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STIMULATION OF SERUM-FREE CELL PROLIFERATION BY COENZYME Q

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SUMMARY: Coenzyme Q added to culture media stimulates the growth of HeLa and Balb/3T3 cells in serum free conditions. The stimulation by coenzyme Q is additive to the stimulation by ferricyanide, an impermeable electron acceptor for the transplasma membrane electron transport. αTocopherylquinone can also stimulate cell growth, but vitamin K_1 is inactive or inhibitory. The response to coenzyme Q and ferricyanide is enhanced with insulin. A contribution to plasma membrane NADH oxidation or modification of the membrane quinone redox balance can be a basis for the growth stimulation.

Activation of transplasma membrane electron transport stimulates the growth of many transformed cell lines in serum free media (1-4). The electron transport can be activated with artificial impermeable oxidants such as ferricyanide. The natural stimulation has been postulated to occur through a ferric transferrin-stimulated NADH oxidase (5). The stimulation by ferric transferrin would be part of the general requirement for ferric transferrin as a serum component required for growth in addition to its role in iron transport (6).

Since coenzyme Q is present in plasma membranes (7,8) and is a part of the plasma membrane electron transport system (9,10), the effect of coenzyme Q on cell proliferation has been investigated.

METHODS: Cell culture: Cells were grown in 25 cm^2 culture flasks with αMEM, and 100 U penicillin and 170 μg streptomycin per ml with CO_2 at pH 7.4 at 37° for 48 hr (11). Cells removed by mild trypinization which was stopped by addition of media with fetal calf serum. Cells resuspended in αMEM were counted on a coulter counter. Viability was greater than 90% as determined by eosin Y exclusion.

RESULTS

Addition of coenzyme Q to the serum free media stimulates the growth of HeLa cells 100% or more as measured by viable cell count after 48 hours (Fig. 1). In nine experiments cell

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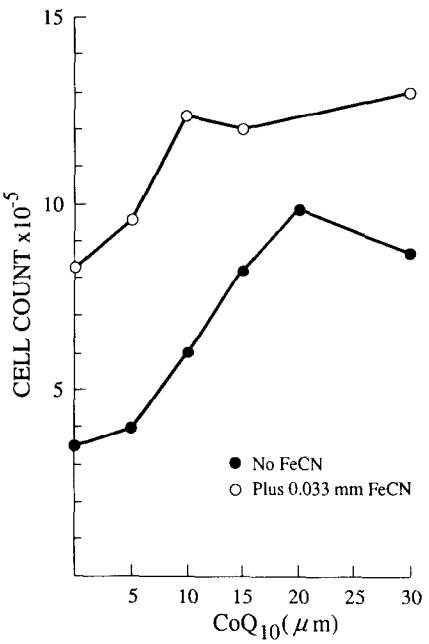

Figure 1. **Stimulation of HeLa cell growth in the absence of fetal calf serum by coenzyme Q_{10} and by coenzyme Q_{10} plus 0.033 mM ferricyanide. Cells cultured in αMEM as described in methods.**

count increased 125 ± 35% when 30 μM or 20 μM coenzyme Q_{10} was added. With 10 μM coenzyme Q_{10} added, the increase was 53 ± 21%.

Ferricyanide stimulates the growth of HeLa cells (12). Coenzyme Q added along with ferricyanide gives an additional stimulation which is almost additive to that of ferricyanide (Fig. 1).

Insulin increases the growth response to ferricyanide (11) but only slightly increases response to coenzyme Q_{10}. The presence of insulin significantly enhances the stimulation by coenzyme Q and ferricyanide combined (Table I).

TABLE I

Effect of Coenzyme Q on HeLa Cell Growth Stimulation in the Presence of Insulin

Addition to Media	Cell Count x 10^{-5}
None	2.7
Coenzyme Q_{10}	4.7
Insulin	3.5
Insulin + Coenzyme Q_{10}	5.3
Insulin + Ferricyanide	8.2
Insulin + Ferricyanide + Coenzyme Q_{10}	14.0

Coenzyme Q at 10 μM, Ferricyanide at 0.033 mM and insulin at 10 μg/ml.

TABLE II

Specificity of Quinone Effects on HeLa Cell Growth in Serum-Free Media

Additions to Media	Cell Growth Increase Percent of Control	
	no $Fe(CN)_6$	+ $Fe(CN)_6$
αTQ 10 μM	140	136
αTQ 20 μM	162	137
αTQ 30 μM	126	141
CoQ_{10} 10 μM	167	132
CoQ_{10} 30 μM	202	163
Vit K_1 15 μM	100	79

Cell count with no addition in cells x 10^{-5} per 25 cm^2 flask without ferricyanide were: αTQ, 2.2, CoQ_{10}, 5.1 and Vit K_1, 1.1; with ferricyanide,0.033 mM,αTQ, 3.2, CoQ_{10}, 8.5 and Vit K_1, 1.7, equivalent volumes of ethanol had no effect. Similar observations for αTQ in four other experiments, for vitamin K_1 in one other.

αTocopherylquinone (αTQ) also stimulates HeLa cell growth at concentrations similar to coenzyme Q. Vitamin K_1 does not stimulate HeLa cell growth and may be slightly inhibitory to ferricyanide stimulation (Table II). The stimulatory effects of αTQ and coenzyme Q are not additive (not shown).

Serum free growth of Balb/3T3 and SV40 transformed Balb/3T3 (SVT2) cells is also stimulated by coenzyme Q. The extent of stimulation is similar to that seen with HeLa cells. As previously shown for Swiss 3T3 cells (3), the Balb 3T3 cells are not strongly stimulated by ferricyanide unless insulin and FGF are present. Ferricyanide does not increase the response to coenzyme Q_{10} with these cells and may inhibit the coenzyme Q effect (Table III). The SV40 transformed Balb 3T3 cells respond more to ferricyanide alone, but the combined effects of coenzyme Q and ferricyanide are not additive without other growth factors.

TABLE III

Comparison of Growth Stimulation in Balb 3T3 Cells and Simian Virus Transformed Balb/3T3 Cells (SV/T2) by Coenzyme Q in Serum-Free Media

Addition	Increase in Cell Count x 10^{-5}	
	Balb 3T3	SV T2
None	1.6	2.3
Coenzyme Q_{10} 10 μM	2.8	4.4
Coenzyme Q_{10} 30 μM	3.1	3.5
Ferricyanide 0.033 mM	1.9	5.3
Ferricyanide 0.033 + CoQ_{10} 30 μM	2.3	4.3

Results representative of four experiments for Balb/3T3 and three experiments with SV/T2.

Two inhibitors of coenzyme Q function in plasma membrane inhibit growth of HeLa cells. Capsiacin at 200 μM inhibits the NADH oxidase of rat liver plasma membrane 98% and inhibits growth of HeLa cells 90% at the same concentration. Chloroquine at 500 μM inhibits the NADH oxidase of plasma membrane 90% (5) and HeLa cell growth 90%.

DISCUSSION

Impermeable oxidants which can be reduced by the transplasma membrane electron transport stimulate the serum free growth of at least eight cell lines (3,13,14). Since there is evidence that the electron transport system depends on coenzyme Q in animal cells (10), a coenzyme Q effect on growth control can be considered. Added coenzyme Q may function as an electron acceptor in the membrane, or it may shift the redox equilibrium of coenzyme Q in the membrane by increasing the concentration of oxidized coenzyme Q in the lipid phase of the membrane. The redox state of plastoquinone in chloroplast membranes is involved in control of phosphorylation of a membrane protein (15), and external oxidants increase phosphorylation of membrane protein band III in erythrocytes (16).

Coenzyme Q in blood serum is from 0.7 to 1.2 μM. The effects observed here are at much higher concentration, but in serum the coenzyme Q is associated with very low density lipoprotein (32). The association with protein may provide for more efficient transfer to the cell surface than addition in ethanol.

The plasma membrane electron transport system is a ligand-activated NADH oxidase which uses oxygen as a natural acceptor. Activation can be by ligands such as diferric transferrin or EGF (5).

Two polypeptides in the plasma membrane at 75 and 36 kDa have been identified as components of the NADH oxidase (5,17). Flavin, coenzyme Q and a chelated form of iron have been identified as prosthetic groups of the oxidase (17,18). Evidence for coenzyme Q function in the oxidase has been obtained by extraction-restoration and reversible analog inhibition (9,10).

Plasma membrane from rat liver contains 0.7 nmoles coenzyme Q per mg membrane protein or about one-half the concentration in liver mitochondria (7). The effect of added coenzyme Q on growth could involve effects on the plasma membrane or mitochondria. Since the plasma membrane oxidase is more related to growth control, it is more likely to be the site where the coenzyme Q acts. The effect of αTQ in growth stimulation also indicates action at the plasma membrane, since αTQ can replace coenzyme Q in the plasma membrane oxidase, but is

inactive in mitochondria (19). The effectiveness of capsiacin and chloroquine in inhibition of plasma membrane NADH oxidase and HeLa cell growth is also consistent with an effect of coenzyme Q at the plasma membrane. Capsiacin binding on plasma membranes is known (20), and it decreases weight gain in adipoise tissue (21). Crude soybean lipid stimulates β lymphocyte growth, and the effect is not based on the phospholipids (22). The stimulation of growth by αTQ may have more significance than a simple replacement for coenzyme Q_{10}. In contrast to mitochondria, plasma membrane contains significant amounts of αTQ. For example, human erythrocyte membranes contain only 0.01 nmoles CoQ_{10}/mg protein and 1.2 nmoles αTQ/mg protein. αTocopherol inhibits muscle cell growth (23) and protein kinase C (24). On the other hand, αTQ has been reported to stimulate the growth of fibroblasts (25). A balance between tocopherol and tocopheryl quinone may exert a redox control function similar to coenzyme Q. Stimulation of growth and oncogene expression by H_2O_2 (26) may be related to a shift from tocopherol and reduced coenzyme Q to the quinone forms. Addition of the quinone forms would also shift the redox balance in the membrane.

The nature of the message transmitted from the redox system in the plasma membrane to the nucleus to activate growth is not defined. Oxidants at the plasma membrane stimulate the Na^+/H^+ antiport to increase internal pH (27,28), give a transient increase in internal calcium ion concentration (29), decrease the cytosolic NADH/NAD ratio (30) and change membrane potential (28). In C3H10T1/2 cells external ferricyanide increases transient expression of the c myc and c fos protooncogenes (31) that are early genes in growth control.

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