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Clausen J E. Tuberculin-induced migration inhibition of human peripheral leucocytes in agarose medium. *Acta Allergol.* **26**:56-80, 1971.
[Medical Department A, Rigshospitalet, University Hospital, Copenhagen, Denmark]

An agarose technique able to demonstrate tuberculin-induced migration inhibition of human peripheral leucocytes is described. The inhibition was well correlated to the sensitivity of the cell donor as expressed by the delayed intracutaneous reaction to tuberculin. [The *SCI*® indicates that this paper has been cited in over 250 publications since 1971, making it the most-cited paper published in this journal to date.]

Jens Erik Clausen
Department of Medicine
Ringsted County Hospital
DK-4100 Ringsted
Denmark

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"My interest in cell-mediated immunity was initiated in 1967 when I was an intern in medicine at Rigshospitalet, University Hospital of Copenhagen. There, I was stimulated by close contact with Gunnar Bendixen and Mogens Søborg, who had adapted the capillary tube technique for the study of cell-mediated immunity in man.[1] Even though the capillary tube technique was valuable in the study of cell-mediated immunity in laboratory animals as well as in man, techniques more sensitive were needed.

"In guinea pig experiments, Carpenter *et al.*[2] observed that splenic and peritoneal exudate cells placed in wells cut into agar medium migrated out beneath the gel. Therefore, I started to develop an agarose migration inhibition technique for *in vitro* demonstration of cell-mediated immunity in man.

"My studies showed that the agarose technique was suited to demonstrate antigen-induced leucocyte migration inhibition and was more sensitive than the capillary tube technique. Furthermore, results obtained by the agarose technique were easier to reproduce. But the test will be useless if all the steps are not thoroughly standardized. It is, for example, very important to use a physiological pH and temperature and to make sure that the cell cultures are incubated in air saturated with water during the culture period. Nevertheless, difficulties may arise because of variations from batch to batch of agarose or culture medium.

"The agarose migration technique has also been used to demonstrate migration inhibitory factor in cell-free supernatants from stimulated leucocyte cultures and to examine the role of the various leucocyte types in the migration reaction.

"A review[3] and my previously published papers were accepted as a doctoral thesis by the University of Copenhagen in 1975. Since 1976, I have been chief physician of a medical department in a small county hospital and therefore unable to continue the immunological studies. In my last study,[4] I demonstrated that the agarose technique was suitable also in investigations of phytohemagglutinin (PHA)-stimulated leucocytes.

"I believe that my paper is frequently cited because techniques to demonstrate the leucocyte migration inhibition reaction as an *in vitro* parameter of cell-mediated immunity had been urgently needed. Even if I am no longer working with the test myself, I am glad to know that the agarose migration technique is still used in the Laboratory of Clinical Immunology at Rigshospitalet, where most of my studies were carried out."

1. **Søborg M & Bendixen G.** Human lymphocyte migration as a parameter of hypersensitivity. *Acta Med. Scand.* **181**:247-56, 1967. (Cited 575 times.)
2. **Carpenter R R, Barsales P B & Ganchan R P.** Antigen-induced inhibition of cell migration in agar gel, plasma clot, and liquid media. *J. Reticuloendothel. Soc.* 5:472-83, 1968.
3. **Clausen J E.** The agarose migration inhibition technique for in vitro demonstration of cell-mediated immunity in man. A review. *Dan. Med. Bull.* **22**:181-94, 1975.
4. ----------, Migration inhibition of human leucocytes mixed with phytohemagglutinin-preincubated leucocytes. *Acta Allergol.* **30**:239-49, 1975.

