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This Week's Citation Classic

Böyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97):77-89, 1968.
[Norwegian Defence Research Establishment, Division for Toxicology, Kjeller, Norway]

A technique for isolation of lymphocytes is described. Blood is layered over a fluid with density of 1.077 g/ml. After centrifugation, red cells and granulocytes have formed a sediment at the bottom, and lymphocytes and monocytes are easily collected from the interface between plasma and the separation fluid. [The *SCI*® indicates that this paper has been cited in over 5,850 publications since 1968.]

Arne Böyum
Division for Toxicology
Norwegian Defence
Research Establishment
N-2007 Kjeller
Norway

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"When the work started in 1961, the goal was to isolate bone marrow lymphocytes for studies of immune reactions following bone marrow transplantation. Fortunately, I was happily unaware of the obstacles ahead, and unfortunately, ignorant of Newton's law of motion. I figured that, with the appropriate gradient design, the slowly sedimenting lymphocytes could easily be picked up after centrifugation. Today, it is with understanding rather than self-irony that I reread in my proposed research protocol that the work would last one to two years. It took six years and I sure remember those hours at the microscope.

"The first two-year period was one of striking discrepancy between effort and progress. No matter how fancy the gradient design, the lymphocytes never behaved as predicted. I even constructed my own monstrous centrifuge. It never worked, and it is still there in the attic to remind me of my scientific infancy. With high polymer compounds as gradient material, the problem was that density and viscosity could not be varied independently. It occurred to me that this difficulty could be overcome using a mixture of two compounds. This turned out to be the first breakthrough. The choice of an X-ray contrast medium to adjust the density was a lucky one. Next, I ended up with different sugar polymers as partners for viscosity control. Moreover, for simplification, I switched from bone marrow to blood.

"A small episode in the lab radically changed the further work. On one occasion, when the gradient was already loaded and the centrifuge being used, while having to wait for a few minutes, I noted that the red cells started to aggregate at the interface, and fell rapidly to the bottom. So, I left the tube on the desk to see what finally happened. This is where a long study of sedimentation in a 1 g gravity field started. (My colleague who used the centrifuge still complains he never got the credit he deserved for his contribution.) I tested out every possible variable, and gradually learned something about the physicochemical mechanisms in a 1 g sedimentation process. This knowledge was then applied to centrifugal techniques. After a total of 3½ years I was able to obtain a pure suspension of mononuclear blood cells, but it took another year to perfect the technique. Altogether, it was a matter of finding the right density and composition of the separation fluid, and a suitable cell concentration. The technique is generally applicable to blood lymphocyte isolation.[1,2] This paper has been highly cited because it has the advantage of being a simple one-step procedure."

1. Böyum A. Separation of blood leucocytes, granulocytes and lymphocytes. *Tissue Antigen.* 4:269-74, 1974.
2. ·····. Isolation of lymphocytes, granulocytes and macrophages. *Scand. J. Immunol.* 5(Suppl. 5):9-15, 1976.