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This Week's Citation Classic

Coulson A S & Chalmers D G. Separation of viable lymphocytes from human blood. *Lancet* **1**:468-9, 1964. [Cambridge Univ., Dept. of Pathology, Cambridge, England]

The paper describes a **technique for separating lymphocytes from human blood using defibrination and gelatine sedimentation. The lymphocytes so separated are viable and respond in tissue cultures. [The *SCI*® indicates that this paper has been cited over 290 times since 1964.]**

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"When as a second year student I went to see Professor Greaves in Cambridge in 1962 and told him I was extremely interested in doing research into lymphocytes in tissue culture he directed me to work with Dr. Chalmers, the University Haematologist. He said we should get along satisfactorily as we were both about the same size. Chalmers and I frequently discussed the optimal system for studying the small lymphocyte's repertoire. We received a lot of helpful contributions from our colleagues in the department during the happy hours spent at the 'Rose' and the 'Fountain' and other nearby hostelries during these discussions. We decided that the small lymphocytes should be isolated cells or at least a pure population in culture, certainly free of contamination by the macrophage/monocyte system which seems to serve as a glorified garbage collecting concern. In addition it would be a help if the medium was defined and the response quantitated in a meaningful way, i.e., not just by protein synthesis, or CO_2, production or DNA synthesis or some other indirect biochemical parameter, but by counting how many cells actually transformed into blast cells. In this way the interaction of lvmphocyte and antigen would approximate a chemical reaction.Another entertaining thought was that in many respects small lymphocytes behaved as though they had evolved from parasitic protozoa, patrolling their host and keeping out all unrecognised material which might herald another parasitic intruder. An extension of this thinking subsequently led to the concept of an intracellular stimulation pathway.

"Initial attempts centered on getting pure small lymphocytes. In those early days the lab was cluttered with columns of glass beads and glass wool, and bottles of assorted dead and dying small lymphocytes. One day Professor Ceppelini stopped by to visit Dr. Coombs. While they were having tea with Chalmers, Ceppelini suggested gelatine might be helpful in lymphocyte separation as it had been used by French workers in the 1930s to this end. I went off in search of some gelatine and finally obtained some from the biochemistry department which was next door to us on Tennis Court Road. The gelatine came in a very grubby can but it had impeccable credentials, coming as it did from the British Clue and Gelatine Research Association (now sadly defunct). When combined with a preliminary defibrination process, gelatine sedimentation worked extremely well.

"Subsequently in the autumn of 1963 Chalmers and I presented this technique at a local Pathology Society meeting and it seemed to evoke a certain amount of interest. Chalmers decided it should be published and arranged this with one of his friends at the *Lancet.* At that time I was sharing a house on Panton Street with a variable number of physics and chemistry research students including my sister. I seem to remember my sister actually typed the paper, rewriting and editing it in the process. When it was published the other members of the house thought it quite amusing that anybody could burst into print with anything less than five years work. Professor Greaves told me that it would be bad form to order too many reprints and that the department would not pay for more than fifty.

" The most probable reasons why the paper has been cited frequently are that the paper and the title were succinct, the method was simple and usually worked, a*nd* it was published fortuitously at a time when lymphocytes immunology and tissue culture were beginning to become bandwagons."