

Dexter T M, Allen T D & Lajtha L G. Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. *J. Cell. Physiol.* 91:335-44, 1977.

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This paper describes a culture system whereby proliferation of multipotent haemopoietic stem cells, differentiation into lineage-restricted progenitor cells, and development of progenitor cells into mature functional blood cells can be maintained for several months. The cultured cells are functionally normal and can reconstitute haemopoiesis *in vivo*. [The SC¹® indicates that this paper has been cited in over 630 publications.]

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As a graduate student and postdoctoral fellow in Laszlo G. Lajtha's department at the Paterson Laboratories, I was studying leukaemia induction *in vivo* at a time when *in vitro* systems were being developed for investigating malignant transformation in fibroblastoid cells. Realising the tremendous advantage that *in vitro* systems have, I attempted to set up such a culture for haemopoietic cells. When the work commenced, short-term clonogenic cultures of haemopoietic cells had already been described,^{1,2} but these cultures favoured differentiation and maturation of already fairly mature haemopoietic progenitor cells. What was really required for my studies on leukaemogenesis was a system where haemopoiesis could be maintained for months. Fortunately, I did not know that many such attempts had been made in the past, all unsuccessful. (A thorough literature search might have discouraged me from undertaking the task, but computers were not accessible then!)

Trying to reproduce *in vitro* the environment conducive to leukaemogenesis *in vivo*, I looked at the possibility of culturing stromal cells from the bone marrow and then adding haemopoietic cells; in other words I was trying to establish *in vitro* the tissue architecture of the sites supporting the growth and differentiation of normal stem cells. The early cultures were terrible, and I can recall being excited when haemopoiesis could be maintained for only two or three weeks. I was, however, lucky in two respects. First, we had several batches of horse serum with remarkable activity for the growth of haemopoietic cells and stromal cells. Second, I reduced the culture temperature to 33° C. In these conditions haemopoiesis could be maintained for several months.

I am often asked why I cultured cells at 33° C. Because of my interest in chemical leukaemogenesis, I thought perhaps that reducing the temperature would slow down cell growth and increase the likelihood of mutagenic lesions being fixed. It didn't, but since the cultures now grew so well, it allowed us to pursue the experiments for which the cultures were developed.³

At the time I did not realise the impact that this work would have on the field. Fortunately, several colleagues in the department had more vision than I, including "Farmer" Ray Schofield and "Lacrosse" Brian Lord; Lajtha, my "boss" and mentor, who allowed me absolute freedom to pursue my interests; and Terry Allen, a collaborator with whom I still work and who continues to share his experience and expertise in dissecting the intracellular relationships in haemopoiesis.⁴ Also, "Bun" McCulloch must be acknowledged for allowing publication in the light of some "unusual" comments from the referees.

The work is highly cited because, like many *Citation Classics*, it describes a system that can be used in various ways,^{5,6} and it has become a standard technique in many laboratories interested in cell differentiation, communication, and transformation.

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