Earle W R, Schilling E L, Stark T H, Straus N P, Brown M F & Sbelton E. Production of malignancy in vitro. 4. The mouse fibroblast cultures and changes seen in the living cells. J. *Nat. Cancer Inst.* 4:165-212, 1943.

This paper describes one of the earliest successful attempts *to* produce "cancer in a test-tube." Fibroblasts derived from the subcutaneous adipose connective tissue of a C_3H mouse were treated *in vitro* with 20-methylcholanthreue. Injection of the cultures into mice produced tumors. The first description of spontaneous malignant transformation of cells in culture was spawned when the control cultures also produced tumors. [The *SCI*® indicates that this paper was cited 601 times in the period 1961-1977.]

Emma Shelton
National Cancer Institute
Department of Health, Education and Welfare
Bethesda, MD 20014

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"This was Wilton Earle's magnum opus. The rest of us, whose names appear after his, lent but our willing hands to his project and he graciously placed us at the beginning of the article rather than the end. To have his article listed as a Citation Classic would be for him an encomium close to the Nobel Prize, for to him quantity was the ultimate essence of life.

"His project was conceived in the days when only a small coterie of scientists was engaged in cancer research, and it was a period when almost all of them believed that if normal cells could be converted to cancer cells by exposure to a pure chemical, the solution to the riddle of cancer was virtually assured. Wilton Earle set about to achieve that goal, constructing on the way an elaborate tissue culture suite rivaled, but not surpassed, only by the former laboratory of Alexis Carrel at the Rockefeller Institute. These were the days when cells were grown in plasma clot and sustained by a soup consisting of an extract of macerated chick embryos and horse serum diluted in a specially formulated saline solution. It was an heroic effort to grow cells in culture. Chickens had to be bled for plasma (and were subsequently eaten by us, now it can be told, because it was World War II and meat was hard to get), horses bled for serum, 10-day-old chick embryos removed from the shell and macerated, and salts weighed out, dissolved and filtered. All this had to be done aseptically, for antibiotics were yet to come. We lost very few of the thousands of cultures to bacterial contamination.

"Wilton realized his goal when the carcinogen-treated cells altered their morphology and growth characteristics and ultimately produced tumors when injected into mice. But his triumph was short-lived for, in spite of his exquisite precautions, which stopped just short of bathing his technicians in acid, some of his control cultures transformed to the malignant state, and the riddle of cancer, instead of being solved, became more arcane than ever. Wilton was convinced until the day he died in 1964 that trace contamination with chemical carcinogen was the explanation for the transformation of the control cells. In spite of all our knowledge, the explanation still eludes us.

"Why has this paper been cited so frequently? Not because of the significance of its contents as outlined above, but because it describes the origin of the famous "L" cells that are now grown in laboratories around the world. Tissue culture techniques have changed so that even high school students can grow them now. At this writing. Strain L is 13,610 days old. Wilton would have loved that."