Permeability of a Nuclear Membrane: Changes during Normal Development and Changes Induced by Growth Hormone

Abstract. *The ion permeability of the nuclear membrane envelope of salivary gland cells (of the midge* Chironomus thummi*) undergoes changes during development. Following the early fourth instar stage, permeability falls to about one-fifth of its base value over a period of 3 to 5 days of development of the animal, and then rises again over the next 2 to 4 days. The falling phase of the change can be reproduced within 1 hour by injections of the growth hormone ecdyson.*

In recent experiments the membrane envelopes of nuclei of certain animal cells were found by electrical techniques to be barriers to ion diffusion (*1*). In the course of these experiments, we were struck by the large variations in ion permeability presented by the various envelopes; the variations were apparently related to cell development (*2*). We have now made a systematic study of nuclear membrane permeability during the development of a given cell type.

We chose the nuclei of salivary gland cells of the midge *Chironomus thummi* for the experiments. The cells are quite transparent and have large interphase nuclei (40 to 70 μ in diameter) clearly visible in vivo. They provide thus a material suitable for direct conductance measurements with intranuclear electrodes, one that does not undergo the disruptive changes associated with cell division over a wide developmental span. Our measurements extended over a large part of this span, from the early fourth instar stage, in which the nuclei are just large enough for the measurements, to the late prepupa stage before the start of gland histolysis.

The procedure was to isolate the gland in a physiological salt solution and to insert two microelectrodes into the nucleus in situ and a third one into the cytoplasm. One of the electrodes served to pass rectangular pulses of current (100-msec duration) between the inside of the nucleus and the outside of the cell across the nuclear membrane envelope and cell (plasma) membrane in series; the other two served to record the resulting resistive voltage drops across these membranes. The resistance of the nuclear membrane envelope (hereafter referred to as "nuclear membrane resistance") could thus be determined directly with a differential amplifier arrangement, as illustrated schematically in the inset in Fig. 1 (*3*).

Figure 1 summarizes the results. The nuclear membrane resistance of unit area increases progressively with development from the early fourth instar to the early prepupa stage, and then declines again. Both the upward and the downward trends of the resistance curve are highly significant (*4*). The resistivity of the nucleoplasm remains constant and, as shown earlier (*1*), is quite negligible compared with that of the nuclear membrane material throughout all stages of development; thus the changes in resistance reflect entirely changes in surface membrane resistance of the nucleus.

These changes in nuclear membrane permeability are paralleled by important changes in cell activity. For instance, the contents of DNA, of total protein, and of certain secretory enzymes of these cells show similar upward and downward trends, with peaks at the same developmental stage as the nuclear membrane resistance (*5*). Moreover, the changes in permeability occur at a time of recognizable change in chromosomal puffing pattern (*6*).

Changes in nuclear membrane resistance of this kind can also be produced by treating the animal with ecdyson. This steroid hormone plays a key role in larval growth (*7*, *8*)

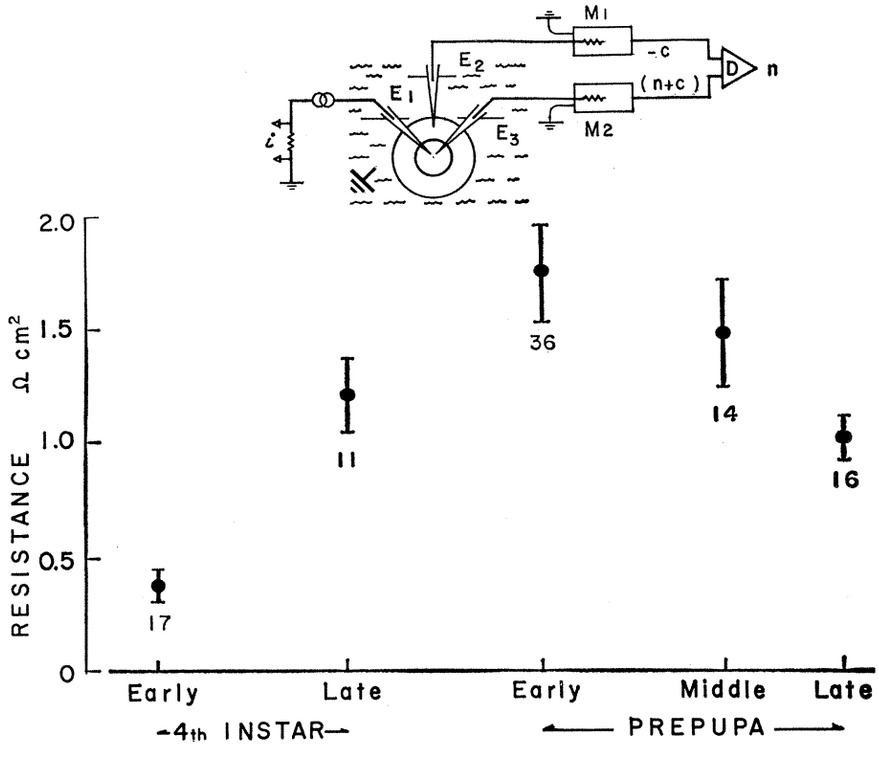

Fig. 1. Changes in nuclear membrane permeability during development. Mean electrical resistance of unit area of nuclear membrane envelopes of *Chironomus* salivary gland cells at various developmental stages. The stages are scaled on abscissa intervals proportional to their average time from egg hatching, to give an idea of the time course of the change in resistance (*12*). Bars subtend standard errors; the numbers on the bars are the numbers of cases examined. (Inset) Diagram of measuring apparatus. Currents are passed between microelectrode E_1 inside the nucleus, and the grounded extracellular fluid. The resulting resistive voltage drops (time $\rightarrow \infty$) across the cell membrane (c) and across the nuclear and cell membranes in series ($n + c$) are measured between microelectrode E_2 (cytoplasm) and extracellular fluid, and, simultaneously, between microelectrode E_3 (nucleoplasm) and extracellular fluid. The voltages are fed through balanced field transistor stages (M_1 and M_2), to match electrode impedance, and through a differential amplifier stage (D) into an oscilloscope on which the resistive voltage drops (n) across the nuclear membrane alone are displayed. The membrane currents (i) are displayed on the second beam of the oscilloscope.

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.

S. Ito*
W. R. Loewenstein
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York

References and Notes

1. W. R. Loewenstein and Y. Kanno, *J. Gen. Physiol.* **46**, 1123 (1963); W. R. Loewenstein, "Protoplasmatologia," *Handbuch der Protoplasmaforschung* (Springer, Vienna, Austria, 1964), p. 26.
2. Y. Kanno and W. R. Loewenstein, *Exp. Cell Res.* **31**, 149 (1963); Y. Kanno, R. F. Ashman, W. R. Loewenstein, *ibid.* **39**, 184 (1965).
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.
5. H. Laufer and Y. Nakase, *J. Cell Biol.* **25**, 97 (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. Wigglesworth, *Symp. Soc. Exp. Biol.* **11**, 204 (1957).
6. U. Clever, *Chromosoma* **13**, 385 (1962); W. Beerman, *ibid.* **5**, 139 (1962); ———, *Am. Zoologist* **3**, 23 (1963); H. Kroeger, *J. Cellular Comp. Physiol.* **62**, Suppl., 45 (1963); H. Laufer and Y. Nakase, *Proc. Nat. Acad. Sci. U.S.* **53**, 511 (1965).
7. P. Karlson, H. Hoffmeister, W. Hoppe, R. Huber, *Ann. Chem.* **662**, 1 (1963); P. Karlson, *Perspect. Biol. Med.* **6**, 203 (1963); U. Clever and P. Karlson, *Exp. Cell Res.* **20**, 623 (1960); U. Clever, *Chromosoma* **15**, 36 (1964): H. A. Schneiderman, *Science* **143**, 325 (1964).
8. U. Clever, *Chromosoma* **12**, 607 (1961).
9. W. J. Burdette, *Science* **135**, 432 (1962).
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).
11. S. J. Socolar and F. Wald, personal communication.
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.
* Visiting fellow from Department of Biology, Kumamoto University, Kumamoto, Japan.

21 September 1965

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