Mitotic Inhibition Induced by Phosphates

It has been shown recently that toluhydroquinone-1:4-diphosphate exerts mitotic inhibition in tissue cultures of chick fibroblasts while 1:4-toluhydroquinone was inactive as mitotic inhibitor[1].

We have started to analyse this result and have investigated whether toluhydroquinone would increase mitotic inhibition in the presence of phosphate. It will be seen from the following table that this is the case. Toluhydroquinone as well as toluquinone develop antimitotic properties, apparently by the mere addition of phosphate at 10^{-3} *M* (pH = 7) to their solutions.

The Table gives the results of 7 tissue-culture experiments, each consisting of 24 cultures. Total mitotic counts have been made in treated and in untreated cultures of each experiment. The number of mitoses in treated cultures is expressed in percentage of the number of mitoses in the controls. These figures subtracted from 100 represent the mitotic inhibitions. The technique applied has been described previously[2].

The observations recorded in the table show again that the mitotic inhibition induced by toluhydroquinone-diphosphate is due to the intact toluhydroquinone-diphosphate molecule and not to its products of cleavage, as toluhydroquinone is inactive as mitotic inhibitor already in the presence of 10^{-4} *M* phosphate and *a fortiori* in presence of 2×10^{-5} *M* phosphate, the concentration obtainable by hydrolysis. The assumption that the work of the cell may raise this concentration to 10^{-3} *M* has no foundation in fact.

On the other hand, it may be necessary to modify the role assigned to toluhydroquinone in the process of mitotic inhibition, induced by toluhydroquinone-diphosphate, as the newly discovered high antimitotic activity of toluhydroquinone in the presence of phosphate allows no more to exclude phosphorylation as leading part in the antimitotic activity of this compound. Phosphorylation conceded, the excess of phosphate, required in this reaction, is easily understood on the basis of the law of mass, as this excess counteracts the breakdown of toluhydroquinone-diphosphate through the phosphatases of the embryo extract[1]. The observation of AUTENRIETH[2] and his collaborators shall be quoted in this connection. They have shown that a multiple of the lethal dose of phenol is well tolerated by animals, if phosphate is administered at the same time.

By adding 10^{-3} *M* phosphate to 10^{-5} *M* toludihydroquinone considerably higher antimitotic values are obtained than by the same addition to 10^{-5} *M* toluquinone. Under these conditions, the dihydric phenol, toluhydroquinone, is of greater antimitotic potency than its oxidation product, toluquinone.

It shall be mentioned that the addition of phosphate during the interaction of sulphydryl compounds with quinones increases the velocity of the reaction apparently at every stage of this complicated process[3]. So far no chemical linkage of phosphoric acid, accounting for these facts, could be detected by spectrophotometric investigations. Thus, it remains to be seen how the biological synthesis of phenolphosphates will contribute to disentangle these involved reactions[4].

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Zusammenfassung

In Gewebskulturen von Hühnerfibroblasten sind Toluhydrochinon und Toluchinon unwirksam. Nach Zusatz von 10^{-3}-*M*-Phosphat werden diese Substanzen antimitotisch wirksam. Der antimitotische Effekt von Toluhydrochinon + Phosphat ist erheblich grösser als derjenige von Toluchinon + Phosphat. Diese Beobachtungen können zur Deutung der antimitotischen Wirkung von Toluhydrochinondiphosphat herangezogen werden.

[1] E. FRIEDMANN, D. H. MARRIAN, and I. SIMON-REUSS, Biochem. Biophys. Acta *13*, 260 (1954).

[2] E. FRIEDMANN, D. H. MARRIAN, and I. SIMON-REUSS, Brit. J. Pharmacol. *3*, 263 (1948).

[1] References under Phosphatases in Bibliography of the Research in Tissue Cultures 1884–1850, Vol. *II*, pp. 1268 and 9 (Academic Press, New York, 1953). – F. MOOG, Proc. Nat. Acad. Sci. *29*, 196 (1942); Biol. Bull. Woods Hole *86*, 51 (1944). – R. J. C. HARRIS, Brit. Emp. Cancer Camp. 31st Annual Rep. p. 393 (1953).

[2] W. AUTENRIETH, Z. physiol. Chem. *25*, 440 (1898).

[3] E. FRIEDMANN (unpublished).

[4] J. ROCHE in *The Enzymes* by I. B. SUMNER and K. MYRBACK (Academic Press, New York), Vol. I, pt. 1, p. 506.

Mitotic Inhibition Caused by Toluhydroquinone and Toluquinone in the Presence of Phosphate

Toluhydroquinone	Toluhydroquinone-1:4-diphosphate	Toluquinone
10^{-5} *M*	10^{-5} *M*	10^{-5} *M*
No mitotic inhibition	Mitotic inhibition: 50%	No mitotic inhibition

Toluhydroquinone	Toluquinone
10^{-5} *M* + Phosphate 10^{-3} *M*	10^{-5} *M* + Phosphate 10^{-3} *M*
Mitotic inhibition: 71 and 84%	Mitotic inhibition: 27 and 13%

Toluhydroquinone
10^{-5} *M* + Phosphate 10^{-4} *M*
No mitotic inhibition

Phosphate 10^{-3} *M*
No mitotic inhibition