Increased relative growth rate of normal rat cells in vitro with crocetin

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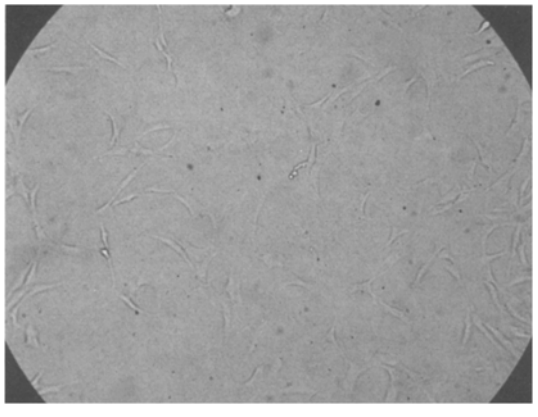
Summary. The carotenoid crocetin (earlier found to increase relative growth of Walker 256 tumor cells and their radiosensitivity), is shown to increase the relative growth of normal Sprague-Dawley rat muscle derived cells in vitro, presumably by increasing oxygen transport.

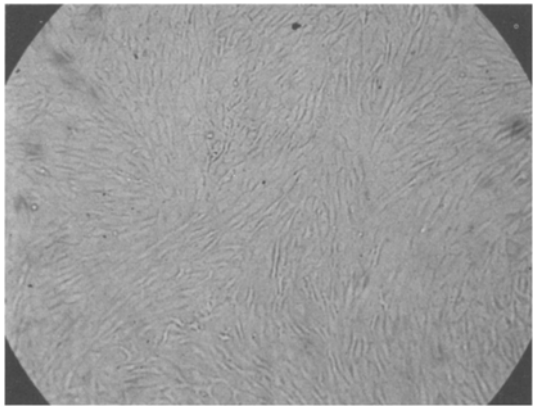
We have previously demonstrated that crocetin, a carotenoid compound, increased the growth rate and radiosensitivity of Walker 256 carcinoma in rats[2]. This paper describes some in vitro effects of crocetin on normal rat cells.

Muscle fragments from the hind limb of a 6-week-old (150 g) male Sprague-Dawley rat were suspended in balanced salt solution and trypsinized according to standard techniques[3]. Cells were resuspended in modified Eagle's medium supplemented with 10% fetal calf serum (Grand Island Biological Company) and assayed for viability by trypan blue exclusion. About 10^5–10^6 viable cells were inoculated into 5 ml stationary flasks (Falcon Flask Company) and incubated at 37 °C in a 5% CO_2 atmosphere.

The flasks reached confluency in about 3 weeks. The fibroblastic monolayer was trypsinized, counted, and cells were inoculated into flasks for 3 experimental groups: control, 0.1 mg/ml crocetin, and 0.001 mg/ml crocetin. This procedure was repeated several times.



a



b

Normal cell cultures after 1 week: *a* without crocetin; *b* with 0.001 mg/ml crocetin.

As shown in the figure, the crocetin-treated cells grew faster than the control cells. These pictures were taken after 1 week of growth. While the culture containing 0.001 mg/ml crocetin had reached confluency, the control cultures were relatively sparse. Flasks treated with 0.001 mg/ml crocetin contained 4 times the number of cells as in the control cultures. The cultures given 0.1 mg/ml crocetin appeared to have reached confluency earlier than 1 week, by which time they were dying from lack of nutrients.

The role in cancer therapy of other carotenoids, such as Vitamin A and its analogs, has been reported by several workers[4,5]. These compounds can act at the cellular membrane level and can cause lysosomal rupture[6,7], especially after the cells have been damaged by some other agent, such as radiation[8–10]. Vitamin A is also reported to alter the immune response[5,11].

It is concluded that crocetin increases the relative growth of normal rat cells in vitro. This may be due to increased oxygen transport, which was shown by Stoker[12] to be an important factor in the regulation of mitosis. The exact mechanism of action, however, is not known. Nevertheless, crocetin shortens the length of time necessary to reach confluent monolayers. This dramatic effect of crocetin should be examined in other cell culture systems. Studies are now in progress to elucidate the precise mechanisms of action of crocetin and to determine its intracellular distribution in normal cells.

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2 E. S. Wilkins, Thesis, University of Virginia, Charlottesville, Virginia, 1976.
3 J. Paul, in: Cell and Tissue Culture. 5th ed. Churchill-Livingston, New York 1975.
4 Editorial, Br. med. J. *2*, 2 (1976).
5 I. F. Tannock, M. D. Suit and N. Marshall, J. nat. Cancer Inst. *48*, 731 (1972).
6 J. Dingle, in: Ciba Found. Symp. Lysosomes. Ed. A. V. S. De Reuck and M. P. Comet. Little Brown and Co., Boston 1965.
7 G. Weissmann, New Engl. J. Med. *27*, 1084 (1965).
8 D. Brandes and E. Anton, Lab. Invest. *15*, 987 (1966).
9 D. Brandes, K. W. Sloan, E. Anton and F. Bloedorn, Cancer Res. *27*, 731 (1967).
10 J. E. Paris and D. Brandes, Cancer Res. *31*, 392 (1971).
11 D. W. Dresser, Nature *217*, 527 (1968).
12 M. G. P. Stoker, Nature *246*, 200 (1973).