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Method for Determination of Chemoattraction in *Tetrahymena pyriformis*

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Abstract. Earlier works presented a variety of techniques for detection of chemoattractants in ciliates. The present agar layer model and the used double-P cutter give an easy, reproducible way of quantitative evaluation of positive chemotaxis. Beside the two-channel chamber, the multi-channel one seems to be also useful in characterization of different taxons or different chemoattractants.

Tetrahymena, *Paramecium* and other protozoa demonstrate a sensitive responsiveness to chemical factors of the environment [3, 10, 14, 16]. Low concentrations (10^{-12} to 10^{-6} M) of biologically active substances can modify the swimming behavior [1, 5, 11] of these cell types, and the direction of movement is usually also changed. Hormones, drugs, or odorants are effective chemoattractant molecules, and their actions are supposed to act on the membrane-receptor level [6, 7]. In this way these unicellular organisms are good model objects to investigate and demonstrate physiological effects by the study of altered chemotactic activity of the cells. There are several easy techniques developed for these investigations. Chronologically old methods applied cellophane or filter paper with the chemoattractant under the cover glass [2] or measured the distribution of cells in a thin vessel filled with a gradient of the test solution [13]. A more recent method is the capillary technique, when two containers are set, the outer contains the test cells and the inner chamber dips into the outer one. The inner one contains the attractant, and the cells can move in through the pores of capillaries [9]. Another method uses a thin layer of agar. In this case the cells are placed on one side and the chemoattractant on the other side of the gelatin. The diffused molecules of the attractant will induce the cells to drill themselves through the gelatin layer [8]. T-mazes were also introduced as special tools to investigate the chemotactic response to at least two substances [15]; its weakness is the time-consuming preparative work and the difficult monitoring of individual cells. A well test apparatus was also introduced to test short-term responses [17]. Finally, self-made equipment was published that uses a capillary, and the two ends of this glass tube are connected to containers with cells or attractant. This equipment is connected to a mechanically driven board, and it is easy to follow the cells not only in mass but individually as well [4, 12]. In this case there is the possibility to get information about the swimming behavior of the cells.

All the above-mentioned techniques are effective and reliable. In some circumstances there are limitations of usage, e.g., it may be hard to follow the movement of cells, not all the cell types are able to drill into the agar, it may be hard to apply the cells into the small capillary, etc.

Materials and Methods

Our agar-plate method also has limitations of application, but its simple technique and reproducible character give it some advantages.

Device setup and use. It requires a Petri dish with 0.5% agar. It is best to prepare it in a microwave oven and cool it for 2 h at room temperature. Two containers are prepared (cut), each has continuous contact with an individual channel. The position of containers and channels is shown on Fig. 1. To gain standard size of containers (15 μl) and channels and to have standard distances between them, we developed a so-called double-P cutter (Fig. 2), which also simplifies the preparative work. After preparing the agar layer, we have two parallel channels (20–20 mm each) with small containers on the distant ends. The next step is to fill the two containers as well the channels; on one side with the test cells and on the other side with the chemoattractant. The suggested density of cells is 5×10^3 cell/ml of log phase *Tetrahymena pyriformis* GL cells in culture

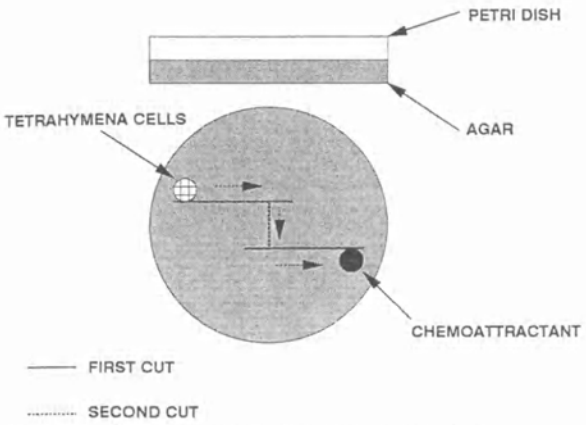
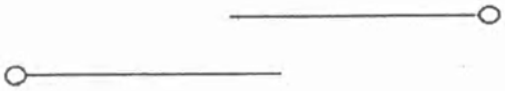


Fig. 1. Scheme of the single-path chemotaxis chamber.



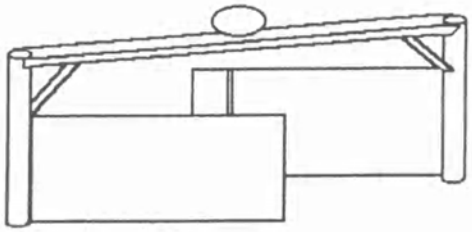


Fig. 2. The structure of the proposed double-P cutter. The upper drawing represents the line-drawing of the edge; the bottom sketch shows the whole structure of the cutter.

medium at 28°C. Until this point the two systems have no connection, and according to diffusion tests by dyes (e.g., low-molecular-size toluidine blue) the applied distances and the concentration of agar do not allow diffusion in the agar during the application period of 10 min preincubation of cells. The start point of the experiment is when we for a second time cut a perpendicular channel (10 mm) to the two parallel ones. This makes the system continuous, the gradient between the two containers will drive the motion of the cells. For this cut it is suggested to use the same material and thickness as the seal was made. According to our experiments, the suggested incubation time is 10 min. To stop chemotaxis, a wide range of fixatives (4% formaldehyde, Bouin, 2.5% glutaraldehyde) could be used. A retrograde schedule of fixation is suggested: first a drop of 3 μl into the container filled with the attractant substance, secondly into the joining channel, and finally into the test-cell containing hole. This way the postexperimental, artificial movement of cells is inhibited. The number of positively responding cells is determined in the container filled with attractant by stereomicroscope in dark field.

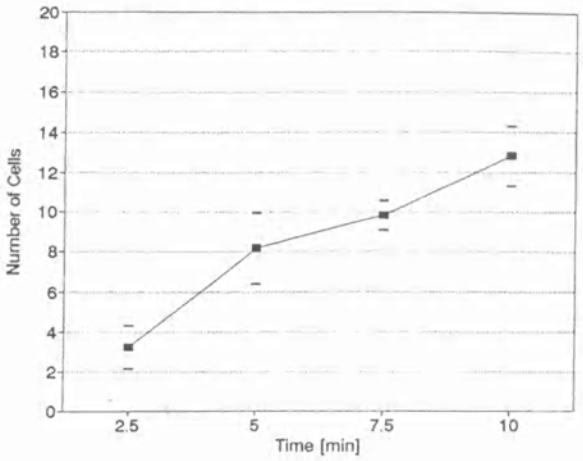


Fig. 3. Time course (2.5–10 min) of chemotaxis of 24-h culture of Tetrahymena cells in the single-path agar chamber. The applied attractant was fresh culture medium.

Results and Discussion

Figure 3 demonstrates a time course study of a 24-h culture of *Tetrahymena pyriformis GL* cells when the attractant was fresh culture medium (0.1% yeast extract in 1% Tryptone, Difco, Michigan, USA).

The above-described method gives the possibility to test two to three groups of cells in a 12-cm-diameter Petri dish. To stabilize the conditions is very easy as well as to test different environmental effects (thermal, magnetic etc). We can follow the movement of the cells by microscope or camera. The disadvantage is that we can evaluate quantitatively only the positive chemotactic responses.

The suggested method has more potential possibilities. From the above mentioned single-channel type it is easy to develop a multi-channel chamber (Fig. 4A), where the two or more directions of migration can be evaluated. In this case the test ciliates are placed into the central container, and different attractants are placed into the bowls around. In another variation we can test differently pretreated cells or different cell lines. In this case the central container is filled with the attractant, and the containers around are filled with the cells. In these forms of test the sure and equal start point requires another tool to cut the channels across at the same time (Figs. 4B and 5).

Obviously, the two presented techniques also have problems, but we hope that their easy reproducibility and their flexible parameters make them useful for protozoologists.

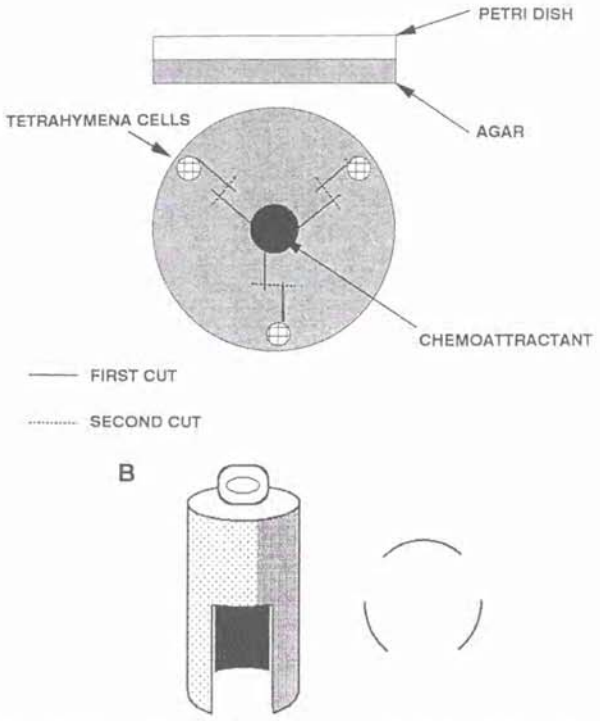


Fig. 4. Scheme of the multi-path chemotaxis chamber (A) and the proposed cutter for simultaneous starting of chemotaxis (B).

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