

# This Week's Citation Classic

Miller R G & Phillips R A. Separation of cells by velocity sedimentation. *J. Cell. Physiol.* 73:191-201, 1969.[Dept. Medical Biophysics, Univ. Toronto, and Ontario Cancer Inst., Toronto, Ontario, Canada]

**A simple and reproducible method for fractionating populations of living cells by velocity sedimentation in the Earth's gravitational field was described. Use of the procedure as an analytical tool rather than as a method for purifying cells was emphasized. [The SC<sup>1</sup>® indicates that this paper has been cited in over 915 publications since 1969.]**

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"After obtaining my PhD in nuclear physics in 1966, I decided to change fields and went to the lab of J.E. Till at the Ontario Cancer Institute to do a postdoc in cell biology. He and E.A. McCulloch were then deeply involved in studies of the hemopoietic stem cell, particularly the factors governing whether it underwent self-renewal or became committed to a particular differentiation pathway. It was felt that a population of 'pure' stem cells would greatly aid these studies and Till suggested I try to develop an appropriate separation procedure. The underlying concept was that cells differing in biological function might also differ sufficiently in various physical parameters that useful separations could be achieved.

"I chose to develop sedimentation separation. Cells are essentially spheres which will fall through a viscous fluid such as saline at a rate determined primarily by their size but also to some extent by their density. I first built an apparatus according to the design of Mel.<sup>1</sup> This evolved, in a number of discontinuous steps, into the

much simpler and more powerful apparatus described in the highly cited article, an apparatus similar to but somewhat simpler than one independently developed by Peterson and Evans.<sup>2</sup>

"Why has this paper become so highly cited? There are, I think, two reasons. First, it describes a cell separation method which many people have found useful. Second, it embodies a new way of thinking about the analysis of cell populations, a way of thinking being developed at the same time (but using different separation procedures) by R.C. Leif in the US, K. Shortman in Australia, and K. Zeiller in the Federal Republic of Germany (reviewed in reference 3).

"By the time the 1969 article was written, R.A. Phillips, another postdoc in the lab, and I had begun to make extensive applications of the procedure (reviewed in reference 4). In the course of these experiments we realized that the primary use of the procedure was not so much in purifying a particular type of cell but in the analysis of differentiating cell populations. Thus, cells of a given type could be characterized with their sedimentation velocity in the same way that a biochemist can use the molecular weight estimated from a Sephadex column to characterize a molecule. To associate a function with a particular cell type, both function and morphology could be assessed independently for each fraction from the separation and a correlation sought between the two. Secondly, the procedure could be used to separate one type of cell from another, an often useful result even if neither cell type is obtained in very high purity. Thirdly, the procedure could be used to purify cells of a particular type. This last, although it was the initial objective, was seldom successfully achieved. Newer methods (e.g., fluorescence-activated cell sorting) can now often achieve this objective if the separation can be directly based on a biological marker for the function of interest (reviewed in reference 5). However, the first two objectives are often still most effectively achieved with the procedure of our 1969 paper (updated in reference 6)."

1. Mel H C. Sedimentation properties of nucleated and non-nucleated cells in normal rat bone marrow. *Nature* 200:423-5, 1963.
2. Peterson E A & Evans W H. Separation of bone marrow cells by sedimentation at unit gravity. *Nature* 214:824-5, 1967.
3. Shortman K. Physical procedures for the separation of animal cells. *Annu. Rev. Biophys. Bioeng.* 1:93-130, 1972.
4. Miller R G, Gorczynski R M, Laflour L, MacDonald H R & Phillips R A. Cell separation analysis of B and T lymphocyte differentiation. *Transplant. Rev.* 25:59-97, 1975.
5. Miller R G & Price G B. Cell separation and surface markers. *Clin. Haematol.* 8:421-34, 1979.
6. Miller R G. Separation of cells by velocity sedimentation. (Bloom B R & David J R, eds.) *In vitro methods in cell-mediated and tumor immunity*. New York: Academic Press. 1976. Vol. 2. p. 283-307.