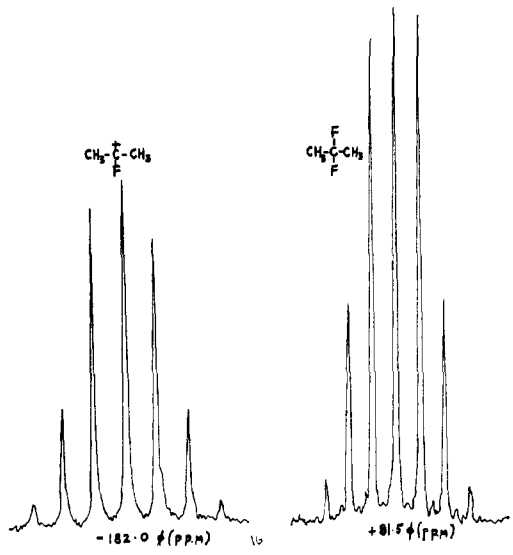

Figure 2.

field doublet of doublets at −1.71 ppm (J_{HH} = 5 Hz; J_{HFvic} = 22 Hz). On the basis of the chemical shift data for the ions III and IV, this *cannot* represent ion V but must be the adduct VI which exchanges SO_3F^- with solvent acid (the resonance of the sulfur-bonded fluorine was not observed).

$$CH_2{=}CHF + HSO_3F \longrightarrow [CH_3\overset{+}{C}HF] \underset{-SO_3F}{\overset{HSO_3F}{\rightleftharpoons}} CH_3CHFSO_3F$$
$$\qquad\qquad\qquad\qquad\quad V \qquad\qquad\quad VI$$
$$CH_3CF_2H + SbF_5 + HSO_3F$$

The same species (VI) was obtained from 1,1-difluoroethane by reaction with a mixture of SbF_5 and HSO_3F in SO_2 at −60° (but SbF_5 alone in SO_2 did not remove fluoride ion under these conditions). Protonation of vinylidene fluoride at −60° gave the adduct VIII whose pmr spectrum showed a triplet at −1.86

$$CH_2{=}CF_2 + HSO_3F + SbF_5 \xrightarrow[SO_2]{-60^\circ} [CH_2\overset{+}{C}F_2] \longrightarrow$$
$$CH_3CF_2SO_3F \xrightarrow[\text{temperature}]{\text{increased}} CH_3CF_3$$
$$\text{VIII}$$

ppm (J_{HFvic} = 15 Hz) and the ^{19}F nmr spectrum showed a triplet at −44.9 ppm (J_{FF} = 9 Hz) arising from the sulfur-bonded fluorine (*cf.* no corresponding resonance observed for VI) and an upfield doublet of quartets at +63.4 ppm (J_{FF} = 9 Hz; J_{HF} = 15 Hz) arising from

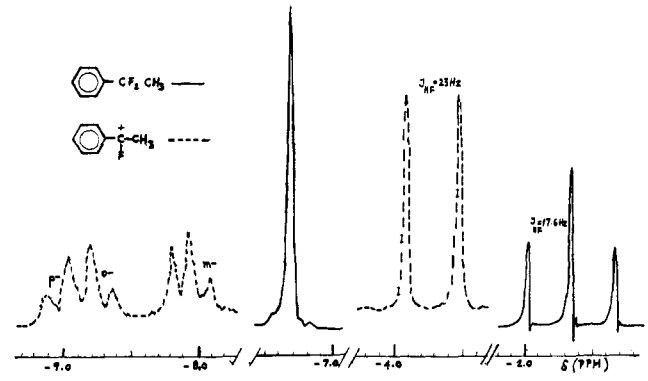

Figure 3.

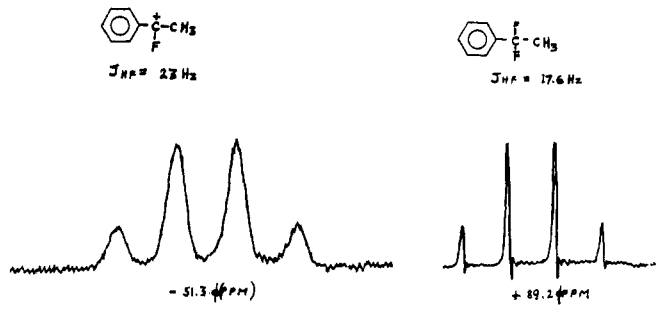

Figure 4.

the difluoromethylene group. Increased temperatures lead to fluorination to 1,1,1-trifluoroethane. These results show that the ions V and VII cannot be observed in the acid systems presently developed and at the same time illustrate how easily 1,1-difluoroethylene, which is normally resistant to electrophilic attack, is protonated in the super acid.

All nmr spectra were obtained on a Varian Associates Model A-56-60A spectrometer equipped with a variable-temperature probe and operated at 60 and 56.4 MHz, respectively. External TMS or CCl_3F were used as reference (sealed capillary tubes).

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(6) National Science Foundation Predoctoral Research Investigator.

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Received December 21, 1966

The Role of the Quinone in Oxidative Phosphorylation in *Mycobacterium phlei*. Evidence against Carbon–Oxygen Bond Cleavage[1]

Sir:

Much evidence exists for the direct involvement of quinone in oxidative phosphorylation,[2] and all the proposed schemes require bond lability at various sites in the molecule. Recent attention has focused on carbon–hydrogen bond cleavage during oxidative phos-

(1) Sponsored in part by Grant AI-04888 from the National Institutes of Health, U. S. Public Health Service.
(2) "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press Inc., New York, N. Y., 1965.

Table I. Oxidative Phosphorylation with Intact and Light-Inactivated Reconstituted[a] Extracts[b] of *M. phlei*

Expt[c]	System	Extract	Quinone added	Duration	ΔP_i, μmoles	O_2, μatoms	P:O
1	Warburg[d]	Standard	None	12 min	10.1	11.4	1.0
		Inactivated	None	12 min	3.9	4.0	1.0
		Inactivated	Phylloquinone-4-^{18}O	12 min	11.8	10.9	1.1
	Macro[e]	Inactivated (76 ml)	Phylloquinone-4-^{18}O	1.5 hr	620		
2	Warburg	Standard	None	12 min	10.0	10.2	1.0
		Inactivated	None	12 min	2.6	1.2	
		Inactivated	Phylloquinone-1-^{18}O	12 min	8.8	7.0	1.3
	Macro	Inactivated (100 ml)	Phylloquinone-1-^{18}O	2.5 hr	1170		
3	Warburg	Standard	None	12 min	8.7	12.6	0.7
		Inactivated	None	12 min	1.8	2.1	0.9
		Inactivated	Phylloquinone-1,4-$^{18}O_2$	12 min	5.2	7.7	0.7
	Macro[f]	Inactivated (91 ml)	Phylloquinone-1,4-$^{18}O_2$	3 hr (N_2) then 3 hr (O_2)	180 750		
4	Warburg	Standard	None	12 min	16.2	15.0	1.1
		Inactivated	None	12 min	8.1	9.2	0.9
		Inactivated	Phylloquinone-1,4-$^{18}O_2$	12 min	14.0	14.2	1.0
		Inactivated + KCN	Phylloquinone-1,4-$^{18}O_2$	12 min	3.0	0	
	Macro	Inactivated + KCN (38 ml)	Phylloquinone-1,4-$^{18}O_2$	24 hr	130		

[a] Extracts (20–28 mg of protein/ml) at 0° were inactivated by 25-min exposure to two 15-w GE black lights (long-wavelength ultraviolet). Phylloquinone (1 mg/ml) was incorporated as an emulsion formed by sonication in a portion of light-inactivated extract at 0°. [b] All systems in each experiment were aliquots of the same bulk cell-free bacterial extract; inactivated systems were aliquots of a light-treated portion of this bulk extract. [c] Substrate was pyruvate in expt 1, 2, and 4, and malate in expt 3. [d] Warburg systems contained components described previously [A. F. Brodie and C. T. Gray, *J. Biol. Chem.*, **219**, 853 (1956)] (extract volume 2.4 ml). [e] Same components as above in amounts proportional to indicated volume; the reaction flask was shaken at 30°. The reaction was followed by periodic phosphate analyses and terminated by centrifugation to separate particles and supernatant and addition of 95% ethanol. [f] Proceeded anaerobically for 3 hr in a nitrogen atmosphere, then aerobically for 3 hr.

Table II. Retention of ^{18}O Label of Quinone during Oxidative Phosphorylation with Extracts of *M. phlei*

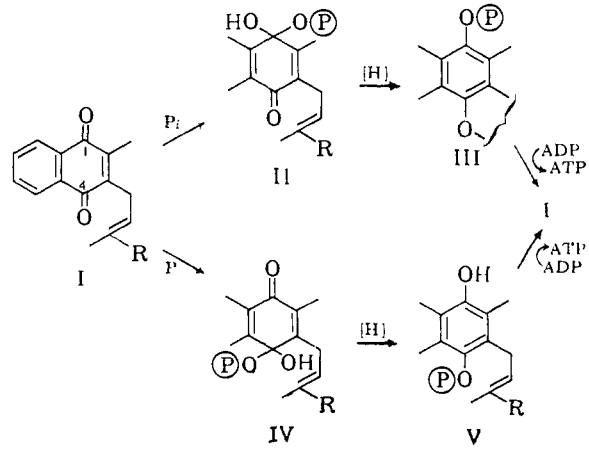
Expt	Quinone isolated[a]	Duration, hr	Phase	% retention of ^{18}O[b]
1	Phylloquinone-4-^{18}O	1.5	Particles	100
			Supernatant	100
2	Phylloquinone-1-^{18}O	2.5	Particles	98.4
			Supernatant	99.3
3	Phylloquinone-1,4-$^{18}O_2$	6	Particles	97.9
			Supernatant	98.7
4	Phylloquinone-1,4-$^{18}O_2$	24	Particles + supernatant	94.9

[a] Purity of quinones isolated was >99.5% as determined by ultraviolet. Both the purity and percentage retention of ^{18}O were determined by comparison with a standard quinone sample which was subjected to purification procedures identical with the test quinones. [b] Standard deviation is ± 0.3%; % ^{18}O: phylloquinone-4-^{18}O, 67%; phylloquinone-1-^{18}O, 72%; phylloquinone-1,4-$^{18}O_2$, 7%.

phorylation,[3] while little has appeared on the question of carbon–oxygen bond lability.[4]

Excluding those schemes already eliminated which require quinone methide intermediates,[3d] a common postulate[2] for quinone (I) participation in oxidative phosphorylation is 1,2 addition of phosphate to the carbonyl function of the quinone (II, IV) followed by reduction to a quinol phosphate (III, V), with or without chromanol formation. Such pathways, or others resulting in loss of the original quinone oxygen to the medium, should be detected by subjecting ^{18}O-labeled quinone to conditions of oxidative phosphorylation.

To answer this question, we have reconstituted oxidative phosphorylation in cell-free, light-inactivated extracts from *Mycobacterium phlei* with uniformly and specifically ^{18}O-labeled phylloquinones and determined the integrity of the ^{18}O label in recovered quinone.



Uniformly labeled phylloquinone was prepared by exchange of natural phylloquinone with $H_2{}^{18}O$ using

(3) (a) D. L. Gutnick and A. F. Brodie, *J. Biol. Chem.*, 240, PC3698 (1965); (b) D. L. Gutnick and A. F. Brodie, *ibid.*, **241**, 255 (1966); (c) W. W. Parson and H. Rudney, *Biochemistry*, **5**, 1013 (1966); (d) C. D. Snyder, S. J. DiMari, and H. Rapoport, *J. Am. Chem. Soc.*, **88**, 3868 (1966); (e) C. E. Horth, D. McHale, L. R. Jeffries, S. A. Price, A. T. Diplock, and J. Green, *Biochem. J.*, **100**, 424 (1966).

(4) The only comments on this subject are references to unpublished observations that inorganic phosphate oxygen does not exchange with quinone oxygen; *e.g.* (a) M. Vilkas and E. Lederer, *Experientia*, 18, 546 (1962), footnote 12; (b) A. F. Brodie in ref 2 above, p 394.

boron trifluoride etherate. The specifically labeled phylloquinones were synthesized from phytol and the corresponding 2-methyl-1,4-naphthoquinones (menadione) which had been selectively labeled with ^{18}O by taking advantage of the 50-fold difference in the acid-catalyzed rate of exchange between the O-1 and the O-4 positions; conversion to phylloquinones proceeded without loss of label. The ^{18}O content ($\pm 0.15\%$) was established by mass spectrometric analysis of the CO resulting from pyrolysis of the quinones at 500°.

The biological system and quinone isolation were as previously described[3d] except that additional chromatography on 5% silver nitrate impregnated Kiesel gel removed any native quinone [MK-9(H_2)] not destroyed by partial light inactivation. All experiments (Table I) displayed phosphate fixation coupled to oxidation. Recovered quinone showed complete retention of isotope (Table II) except for a slow loss roughly proportional to the exposure time of quinone to the medium, which probably occurred *via* simple exchange of the carbonyl functions with water. That such exchange is not related to oxidative phosphorylation is established by the parallel exchange in the KCN-treated system (expt 4) where no consumption of oxygen occurred.

Notable is the absence of exchange in the anaerobic–aerobic expt 3. The effect of this cycle should be to form and then oxidize any reduced species,[5] thus increasing the fraction of phylloquinone involved in oxidative phosphorylation relative to a possible small fraction in the steady-state aerobic case. Since no enhancement of exchange occurred, such a phosphorylated intermediate, if formed, was not formed by any of the schemes shown.

Our evidence for the absence of quinone oxygen exchange says that quinone involvement in oxidative phosphorylation must proceed with the original carbon–oxygen bonds remaining intact. Since this is a negative result, conceivably such exchange did occur but to an undetectably small extent, *i.e.*, less than 0.3%, the accuracy of our analysis. This limitation, plus the anaerobic–aerobic experiment, make it extremely unlikely that oxygen exchange is involved in the quinone's role in oxidative phosphorylation.

(5) P. J. Russel and A. F. Brodie, *Biochim. Biophys. Acta*, **50**, 76 (1961); D. Gutnick, T. Watanabe, and A. F. Brodie, *Federation Proc.*, **25**, 530 (1966); T. Watanabe and A. F. Brodie, *Proc. Natl. Acad. Sci. U. S.*, **56**, 940 (1966).

(6) National Institutes of Health Predoctoral Fellow.

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Received October 20, 1966

Pyrolysis of Biphenylene[1]

Sir:

Biphenylene, upon electron impact,[2] gives a relatively abundant ion at *m/e* 76 (13.4% relative intensity). By comparing the ratios of intensities of the *m/e* peaks at 76 (13.4) to 152 (100) and 76.5 (1.3) to 153 (12.9), it is estimated that the contribution of doubly charged ion at *m/e* 76 is about 75%. The balance, *i.e.*, 25%, is an ion of mass 76 and presumed to be benzyne.[3]

(1) Supported in part by the Case Research Fund.

(2) L. Friedman, N. Ingber, and D. F. Lindow, unpublished data.

In attempts to generate benzyne thermally[4] 1:1 mixtures of biphenylene[6] and anthracene were pyrolyzed at ~440° in a sealed Vycor tube (liquid phase) or in the gas phase.[7] It was assumed that formation of triptycene[8] would be indicative of the presence of benzyne. In these experiments little, if any, biphenylene was recovered, and triptycene was not detected.

The sealed tube experiments in the presence or absence of anthracene gave only one major product. This was found to be tetraphenylene[9,10] (tetrabenzocyclooctatetraene) on the basis of melting point (232–233°, cor) and infrared, ultraviolet, and mass spectra. A minor by-product is biphenyl.[11]

Tetraphenylene was obtained in 96% yield by pyrolysis (400°) of biphenylene in an evacuated sealed tube.[12,13] The effects of time and temperature on the course of the reaction are listed in Table I. Lower

Table I. Pyrolysis of Biphenylene (Sealed Tube)

Run	*T*, °C	Time, hr	Tetraphenylene, %	Biphenyl, %	Biphenylene, %
1	395–408	1	96	4	0
2	430–445	0.5	85	3	0
3	430–445	18	66	5	0
4	330–350	1	5	0	95
5	330–350	6	11	1	76
6	330–350	30	11	6	17

yields at higher temperatures and extended reaction times indicate thermal destruction of tetraphenylene (*cf.* runs 1–3). Lower temperatures result in decreased conversion of biphenylene; however, by extending the

(3) (a) R. S. Berry, J. Clardy, and M. E. Schafer, *J. Am. Chem. Soc.*' **86**, 2738 (1964). For other examples of electron-impact fragmentation leading to *m/e* 76 ions (presumably benzyne) see: (b) F. W. McLafferty and R. J. Gohlke, *Anal. Chem.*, **31**, 2076 (1959); (c) E. K. Fields and S. Meyerson, *Chem. Commun.*, 474 (1965), *J. Org. Chem.*, **31**, 3307 (1966); (d) R. F. C. Brown and R. K. Solly, *Australian J. Chem.* **19**, 1045 (1966).

(4) Electron-impact and thermal fragmentation may or may not lead to similar results. For example, the results from electron-impact and thermal fragmentation of phthalic anhydrides[3c,5] and indantrione[3d] parallel each other while those from anthraquinone and fluorenone do not.[3d]

(5) M. P. Cava, M. J. Mitchell, D. C. DeJongh, and R. Y. Van Fossen, *Tetrahedron Letters*, 2947 (1966).

(6) Obtained in ~30% yield from benzenediazonium-2-carboxylate: L. Friedman and A. Seitz, in press.

(7) Reaction effected in an oven (helium atmosphere) connected directly to a dual column (7 ft × 14 in. o.d. 3% GE SF 1093 on acid-washed DMCS-treated Chromosorb G, 70–80 mesh) F & M gas chromatograph. The authors acknowledge with thanks Dr. Stephen S. Hirsch and Mr. Gazie K. Ragep, Chemstrand Research Corp., for performing these experiments.

(8) Under these conditions triptycene remains essentially unchanged.

(9) G. Wittig and G. Lehmann, *Chem. Ber.*, **90**, 875 (1957). In an excellent paper on biphenylene chemistry, it was stated that "diphenylene is extraordinarily stable—it is formed at 350°." However, in the discussion J. Chatt reported that biphenylene and bis(triphenylphosphino)nickel dicarbonyl at 100° for 7 hr gave tetraphenylene (~10%) as the only isolable product: W. Baker and J. F. W. McOmie, "Diphenylene and the Cyclobutadiene Problem," in the Chemical Society Symposia, Bristol, 1958, Special Publication No. 12, The Chemical Society, London, 1958, pp 49–67.

(10) Vapor-phase pyrolysis gave, in addition to tetraphenylene and biphenyl, triphenylene and significant amounts of a large number of as yet unidentified hydrocarbons.

(11) (a) Another possible by-product is *o*-quaterphenyl which was not separable from tetraphenylene *via* glpc. On the basis of infrared analysis (ultraviolet analysis does not distinguish between them) not more than 1% could be present in the crude tetraphenylene. (b) Mass spectral data indicates that hexaphenylene may be present (<1%).

(12) Large-scale (20 g) pyrolysis of biphenylene was conducted in a small (40 ml) stainless-steel bomb at 375° for 1 hr.

(13) Small amounts (~1%) of tetraphenylene were also obtained by photolysis of biphenylene (0.066 *M*) in hexane at 2538 and 3500 A. Solid films were unaffected.