COENZYME Q. XXVIII. ACTIVITY OF THE COENZYME Q GROUP IN SPERM MOTILITY

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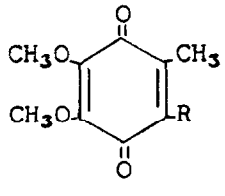
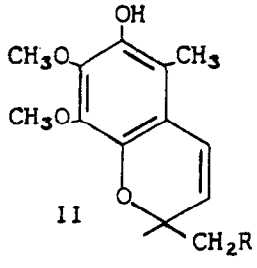
We have found that the coenzyme Q group, particularly a chromanol, are biologically active in maintaining sperm motility. Whether or not these data are of basic significance remains for further research. The motility of chicken sperm cells persists about four times as long as controls if the medium is supplemented with a compound from the coenzyme Q group.

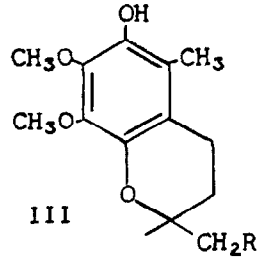
The coenzyme Q group is defined as the 2,3-dimethoxy-5-methyl-6-substituted benzoquinones (I), the corresponding 6-chromenols (II), and 6-chromanols (III). The naturally occurring



I R = (isoprenoid)



II



III

members of the quinone series (I) have a recognized role with succinoxidase in cellular respiration; the quinones and corresponding 6-chromanol (III) are of current interest in studies on oxidative phosphorylation.

In motile spermatozoa, the energy of undulation is released by ATPase (Mann, 1955), and the energy expended by the cell during such motion is replenished (Gray, 1955). Thus, resynthesis of ATP must take place in the sperm cell, presumably either by glycolysis or by coupling reactions with respiratory enzyme systems.

The electron microscope studies of Nelson (1958-59) showed the association of ATPase and succinic dehydrogenase with microstructures in rat sperm flagella. His studies led us to presume an important role for succinoxidase in sperm motility and to test the effect of the coenzyme Q group on sperm motility.

Coenzyme Q_{10} has been identified (Linn et al., 1959) in extracts of chick tissues, but its solubility in aqueous media is so low that we have confined our studies primarily to the more soluble, lower molecular weight compounds.

Aliquots of ethanol solutions containing compounds of the Q group were placed in sterile 10 x 75 mm. tubes and 0.25 ml. of medium added to each aseptically. The medium (Wilcox, 1960) consisted of aseptically collected and blended egg white, antibiotics and presterilized phosphate buffer and fructose solutions. Semen samples were collected under ordinary room illumination (cf. light influence, Hamner and Williams, 1961) from adult male chickens of proven fertility and diluted at once with four volumes of sterile unsupplemented medium. After being gently mixed, 0.25 ml. of the semen-medium mixture was added to each tube. The racks of tubes were covered with sterile aluminum foil, gently agitated, and placed in the dark at 10° C. for the storage period of 5 to 7 days. With the exception of the first experiment, the final concentration of ethanol in all tubes was 1% or less, and in all experiments the negative controls contained corresponding concentrations of ethanol. Less than twenty minutes was required for all operations from the commencement of the semen collection to the placement of the racks in the storage chamber. After the storage period, each tube in an experiment was removed individually from the racks, incubated 4 minutes in a 37° water bath, and the sperm observed in hanging drop preparations in a microscope stage incubator. Estimates of the percent of cells still actively motile in the coded samples were made at 440 diameter magnification and recorded indepen-

dently by each of three or four observers. The figures in Table I are the unweighted arithmetic means of these individual estimates.

TABLE I

Sperm Motility after 5-7 Days

Substance	Concentration μg./ml.	% Motile Cells
Control (Ethanol)		0 (all expts.)
Coenzyme Q_2	10γ	35 (5)[1]
	30γ	9 (5)
Hexahydro Q_4	10γ	0 (3)
	30γ	19 (5)
Hexahydro Q_4 chromenol	10γ	34 (2)
	30γ	41 (2)
Hexahydro Q_4 chromanol	1γ	0 (1)
	3γ	55 (1)
	10γ	53 (5)
	30γ	58 (8)
α-Tocopherol	10γ	27 (5)
	30γ	39 (9)

[1] No. experiments

To study the duration of motility, replicate samples of diluted semen supplemented with 30γ/ml. of the chromanol of hexahydrocoenzyme Q_4 were prepared and observed after 7-21 days storage. Half of the cells were still motile after 11-14 days and some motile cells were observed after 20 days storage. Control cells were totally immotile after 4 days.

Of all the compounds in Table I, the 6-chromanol of hexahydrocoenzyme Q_4 has shown activity most consistently. Although compounds of structures I, II, and III have each shown activity, some structural specificity for motility maintenance may be indicated. For example, the benzoquinones, 6-chromenols and 6-chromanols might be enzymatically interconvertible, and the resulting 6-chromanol might exhibit activity by participation in oxidative synthesis of ATP. Other investigators have proposed such a role for chromanols in oxidative phosphorylation. Alternatively, these compounds may act by pre-

venting the accumulation of inhibitory lipid peroxides in the sperm suspensions.

We have also examined vitamin K_1 at the 10γ and 30γ levels. Results have been very erratic, with an overall average of 10% motile cells in nine trials.

Although we have insufficient information to establish the mode of action, one mechanism apparently can be excluded. A technique for extending sperm motility and fertility through reversible metaboli inhibition has recently been demonstrated (Blackwood, 1960). If the Q group were to act by inhibition, we would expect to observe decrease motility, fructose conversion or oxygen consumption. In comparisons o controls and the chromanol of hexahydrocoenzyme Q_4, we have found no time at which motility or fructose disappearance rate is greater in the controls. Preliminary oxygen consumption data also gave no evidence of inhibition by the chromanol at 37°.

These studies are being extended to other coenzyme Q containing species, particularly man. It is conceivable that the coenzyme Q group may be significant in human fertility and sterility.

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