

Nowell, Peter C. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Research* 20:462-6, 1960.

Mucoprotein plant extract, phytohemagglutinin (PHA), was found to be a specific initiator of mitotic activity. The paper suggested that the mitogenic actions of PHA did not involve mitosis *per se* but the alteration of circulating monocytes and large lymphocytes to a state in which they are capable of division. [The *SCI*[®] indicates that this paper was cited 917 times in the period 1961-1975].

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"The PHA story is another triumph for the Princes of Serendip. Upon joining the Penn faculty in 1957, I began working with short-term cultures of human leukemic cells, using a method developed by Edwin Osgood.¹ His technique employed PHA, a lectin extracted from Navy beans, to agglutinate and remove erythrocytes in preparing leukemic cells for culture. My technician and I traveled across town one day to obtain some leukemic blood, but found the patient was in remission. Rather than waste the trip, we cultured the leukocytes anyway, and to our surprise, found many mitoses. Culture of our own blood promptly followed, confirming the suspicion that normal leukocytes were proliferating in our cultures.

"My major interest then involved attempts to cause leukemic cells to differentiate in culture, but some time was spent on chromosome studies in leukemia, with David Hungerford, and on efforts to explain the growth of normal leukocytes in culture. The cytogenetic work produced the Philadelphia chromosome, but the studies of normal cells seemed initially less rewarding.

Variables in the culture system were eliminated one by one, and eventually PHA emerged as the initiator of mitosis. I agreed with one reviewer of the manuscript who indicated it was an interesting observation, but of no obvious significance. PHA-stimulated cultures did soon become widely used for human chromosome studies, although the chief supplier of PHA created a major crisis by marketing a non-mitogenic product just as many laboratories began to use it. Interestingly, the mitogenic component of PHA still has not been completely identified.

"I did not recognize that the responding cell was a small lymphocyte, believing large lymphocytes and monocytes were more likely candidates. Unfortunately, I chose blood from chronic lymphocytic leukemia to test the response of small lymphocytes, a population which we now know to consist largely of non-responsive B cells. Only when workers such as Gowans² demonstrated the proliferative capacity of the small lymphocyte *in vivo*, and its central role in immune responses, were others stimulated to use lymphocyte cultures triggered by PHA (and eventually many other agents) as *in vitro* models for studying various aspects of immunity and also the general problem of mitogenesis in resting mammalian cells. These investigations continue today in many laboratories, including my own.

"Now I write expensive, and I trust, adequately-focused grant proposals. The PHA story evolved in a laboratory which cost \$5,000 annually to run, including technician, and in which the research goals were fuzzy, at best. One can only hope that the present research-academic establishment will continue to include enough money and time to allow young faculty the luxury of following up the *unexpected* result, which provides much of the excitement and many of the new directions in biomedical research."

1. Osgood E E & Krippaehne M L. The gradient tissue culture method. *Experimental Cell Research* 9:116-27, 1955.
2. Gowans J L, Gesner B L & McGregor, D D. The immunological activity of lymphocytes. *CIBA Foundation study group No. 10: Biological activity of the leucocyte.* (Wolstenholme G E W & O'Connor M. ed.) Boston: Little, Brown & Co., 1961, pp. 32-40.