THE TOXICITY OF L-ASCORBIC ACID TO L929 MOUSE FIBROBLAST CULTURES

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On the basis, inter alia, of its reported ability to stimulate the growth of fibroblasts and the production and deposition of collagen, it has been suggested by Lacroix et al (1988) that ascorbic acid might be of value in postsurgical therapy and in wound healing. Our initial attempt to investigate the effect of L-ascorbic acid on mouse L929 fibroblast cultures resulted in a marked inhibition in fibroblast growth rate to 26% that of control cultures after 3d and to 5% of control after 6d using the same concentration we have previously used (11mM) to study the effects of simple sugars in the same system (Schmidt et al 1989). To gain a better understanding of these apparently contradictory results, we carried out a dose / response study and measured changes in glutathione status of our L929 cells after exposure to different concentrations of ascorbic acid.

Three $21cm^2$ culture dishes were each inoculated with 10^5 cell / ml for each concentration of ascorbic acid (0, 0.5, 1.0, 2, 5, 8, and 11mM) and allowed to grow to confluence for 7d. Glutathione/glutathione disulphide assays (Griffith 1980) were carried out in duplicate on the cells in each dish after separation from the culture medium and washing with normal saline. Results were recorded as mean values ± standard deviations.

At low ascorbic acid concentration (0.5mM) we observed a significant elevation of glutathione level in cells from confluent cultures to a value of ~160% relative to control; as the concentration of ascorbic acid was increased from 1mM to 5mM we observed a dose-dependent decrease in glutathione levels from the high recorded for 0.5mM ascorbic acid, reaching control levels at about 5mM and becoming depleted relative to control at levels in excess of 5mM.

At low concentrations, ascorbic acid evidently contributes to the net total of reducing equivalents and therefore has a sparing effect on glutathione (Boorsook et al 1937). We may suppose that a direct or indirect induction of glutathione synthesis may also occur. As the concentration of ascorbic acid was increased a paradoxical oxidative stress condition arose, as evidenced by the depletion of glutathione, which was associated with some loss of viability and a failure to divide. An analogous course of biochemical events has been documented for glucose-6-phosphate dehydrogenase (G6PD)-deficient red blood cells when exposed to ascorbic acid in the presence of air and this is believed to arise from the generation of hydrogen peroxide by oxidation of the ascorbic acid. The hydrogen peroxide in turn depletes glutathione by converting it to glutathione disulphide (GSSG) through the action of glutathione peroxidase. Such (G6PD)-deficient red blood cells fail to resynthesise glutathione from GSSG and succumb to the resulting oxidative stress condition by lipid peroxidation and hence cell lysis (Mager et al 1980).

We may conclude that whilst low levels of ascorbic acid may well enhance growth of fibroblast cultures, higher levels can perturb the glutathione status of the cells unfavourably and lead to inhibition of growth.

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Boorsook H. et al (1937) J. Biol. Chem. 117: 237-279
Griffith O.W. (1980) Anal. Biochem. 106: 207-212
Lacroix B. et al (1988) Internat. J. Vit. Nutr. Res. 58: 407-413
Mager J. et al (1980) In: Liener I.E. (ed.) Toxic Constituents of Plant Foodstuffs 2nd edn. New York: Academic Press pp 265-294
Schmidt R.J. et al (1989) J. Pharm. Pharmacol. 41: 781-784