This paper describes a procedure for the growth of single isolated tissue cells and the establishment of NCTC clone 929-L. By restricting the volume of culture medium and preconditioning the medium, conditions were devised that allowed the continued proliferation of single isolated cells of this line. [The SCI® indicates that this paper has been cited over 310 times since 1961.]

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"W.R. Earle would be pleased to know that our paper with CD. Likely has been included on the list of most-cited papers. The frequent citation undoubtedly stems from the wide use of this cloned line in many fields of investigation.

"When I first went to the National Cancer Institute in 1947, the growth of single isolated cells was a challenging problem. A theory proposed to explain the many unsuccessful attempts was that the fixed tissue cell has a limited autonomy as a physiological unit and that exchange between cells in a tissue by bridges or contacts is required for initiation of proliferation. Our working hypothesis was that even the best culture media were so inadequate as to need extensive modification by the living cell before they would be suitable for single cell growth. We attempted to reduce the volume of culture medium bathing the single cell to that volume which the cell can adjust, as calculated from a mass culture. A capillary tube appeared to be the most practical culture vessel to allow handling of such a limited volume of medium without evaporation, pH change, or bacterial contamination. By immersing the capillary tube in a larger vessel of culture medium, a slow renewal of culture medium could be effected by diffusion, and ultimately the cells could migrate through the open ends of the capillary into the larger vessel. When emerging from the tube, a preconditioned medium was found necessary.

"Single cells were isolated by means of a capillary pipette. The terminal capillary tube was heat-sealed at each end, and scanned for well-separated cells. Segments approximately 5-8 mm long, each containing a single cell, were cut from the tube and inserted in a culture vessel. One technical problem, movement of fluid in the capillary tube during cutting, was solved later by using precut 8 mm-long capillaries. With this capillary technique, we cloned cells from freshly isolated normal and malignant tissues of several species.

"Single cell isolation techniques have not been widely used in recent years, for many investigators have been satisfied with colony isolates as clones. However, the plating of single cell suspensions without scoring for single cells and without rigid segregation of such cells from other colonies or cells dislodged in the fluid medium provides no assurance that the colony is, in fact, a clone. The capillary tube is a useful microvessel that has certain advantages over other more recently introduced techniques. Besides protecting the cell from excessive evaporation and pH changes, the tube can be easily rolled over to examine all surfaces for contaminating cells."