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

Nuclear membrane resistance (ohm-cm²)		Cases (No.)
Mean	Standard error	
	*Control animals**	
0.72	0.09	18
	*Ecdyson-treated animals**	
1.38	0.1	28

* 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 (*Caliphora*) units (0.2 to 0.33 μg) of a crystalline preparation of ecdyson into the larvae (*10*) and measured resistance of the nuclear membrane envelope 1 to 5 hours thereafter. Larvae from a developmental stage with nearly minimal nuclear membrane resistance (early fourth instar) were used in these experiments. Control groups from the same stage and from the same larval batch were injected with 2 μl of physiological solution. The magnitudes of the effects varied in different nuclei, but all but one of 28 ecdyson-treated nuclei we examined presented specific membrane resistances greater than those of the average controls. The mean resistance roughly doubled after ecdyson treatment; the difference is highly significant (Table 1).

The rise in mean nuclear membrane resistance due to ecdyson treatment is comparable in magnitude to that occurring during a period of 5 to 7 days in normal development (20°C), from the base level of resistance at the fourth instar to nearly the peak resistance at the early prepupa stage (Fig. 1). Rises obtained in measurements made 1 hour after hormone injection (which was about as soon as the measurements could be made) were roughly the same as after 2, 3, or 5 hours. Apparently the major change in nuclear membrane resistance takes place during the first hour after hormone injection. Interestingly, the first recognizable changes in chromosomal "puffing" pattern also occur within the first hour following injection of ecdyson (*8*).

Resting potentials (at zero current) across the cell and across nuclear membranes were measured routinely. No significant changes in resting potentials of cell membrane potentials were seen either during development or as a result of ecdyson 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 (*1*) in these cells were too small (2 to 5 mv) in relation to their individual fluctuations to show significant differences.

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3. The physiological solution had the following composition (values are millimoles): NaCl, 87.2; KCl, 2.73; $CaCl_2$, 1.28; $NaH_2PO_4 \cdot Na_2HPO_4$ buffer, 10, adjusted to *p*H 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 larvae. [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 0.001, except for the points of the early and mid-prepupa stages, which differ at a level of 0.004.
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10. We are indebted to Prof. Peter Karlson, Marburg, for a gift of ecdyson. The 2 μl of ecdyson 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 definition of hormone activity units, see P. Karlson, *Ann. Sci. Nat. Zool.* **18**, 125 (1956).
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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).
13. Supported by grants from the NIH and NSF.
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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 (*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

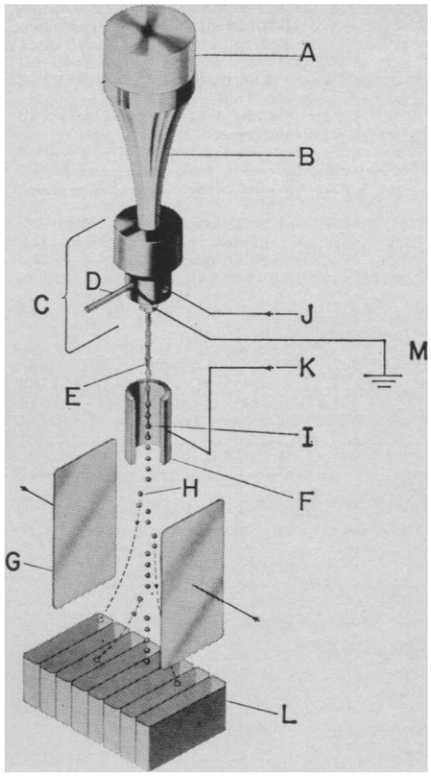

Fig. 1. Cell separator.

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 μ^3, respectively) before and after separation in physiological saline. The apparatus was adjusted to separate all cells of volume greater than approximately 80 μ^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.

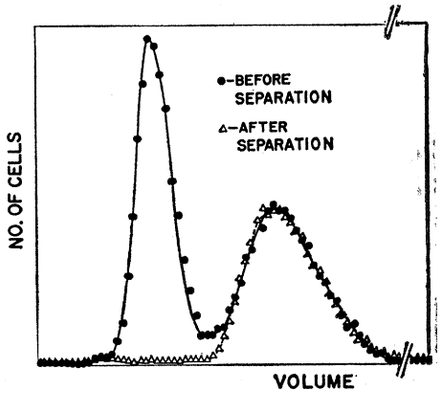

Fig. 2. Distribution by volume of mouse and human erythrocytes before and after separation.

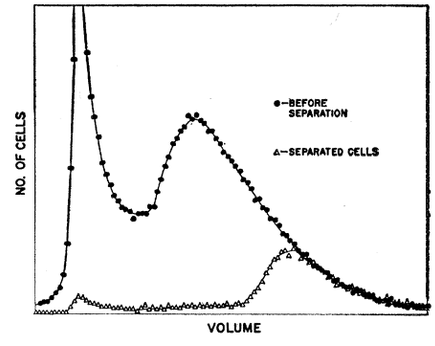

Fig. 3. Distribution by volume of mouse lymphoma cells before and after separation.

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6. I thank Mr. R. B. Glascock 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.

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