

# This Week's Citation Classic

MacKinney A A, Jr., Stohlman F, Jr. & Brecher G. The kinetics of cell proliferation in cultures of human peripheral blood. *Blood* 19:349-58, 1962. [Natl. Inst. Arthritis and Metabolic Diseases, Natl. Insts. Health, Public Health Serv., US Dept. Health, Education, and Welfare, Bethesda, MD]

**Human peripheral blood cells in tissue culture increased their synthesis of DNA beginning at 24 hours and peaking at 72 hours. Morphologic and kinetic analysis of the cells using tritiated thymidine and colchicine indicated that small lymphocytes were induced to divide. [The SC<sup>®</sup> indicates that this paper has been cited in over 325 publications since 1962.]**

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January 14, 1983

"The mature lymphocyte's capacity for division had been the subject of controversy for 50 years. Growth of thymus<sup>1</sup> or lymph nodes in some tissue culture experiments had been described, but a reproducible method for peripheral blood cells did not emerge. Skeptics were convinced that the peripheral blood lymphocyte was, like the erythrocyte and granulocyte, incapable of division.

"In the 1950s, plant lectins, e.g., phytohemagglutinin (PHA), had been used to sediment red cells from peripheral blood. Purified white cells from these preparations were capable of dividing *in vitro*. Nowell<sup>2</sup> showed that the plant lectin PHA was critical to cell growth. Hungerford<sup>3</sup> exploited this technique to study human chromosome abnormalities.

"In 1960, I was working for Fred Stohlman and George Brecher as a hematology research associate at the National Institutes of Health after four years of internal medicine residency. Stohlman returned from a conference in Edinburgh, Scotland, with news about this new peripheral blood tissue culture technique. In our discussion of this discovery, Brecher remarked that the central question was which kind of white blood cell was growing. It was a most attractive problem and I was delighted with the chance to work on it

"By morphologic analysis of the five different white cells in the blood, we noted striking changes in the two most abundant forms: granulocytes disappeared by 24 hours, while lymphocytes became swollen and irregular, displayed nucleoli, and were changed into clusters of rather malignant-looking, large, mononuclear cells by 72 hours.

"Using tritiated thymidine and autoradiography to identify cells in DNA synthesis, we found a rapid increase in the number of lymphocytes showing the nuclear thymidine label beginning at 24 hours, with a peak of about 50 percent of the cells in DNA synthesis at 72 hours. Colchicine (used to arrest cells in metaphase) showed that the first divisions occurred at 40 hours. In other experiments, rare cells which were in division at the outset of the culture were tagged and their contribution to the large pool of replicating cells was excluded. We concluded that the replicating cells were derived from a relatively large population of previously nondividing lymphocytes. These data were quickly confirmed.<sup>4,5</sup>

"Initially, lymphocyte culture was a biological oddity since plant lectins could hardly be regarded as normal initiators of cell division. But antigens, bacterial and viral,<sup>6,7</sup> as well as foreign lymphocytes,<sup>8</sup> were soon added to the list of stimuli, and lymphocyte culture became a major tool of immunology. It was now possible to study lymphocyte competency and diversity with a high degree of sensitivity. It was found that cells of thymic origin (T lymphocytes) responded to PHA, while cells destined to make immunoglobulin (B cells) responded to other plant lectins that could induce immunoglobulin synthesis in culture. There are now more than a dozen cells which fit under the umbrella term 'lymphocyte' and the variety of T lymphocytes continues to grow. Lymphocyte tissue culture is a standard technique of the geneticist, transplant surgeon, immunologist, virologist, oncologist, and cell biologist. It has been estimated that over 10,000 papers have emerged from our apparently innocent observation Brecher predicted that 'we should get a little mileage out of this paper.' None of us could have imagined the scope of the present scientific effort."

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4. Carstairs K. Transformation of the small lymphocyte in culture. *Lancet* 2:984, 1961.
5. Cooper E H, Barkhan P & Hale A J. Letter to editor. (Mitogenic activity of phytohemagglutinin.) *Lancet* 2:210, 1961.
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