# Short Communications

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## On the lipid components of the respiratory chain

NASON[1] was the first to introduce treatment with organic solvents as a method of studying the possible role of lipids in the respiratory chain. On the basis of the inactivation brought about by shaking a respiratory-chain preparation with isooctane and the reactivation by the subsequent addition of α-tocopherol, he proposed that the latter compound was a component of the respiratory chain. It now seems clear, however, that the inactivation is caused by the inhibitory action of isooctane dissolved in the mitochondrial lipid and that α-tocopherol reverses the inhibition by removing the isooctane[2–8].

The same objection could be raised against the conclusion drawn by CRANE and his coworkers[9–11] on the reactivation by ubiquinone (coenzyme Q) of isooctane-extracted respiratory-chain preparations. However, later work from the same laboratory[12–15], in which acetone was used instead of isooctane, is free from this objection and has provided important support for the view that this quinone is a component of the respiratory chain. The procedure introduced by these authors appeared suitable for the study of two problems of interest to us: the possible role of ubichromenol (the isomer of ubiquinone discovered by MORTON[16]), and the possibility that ubiquinone might be identical with the BAL-labile[17] or the antimycin-sensitive factor.

*Effects of ubichromenol on acetone-extracted heart-muscle preparation*

Table I shows the effects of various additions on the succinate oxidase activities of the acetone-extracted lyophilized preparation. In the presence of cytochrome *c*, either ubiquinone or the acetone extract, which contains about 80 % of the ubiquinone originally present in the heart-muscle preparation[15], and especially the two together, greatly stimulated the activity of the acetone-extracted preparation. The degree of reactivation by ubiquinone plus the acetone extract is comparable with that reported by AMBE *et al.*[15] for a electron-transfer particle. Both ubichromenol and α-tocopherol were inactive. In fact, these compounds appeared to inhibit activation by the acetone extract, although they were without action on the non-extracted preparations.

It may be concluded that ubichromenol is not an active component of the non-phosphorylating respiratory chain of the heart-muscle preparation; nor can it be converted to ubiquinone by this preparation. This does not exclude the possibility that it may play a rôle in phosphorylating preparations. However, LAIDMAN AND MORTON[19] found no conversion to ubiquinone of ubichromenol injected intramuscularly into the rat.

The inactivity of α-tocopherol in this system is in accord with the results of the Madison group[12,13,15]. Acid-reduced ubiquinone-50 (ubichromanol or a derivative thereof[20]) was also found to be inactive. The product obtained by oxidizing with $HAuCl_4$ the acid-reduced ubiquinone-50 had about one half the activity of ubiquinone when measured in the presence of cytochrome *c* and the acetone extract (*cf.* ref. 15).

TABLE I

EFFECT OF DIFFERENT LIPIDS ON SUCCINATE OXIDASE ACTIVITY OF ACETONE-EXTRACTED HEART-MUSCLE PREPARATION

A Keilin and Hartree heart-muscle preparation[18], suspended in 0.1 M potassium phosphate buffer (pH 7.4), was lyophilized and 50-mg aliquots of the dried material were extracted with 10 ml pure acetone for 30 min at 4° (*cf.* ref. 14). The acetone extract was poured off and the enzyme preparation stirred up twice with acetone at 4° (*cf.* ref. 12). After drying under vacuum, the powder was treated in a Potter and Elvehjem homogenizer with 2.5 ml 0.1 M phosphate buffer (pH 7.4). A sample of the lyophilized preparation was similarly homogenized. The acetone extract was evaporated under vacuum and the residue dissolved in ethanol. The succinate oxidase activity was determined manometrically at 37° in a medium containing 33 mM potassium sodium phosphate buffer (pH 7.4), 0.33 mM EDTA, 27 mM sodium succinate and 1.7% ethanol. The heart-muscle preparation (about 1 mg protein) was added to the reaction mixture containing the phosphate buffer, EDTA and the ethanolic solution of the lipids (including the acetone extract), and the mixture allowed to stand 30 min at room temperature. Where indicated, 7.3 $\mu$M cytochrome *c* was then added, and the flasks attached to the manometers and placed in the bath at 37°. After equilibration for 10 min, the reaction was started by adding succinate from the side-arm, and the rate of $O_2$ uptake was measured between 20 and 30 min after adding the succinate. Ubiquinone-(50) was isolated from horse-heart muscle[22]. Ubichromenol was prepared by isomerization on alumina[20]. α-Tocopherol was obtained from Hoffmann–La Roche and Co.

| *Addition* | $Q_{O_2}$ ($\mu$l $O_2$/mg protein/h) | | | |
|---|---|---|---|---|
| | *Original preparation* | *Lyophilized* | *Extracted* | *Extracted + acetone extract*** |
| None | 466 | 273 | 14 | 41 |
| Cytochrome *c* | 546 | 404 | 57 | 246 |
| Ubiquinone(50)* | 461 | 265 | 22 | 41 |
| Ubiquinone(50)* + cytochrome *c* | 519 | 388 | 145 | 496 |
| Ubichromenol(50)* | 456 | 265 | 11 | 27 |
| Ubichromenol(50)* + cytochrome *c* | 485 | 377 | 49 | 93 |
| α-Tocopherol* | 429 | 254 | 19 | 22 |
| α-Tocopherol* + cytochrome *c* | 505 | 390 | 41 | 134 |

* 67 $\mu$g/ml.
** Equivalent in amount to the heart-muscle preparation present.

*Study of possible relationship of ubiquinone with the antimycin- and BAL-sensitive factors*

The possibility that ubiquinone is identical with the BAL-sensitive factor has already been considered in this laboratory. DEUL[21] showed that treatment of heart-muscle preparation with BAL in the presence of oxygen[17] had no effect on the ultraviolet-absorption spectrum of an ethereal extract of the preparation, nor on the amount of ubiquinone determined by the $KBH_4$-difference method. These studies did not exclude the possibility that the BAL treatment might affect the polyisoprenoid side chain of ubiquinone in such a way as to affect its function as a hydrogen or electron carrier, without having any effect on the ultraviolet-absorption spectrum. The ability of an acetone extract to reactivate an acetone-extracted heart-muscle preparation enabled a test of this possibility.

Table II shows that an acetone extract of a BAL-treated heart-muscle preparation was as effective as the extract of a normal preparation in reactivating an acetone-extracted normal preparation. Neither extract reactivated an acetone-extracted BAL-treated preparation. It appears clear then that ubiquinone is not the BAL-sensitive factor. It seems probable, in fact, that the BAL-sensitive factor cannot be extracted by dry acetone under the conditions used. In similar experiments, it

TABLE II

EFFECT OF BAL TREATMENT ON ABILITY OF ACETONE EXTRACT OF HEART-MUSCLE PREPARATION TO REACTIVATE ACETONE-EXTRACTED HEART-MUSCLE PREPARATION

*BAL treatment.* 5 ml heart-muscle preparation was incubated according to the "general procedure" of SLATER[17] and 90 ml cold 0.1 M phosphate buffer (pH 7.4) were added. After removing 3 ml for measurement of the enzyme activity, the remainder was made up to 300 ml with buffer and the suspension centrifuged in the Servall centrifuge at 19000 × *g* for 10 min. The sediment was homogenized in 100 ml buffer and recentrifuged. The washing was repeated twice, and the precipitate was finally suspended in 5 ml water, lyophilized and extracted with acetone as described in Table I. Succinate oxidase was determined as in Table I, with added cytochrome *c*.

| | $Q_{O_2}$ (μl $O_2$/mg protein/h) | |
|---|---|---|
| | *Normal preparation* | *BAL-treated* |
| Original suspension | 252 | 76 |
| Lyophilized | 220 | 52 |
| Acetone-extracted | 32 | 0 |
| Acetone-extracted + Extract I* | 86 | 0 |
| Acetone-extracted + Extract II* | 80 | 0 |

* Extract I, acetone extract of normal preparation; Extract II, acetone extract of BAL-treated preparation.

was found that inhibition by antimycin could not be reversed by lyophilization and extraction with acetone, followed by addition of the acetone extract of a normal preparation.

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