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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Resistance (ohm-cm²)</th>
<th>Cases (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals</td>
<td>0.72</td>
<td>18</td>
</tr>
<tr>
<td>Ecdyson-treated animals</td>
<td>0.12</td>
<td>28</td>
</tr>
</tbody>
</table>

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

and varies in concentration in the body fluids during development (9). We injected 2 μl of physiological solution containing 20 to 33 activity (Calliphora) units (0.2 to 0.33 μg) of a crystalline preparation of ecdyson into the larvae (10) and measured resistance of the nuclear envelope 1 to 5 hours thereafter. Larvae injected presented specific membrane resistance due to ecdyson treatment; the difference is highly significant. The mean resistance of the larvae (10) and measured resistance of the nuclear envelope 1 to 5 hours thereafter is about as soon as the measurements were started and ecdyson-presented specific membrane resistance due to ecdyson treatment is the difference.

Electronic Separation of Biological Cells by Volume

Abstract. A device capable of separating biological cells (suspended 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 (1) by way of a tube (2) and emerges as a high-velocity fluid jet (3) (jet diameter, 36 μ; velocity, 15 m/sec). A piezoelectric crystal (4), driven at a frequency of 72,000 cps/sec, produces vibrations which pass down the Lucite rod (5) 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 bunches 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...
K relative to point M, which is in contact with the emerging stream. As the droplet separates, it carries away a charge proportional to the instantaneous charge on the column of liquid. In this way one or more droplets may be charged. The charged droplets are then deflected (H) on entering the electrostatic field (7000 volts per centimeter) between the deflection plates (G). A series of collection vessels (L) receive the deflected droplets.

The sequence of events leading to separation is as follows. Cell volume is sensed as the cell passes through a Coulter aperture (I) within the droplet generator (C). An electric pulse proportional to cell volume is obtained at J. The cell then emerges in the jet and arrives at the separation point (I) within the charging collar (F) 250 μsec later. The size of the charging pulse needed to deflect droplets into the proper vessel is electronically determined from the cell volume pulse. Approximately 200 μsec later the charging pulse is applied to the charging collar (K); the cell is caught in a forming droplet; the droplet is charged and then deflected by the electrostatic field into the appropriate collection vessel. The 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 μm³, respectively) before and after separation in physiological saline. The apparatus was adjusted to separate all cells of volume greater than approximately 80 μm³. 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.

M. J. Fulwyler
Biomedical Research Group,
Los Alamos Scientific Laboratory,
University of California,
Los Alamos, New Mexico

References and Notes
6. I thank Mr. R. B. Glasscock for advice, design, and construction of electronic components and Mr. J. R. Coulter for suggestions and for fabrication of mechanical parts of the device. Work performed under the auspices of the U.S. Atomic Energy Commission.

22 June 1965