Electronic Separation of Biological Cells by Volume

Abstract. A device capable of separating biological cells (suspending in a conducting medium) according to volume has been developed. Cell volume is measured in a Coulter aperture, and the cells are subsequently isolated in droplets of the medium which are charged according to the sensed volume. The charged droplets then enter an electrostatic field and are deflected into a collection vessel. Mixtures of mouse and human erythrocytes and a large volume component of mouse lymphoma cells were separated successfully. In tests with Chinese hamster ovary cells essentially all cells survived separation and grew at their normal rate.

A device has recently been developed which physically separates particles, including biological cells, on the basis of electronically measured volume. Figure 1 is an illustration of the cell separator. A cell suspension (under 4 atm pressure) enters the droplet generator (C) by way of a tube (D) and emerges as a high-velocity fluid jet (E) (jet diameter, 36 μ; velocity, 15 m/sec). A piezoelectric crystal (A), driven at a frequency of 72,000 cy/sec, produces vibrations which pass down the Lucite rod (B) into the liquid within the droplet generator. The shape of the rod (catenoidal) serves to amplify the magnitude of the vibrations within the liquid. The velocity fluctuations of the emerging liquid produce bunching of the liquid column. Surface-tension forces cause the disturbances to grow until the jet is broken into 72,000 very uniform droplets each second.

Droplets are charged as they pull away from the charged liquid column. A charge is produced on the liquid column by applying a voltage at point

Table 1. Effects of ec dysion on nuclear membrane permeability. The difference is significant at .001 level.

<table>
<thead>
<tr>
<th>Case</th>
<th>Nuclear membrane resistance (ohm-cm²)</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals</td>
<td>0.72</td>
<td>0.09</td>
<td>18</td>
</tr>
<tr>
<td>Ec dysion-treated animals</td>
<td>1.38</td>
<td>0.10</td>
<td>28</td>
</tr>
</tbody>
</table>

* Control and ec dysion-treated groups are from the same batch of animals of the early fourth instar stage.

No significant changes in resting potentials of cell membrane potentials were seen either during development or as a result of ec dysion treatment. The changes in nuclear membrane resistance are thus not due to changes in ion concentration in the cytoplasm, such as K⁺ or Cl⁻ ions; the resting potential of the cell membrane is sensitive to such ion changes in these gland cells (11), as it is in many others. Resting potentials of the nuclear membrane envelopes (I) in these cells were too small (2 to 5 mv) in relation to their individual fluctuations to show significant differences.

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References and Notes


3. The physiological solution had the following composition (values are millimoles): NaCl, 87.2; KCl, 2.73; CaCl₂, 1.28; NaH₂PO₄, buffer, 10, adjusted to pH 6.3. Care was taken to avoid injury to the cells during isolation and manipulation. Nuclear and cell transparencies, nuclear and cell membrane resting potentials and resistances, and current leakage were routinely checked. Only material satisfactory with regard to all of these indexes was used for the experiments. Developmental stages were classified according to characteristics of size and infolding of imaginal discs, and of size and color of meso- and metathorax of the larva. (For morphological description see K. Strenzke, Arch. Hydrobiol. 18, Suppl. 207 (1950); ibid. 56, 1 (1959).) For details of electrical techniques see 1.

4. Successive points on the curve differ at a level of significance better than .001, except for the points of the early and mid-prepupa stages, which differ at a level of .004.

5. Laufer and Y. Nakase, J. Cell Biol. 25, 95 (1965); H. Laufer, personal communication. For changes in other cell types, as revealed by histochemical techniques and a general study of hormonal effects, see V. B. Wigleyworth, Sympos. Soc. Exp. Biol. 11, 204 (1957).


10. We are indebted to Prof. Peter Karlson, Marburg, for a gift of ec dysion. The 2 al of ec dysion solution was injected into the ventral aspect of the penultimate segment of the larva by means of a glass micropipette of about 5-μ tip diameter. For a description of hormone activity units, see P. Karlson, Ann. Sci. Nat. Zool. 18, 125 (1965).


12. The development is not synchronous in a given larva batch. The time ranges, in days after egg hatching, were as follows for each stage: fourth instar stage, early, 13 to 17; late, 15 to 20; prepupa stage, early, 18 to 20; middle, 20 to 22; and late, 20 to 24 days (20°C).

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method of forming, charging, and deflecting droplets is a modification of that developed by Sweet as an ink writing oscillograph (2).

Figure 2 shows the volume distribution of a mixture of mouse and human red blood cells (volumes approximately 50 and 100 $\mu$m$^3$, respectively) before and after separation in physiological saline. The apparatus was adjusted to separate all cells of volume greater than approximately 80 $\mu$m$^3$. The closed circles represent the volume distribution of the unseparated mixture; the triangular data points represent the volume distribution of the separated cells.

Figure 3 shows a volume distribution of mouse lymphoma cells (3) suspended in standard growth medium. That portion of the distribution, before separation, which rises out of the top does not represent cells of small volume but rather debris present in the growth medium. In this experiment the larger (presumably older) cells were separated from the randomly growing culture. The second curve (triangles) is the volume distribution of the separated cells.

Viability of the cells after separation is important in many applications of this device. To establish what fraction of the cells survives separation, several experiments were performed with Chinese hamster ovary cells (4). Growth rate, mitotic index, ability to incorporate tritiated thymidine into DNA, and permeability to trypan blue were used as criteria of survival. In no case was viability of the separated cells less than 96 percent. Cells, grown and passed through the separator in Ham's F-10 medium (5), exhibited a mean generation time (21 hours) identical with that of a nontreated control.

The present system can analyze from 500 to 1000 cells per second, and up to 50 percent may be separated.

The separations described here were made with a simple two-vessel collection system, one for the charged and deflected droplets and the other for the uncharged droplets. Because of the primitive nature of this first system, droplets were charged in groups of seven. Reduction of this number to four or fewer with forthcoming mechanical and electronic improvements is feasible.

In principle, the system is capable of separating minute particles (biological or nonbiological) according to other electronically measurable characteristics such as optical density, reflectivity, or fluorescence. It may be possible also to measure simultaneously two (or more) characteristics of a cell and to make separation dependent on the ratio of such characteristics.

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References and Notes

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