

This Week's Citation Classic

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. Sci.* **46**:375-96, 1968. [Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia]

Lymphocytes were separated according to their buoyant density, by centrifugation to equilibrium in continuous gradients of albumin. The procedure gave good resolution, high reproducibility, and good recovery of biologically active cells. Lymphocytes were separable into a series of discrete density subpopulations. [The *SCI*[®] indicates that this paper has been cited over 185 times since 1968.]

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"In 1964 when I began attempts to purify cells from the heterogeneous populations in lymphoid organs there was no technology available for cell separation: the best that could be done was a preliminary sorting of blood elements. After failing in attempts at affinity chromatography, I turned to methods that would separate on the basis of physical parameters, such as density. There was nothing new in the principle, the challenge being to reproducibly band and to recover in an active form labile entities whose density varied with environmental conditions such as osmolarity or pH, and which tended to aggregate, to 'stream' and to stick to the walls of the centrifuge tube. It was a bioengineering exercise.

"At the time most biologists would have considered cells as variable and

imprecise entities. Encouraged by the work of Leif and Vinograd,¹ who used the analytical precision of physicists in their study of erythrocytes, we found that lymphocytes could also be studied this way, their physical parameters being specified with amazing precision.

"The main observation from our analytical approach was that lymphoid populations consisted of many physically separable subsets. Nowadays no immunologist working with the multiple subgroups of T and B cells would be surprised by this finding, but at the time everyone hoped life would be simpler. The possibility of experimental artifacts was then investigated by ourselves and others, but the published procedure had been well controlled and no artificial source of multiple peaks has been substantiated. Subsequent work has shown the main source of heterogeneity to be distinct metabolic or activation states of lymphocytes, probably occurring within each of the several lymphocyte subclasses.

"The frequency of citation of this article certainly does not reflect frequency of usage of the full procedure since the tedious analytical approach with 20-30 fractions is just too much hard work for most immunologists. However it has served as a basic reference for density separation of cells, especially since problems of general importance in cell separation, such as osmolarity control and cell aggregation, were considered in some detail. I must have accounted for a substantial proportion of the citation count myself, since in the past we have made extensive use of the technique to study lymphocyte differentiation. Nowadays even I think it is too much hard work."

1. **Leiff R C & Vinograd J.** The distribution of buoyant density of human erythrocytes in bovine albumin solution. *Proc. Nat. Acad. Sci. US* **51**:520-8. 1964.