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This Week's Citation Classic

Marbrook J. Primary immune response in cultures of spleen cells. *Lancet* **2**:1279-81, 1967. [Walter and Eliza Hall Inst. Med. Res., Melbourne, Australia]

Spleen cells from normal unimmunized mice were cultured on dialysis membranes with heterologous erythrocytes above a reservoir of culture medium. Antibody-secreting cells appeared in significant numbers with a peak response after four to five days. [The *SCI*® indicates that this paper has been cited over 365 times since 1967.]

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"I went to the Walter and Eliza Hall Institute in Melbourne in 1966 with the idea that I should learn enough about immune cells to consider studying the molecular biology of antibody production. To contemplate using radioactive precursors of nucleic acids and proteins it was essential to move away from *in vivo* studies. When I joined Jacques Miller's group, he was keen to establish assays for small numbers of immunocompetent cells, particularly in relation to the effects of thymectomy. The idea was to culture lymphoid cells and use the haemolytic plaque assay to measure responsive cells.

"To obtain an *in vitro* immune response involved several miserable months in which immune spleen cells were cultured with sheep erythrocytes in every culture system I could find. We were able to modify the Bradley-Metcalf bone marrow culture system in soft agar to detect the secretion of haemolytic antibody secreting cells.[1] Although viable cells formed clusters in liquid medium there was a consistent loss of cell viability at high cell concentrations. The success with growing cells in agar suggested that cultures at high cell densities needed to be associated with a reservoir of nutrients. The first burst of haemolytic plaque-forming cells was generated when a single spleen cell suspension was placed in a dialysis bag with sheep erythrocytes, the bag being immersed in medium. It was from this experiment that the culture vessel was developed. It was obvious in 1966 that culture techniques were becoming mandatory for immunological studies so that the development of 'primary' responses *in vitro* was timely.

"In retrospect, the development of the work owed a lot, not only to encouragement from numerous colleagues at the Hall Institute, but also to the scientific climate that they generated. The work of Miller and Mitchell[2] on T and B cell collaboration and the cell separation techniques developed by Shortman[3] led naturally to detailed investigations of cell collaboration and B cell differentiation. It is clear that the site influences citations. If the 'collection of citations' can be compared to angling, it is a pursuit that flourishes in a busy stream, particularly if the stream is also a migratory route."

1. **Robinson W A, Marbrook J & Diener E**. Primary stimulation and measurement of antibody production to sheep red blood cells *in vitro*. *J. Exp Med.***126**:347-56, 1967.
2. **Miller J F A P & Mitchell G F.** Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes.*J. Exp. Med.* **128**:801-20, 1968.
3. **Shortman K.** The separation of different cell classes from lymphoid organs. II. The purification and analysis of lymphocyte populations by equilibrium density gradient centrifugation. *Aust. J. Exp. Biol. Med. Set.* **46**:375-96. 1968.
[Citation Classic. *Current Contents/Life Sciences* (14):12, 7 April 1980.]