Our observations not only support the theory that clonal selection is fundamental to the immune response, but also indicate that selective destruction of clones proliferating in response to transplantation antigens may prove useful for surmounting the histocompatibility barrier.

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References and Notes

1. B. Bain and L. Lowenstein, in *Histocompatibility Testing, Conference of Histocompatibility Testing, Washington, D.C., 1964* (NAS-NRC Publ. 1229, National Academy of Sciences, Washington, D.C., 1965), p. 179.
2. D. B. Amos and F. H. Bach, *J. Exp. Med.* **128**, 623 (1968). In the one-way MLC reaction, one donor's cell population is first treated with the antineoplastic agent mitomycin C (25 μg/ml) which renders that population unable to synthesize DNA. Thus, only one donor's cells respond in this system.
3. F. H. Bach, R. J. Albertini, P. Joo, J. L. Anderson, M. M. Bortin, *Lancet* **1968-II**, 1364 (1968).
4. R. W. Dutton and R. I. Mishell, *J. Exp. Med.* **126**, 443 (1967).
5. All tissue culture supplies were obtained from Grand Island Biological Co., and labeled thymidine was obtained from New England Nuclear Corp. The cells were cultured in sterile, aged, tightly capped Falcon plastic tubes (16 by 125 mm). Slides were prepared for autoradiography by fixation in methanol; they were then dipped in NTB-3 (Eastman Kodak), exposed for 2 weeks, and stained with acid Giemsa. Samples for radioactivity counting were solubilized with NCS (Nuclear-Chicago), dissolved in Liquafluor (New England Nuclear), and counted in a Packard Tricarb scintillation counter (model 3375, Packard Instruments) which had been standardized for counting of ^{3}H and ^{14}C at the same time.
6. J. E. Curtis, E. M. Hersh, J. E. Harris, C. McBride, E. J. Freireich, *Clin. Exp. Immunol.* **6**, 473 (1970).
7. S. E. Salmon, W. F. Whitmore, R. S. Krakauer, unpublished observations.
8. G. W. Santos, P. J. Burke, L. L. Sensenbrenner, A. H. Owens, Jr., in *Pharmacological Treatment in Organ and Tissue Transplantation, Proceedings of the International Symposium on Pharmacological Treatment in Organ and Tissue Transplantation, Milan, February/March, 1969*, A. Bertelli, Ed. (Excerpta Medica International Congress Series No. 197, Amsterdam, in press).
9. S. E. Salmon, B. A. Smith, R. I. Lehrer, S. N. Mogerman, H. R. Shinefield, H. A. Perkins, *Lancet* **1970-II**, 149 (1970).
10. HL-A typing was performed by the fluorochromatic cytotoxicity method. We thank Dr. H. A. Perkins, Research Director, Irwin Memorial Blood Bank, San Francisco, for HL-A typing our subjects. Supported in part by PHS grant CA-11067. R.S.K. and W.F.W. were summer student clinical cancer trainees (PHS/NCI T12, CA-08054).

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Uncoupling Cell Junctions in a Glandular Epithelium by Depolarizing Current

Abstract. *The high electrical conductance linking adjacent border cells in* Chironomus *salivary gland is depressed reversibly when current is passed outward from one of the cells, though not when current is passed inward. This "uncoupling" is closely associated with an electrically induced increase in conductance in the (nonjunctional) membrane of that cell.*

Several different treatments can depress the high electrical conductance that characterizes cell junctions in a wide variety of tissues (*1–6*). During a study of such cell "uncoupling" in the salivary gland of *Chironomus* by cooling and by certain metabolic inhibitors, we noted that the loss of electrical coupling was generally accompanied by a decrease in cell membrane potential (*6*). This prompted us to inquire whether a simple shift of membrane potential can uncouple these cells. In four kinds of nerve cell linkages the conductance of junctions is known to change transiently in response to electric fields or currents impressed between cell interior and exterior (*1, 2, 7, 8*). We now find that cell junctions in *Chironomus* salivary gland show a reversible decrease in conductance, with some distinctive features, during passage of outward current from a cell.

Salivary glands of mid-fourth-instar larvae of the midge *Chironomus thummi* are used. To test for junctional coupling, we pass a step of electric current between the inside of one of the large border cells and the outside; meanwhile we record the potentials in this cell (E_I) and in a contiguous one of the same type (E_{II}). Comparison of the net changes of potential (V_I and V_{II} in Fig. 1D) as a steady state is reached shows the degree of coupling (*9*). Details of the method appear elsewhere (*6*).

Figure 1A shows the effect of depolarizing (that is, outward) current steps of different sizes. With small currents, E_{II} follows E_I closely in time course and magnitude, giving the voltage response typical of a linear, time-invariant resistance-capacitance network. In displaying such good electrical coupling, the cells show that they are linked by junctions of high conductance (*10*). But when the currents exceed about 10^{-7} ampere, the coupling falls markedly after some delay, and remains depressed while the current continues. First, an inflection appears in the E_I trace; E_I shoots up to a peak, then subsides to a steady value substantially larger than the normal values seen with slightly smaller current. E_{II}, however, follows the rise of E_I only briefly, then declines to a steady level smaller than is seen with slightly smaller current. The greater the current, the earlier these events develop. The larger outward currents thus diminish V_{II} even though V_I increases. These features are seen consistently in fresh preparations (37 cases). The general result is brought out especially clearly in Fig. 1B, which gives the steady-state current-voltage characteristics for V_I and V_{II} with outward current. The failure of V_{II} to keep pace with V_I as the current is increased reflects a substantial fall in junctional conductance during the larger currents. The alternative possibility, that the membrane conductance of cell II increases substantially, is untenable since no such change is evidenced in the time constant of E_{II} at cutoff of a steady current (*11*).

The course of E_I during large depolarizations is not that of an action potential. As Fig. 1D shows, we find no response capable of sustaining itself once the driving current is stopped. Tests (not shown) with small 140-msec square pulses of current superimposed on the uncoupling current step confirm that the secondary rise of E_I reflects a a rising input resistance and the later decline, a partial subsidence of the input resistance of the cell system. Testing with a small current pulse, after cutoff of the large depolarizing current, reveals that normal coupling is restored within a few tenths of a second.

When applying hyperpolarizing current, we have seen no change in coupling (Fig. 1C), even with currents and potential shifts that would more than suffice to uncouple were they depolarizing. The steady-state hyperpolarizations have ranged up to the largest that the cells can sustain, about 100 mv negative from resting potential.

A pair of contiguous cells behaves symmetrically with respect to an outward current passed from either cell. This is evident from the experiment of Fig. 1C. Here a current lead and a voltage probe are present in each of the two cells. A depolarizing current of given size in either cell uncouples in roughly the same length of time. The symmetry seen here has been confirmed in each of three trials (*12*). Since a junction's susceptibility to uncoupling

by cell polarization is bilaterally symmetric, but asymmetric with respect to electric polarity, uncoupling is not attributable to transjunctional current or potential, or at any rate not to this alone. Thus the junction is responding to a change in the state (for example, the potential) of either or both of the cells it links (*13*). In distinguishing between a depolarization on one side and an equal hyperpolarization on its other side, and in failing to discriminate the direction of transjunctional current or potential, a pair of contiguous border cells in *Chironomus* salivary gland behaves quite differently from the bilaterally asymmetric cell junctions observed in certain nerve cells (*1, 8*), junctions analogous to the two-terminal rectifier elements of electrotechnology (*14*).

Smith *et al.* have found that retinula cells in the lateral eye of the horseshoe crab uncouple when either of them is depolarized (*2*); but these cells uncouple under hyperpolarization too, even more readily. The symmetry seen in the salivary gland also resembles that reported by Baylor and Nicholls for touch cells of the leech (*7*), but in the latter it is hyperpolarization rather than depolarization that uncouples.

The change in the state of cell I causing junctional conductance to decrease may be a change in cytoplasmic composition. This could come about by means of an alteration of the nonjunctional membrane upon depolarization, namely, either by change in permeability of the membrane, or, perhaps more readily, by release of membrane-held ions or other molecules to the cytoplasm (*15*). There is, in fact, evidence that the nonjunctional membrane of cell I is altered: in Fig. 1D, E_1 shows a reduced decay time—a rapid initial phase of repolarization—if the current is stopped after the upward in-

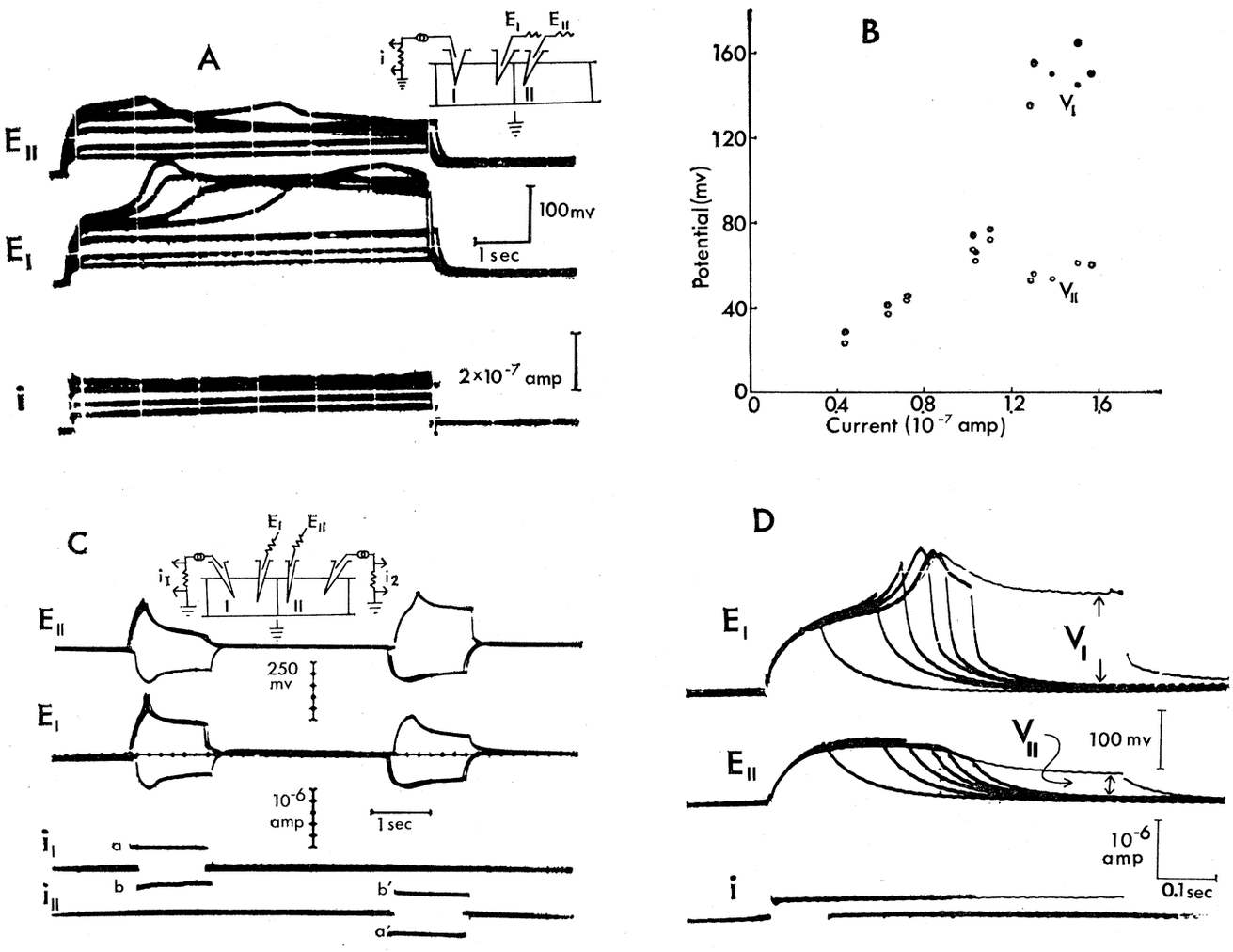

Fig. 1. Uncoupling by outward current. All traces photographed from storage oscilloscope. Upward deflections indicate depolarization. (A) Membrane potential (E_I and E_{II}) as a function of time in two contiguous cells (I, II; inset) when step of current (*i*) flows from cell I interior to exterior. For summary description, see text. (B) Steady-state potentials V_I and V_{II} as functions of outward current. Uncoupling is shown by failure of V_{II} to follow rise of V_I (see text). Same experiment as above; includes data from the six trials in preceding figure that approximate steady state, and from additional trials whose oscilloscope records are not shown. Baseline for ordinates is resting potential (−45 mv). (C) Symmetry of uncoupling by depolarizing from either cell of a contiguous pair. Here current electrodes are in both cells I and II (inset). Record shows three sweeps superimposed on each of the four traces. During first sweep, i_I depolarizes (*a*), then i_{II} hyperpolarizes (*a'*). On the second sweep, 4 minutes later, i_I hyperpolarizes (*b*), then i_{II} depolarizes (*b'*). Finally, 4 minutes later, the sequence of the first sweep is repeated. A depolarizing current of given size in either cell I or cell II uncouples in about the same length of time, but hyperpolarizing either cell fails to uncouple. "Sag" seen here in hyperpolarizing potentials is observed when the early shift of potential exceeds about 100 mv; on cessation of current, such a hyperpolarizing response is followed by long-lasting after-depolarization in both cells, both the sag and the overshoot becoming quite large for large currents. (D) Repolarization upon termination of current. Decay of response starts promptly when current pulse ends. Note fast and slow phases of E_I decay after longer pulse durations. Electrode arrangement as in (A).

flection of E_I. The later the current is stopped, the faster repolarization begins. But the effect is already significant soon after the inflection. At the second earliest cutoff in Fig. 1D the initial logarithmic decay rate has already doubled. This indicates that the development of uncoupling is associated with an increased nonjunctional membrane conductance in cell I. As E_I nears its steady state, the time constant of the fast phase of repolarization levels off at about 10 msec. (This phase is followed by a second, with a time constant of about 0.1 second, and sometimes even by a third phase, lasting many seconds). At rest, the time constant is about 50 msec (*16*). The resultant picture of changes during depolarization, then, is that the junctional conductance falls as the membrane conductance of cell I rises; the former effect always dominates the change of input resistance, but less strongly in the steady state than earlier.

Two characteristic features mark the development of uncoupling during depolarization. (i) Depending on the intensity of the depolarizing current step, uncoupling appears after a delay ranging from 0.1 second to more than 40 seconds. (ii) If essentially complete repolarization for a short time is permitted before uncoupling has developed, the attained advance of the mediating process (or processes) is not entirely undone and is resumed on further depolarization. The latter feature emerges most strikingly when we uncouple by repeated application of a current pulse that is too brief to uncouple on single application (Fig. 2). This kind of facilitation of uncoupling by earlier depolarization is also seen consistently when that depolarization has evoked uncoupling. Unlike the uncoupling, the facilitation can persist for as long as 3 minutes after repolarization, its persistence increasing with the size or duration of the depolarization.

The data in Fig. 1B suggest the possibility of an electrical threshold for uncoupling, but although we have seen uncoupling initiated by small current (1.0×10^{-7} ampere) take as long as 40 seconds to develop, we have not yet made a systematic study with depolarizations that may be liminal for uncoupling. It has not been possible to identify any threshold of potential as the necessary and sufficient condition for prompt start of a junctional resistance rise. We considered that one criterion for onset of uncoupling might be the first inflection in the ascending E_I trace. This inflection, however, occurs not at one definite potential but at an E_I value that increases with the size of the applied current. Thus, for example, for a preparation with a normal cell resting potential of -55 mv, the depolarization to inflection in cell I ranged from 80 mv at a current of 0.1 μa to 200 mv at 0.5 μa. A somewhat similar current dependence is found in the E_I level synchronous with the peak of E_{II}.

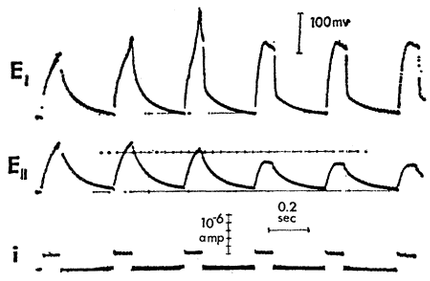

Fig. 2. Development of uncoupling by repeated depolarizations. Between successive pulses, cells repolarize almost completely (same cells as Fig. 1D).

Our results indicate that depolarization actuates a cumulative secondary process that mediates uncoupling; and they suggest an intimate relation, and possibly a causal one, between a nonjunctional membrane change and the junctional uncoupling elicited by depolarizing current.

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References and Notes

1. E. J. Furshpan and D. D. Potter, *J. Physiol. (London)* **145**, 289 (1959). The crayfish giant motor synapse was found in this study to behave as a rectifier having almost maximum resistance at zero potential difference but nevertheless showing some rise in resistance on presynaptic hyperpolarization or postsynaptic depolarization.
2. T. G. Smith, F. Baumann, M. G. F. Fuortes, *Science* **147**, 1446 (1965); T. G. Smith and F. Baumann, *Progr. Brain Res.* **31**, 313 (1969).
3. M. Nakas, S. Higashino, W. R. Loewenstein, *Science* **151**, 89 (1966); Y. Asada, G. D. Pappas, M. V. L. Bennett, *Fed. Proc.* **26**, 330 (1967); B. Rose and W. R. Loewenstein, *Biochim. Biophys. Acta* **173**, 146 (1969); O. Hauswirth, *J. Physiol. (London)* **201**, 42P (1969); B. W. Payton, M. V. L. Bennett, G. D. Pappas, *Science* **165**, 594 (1969).
4. W. R. Loewenstein, *Ann. N.Y Acad. Sci.* **137**, 441 (1966).
5. ———, M. Nakas, S. J. Socolar, *J. Gen. Physiol.* **50**, 1865 (1967).
6. A. L. Politoff, S. J. Socolar, W. R. Loewenstein, *ibid.* **53**, 498 (1969).
7. D. A. Baylor and J. G. Nicholls, *J. Physiol. (London)* **203**, 591 (1969).
8. A. A. Auerbach and M. V. L. Bennett, *J. Gen. Physiol.* **53**, 211 (1969).
9. In some earlier reports from this laboratory [for example (*6*)], in which only steady states were considered, E symbolized resting potential; V, however, retains its former meaning.
10. W. R. Loewenstein, S. J. Socolar, S. Higashino, Y. Kanno, N. Davidson, *Science* **149**, 295 (1965).
11. With respect to its conductive properties, the coupled cell system of the salivary gland may be regarded, approximately, as a network of resistors each of which represents the lumped conductance of a cell nonjunctional membrane or of a cell junction. The nonjunctional resistors form a chain (or a two-dimensional network) [see text, (*5*), and (*12*)]. From a common external point representing the extracellular medium, nonjunctional resistors extend, respectively, to each point where junctional resistors meet, each of these meeting points corresponding to the interior of a cell. The interior of cell II may then be viewed as a point on a potential divider that bridges from the cell I interior to the external medium, shunting the nonjunctional membrane of cell I. For this network, the analysis in the text then follows, no matter what changes are assumed to be occurring in cell I.
12. Recent work in this laboratory (B. Rose, *J. Membrane Biol.*, in press) has shown that the border cells have low-resistance coupling not only via their common junctions, but also via their junctions with the thin cells that bound the lumen laterally. [The morphology has also been studied by J. A. Kloetze and H. Laufer, *J. Ultrastruct. Res.* **29**, 15 (1969)]. However, the observed loss of coupling (Fig. 1) is so great that it appears necessary that both of the aforementioned pathways suffer large rises in resistance during the depolarization.
13. In reporting symmetric uncoupling by hyperpolarization in the leech touch cells, Baylor and Nicholls have suggested the need for a mechanism proceeding from the change in potential of a single cell (*7*).
14. After this report had been written, we learned that other rectifying junctions, between sensory cells and motoneurons in the leech, have been reported by J. G. Nicholls and D. Purves, *J. Physiol. (London)* **209**, 647 (1970).
15. The process mediating uncoupling might be one that has been proposed as common to several uncoupling procedures, the release of Ca^{2+} ions into the cytoplasm (*4*, *5*). In line with this view, Loewenstein proposes potential-dependent release and uptake of Ca^{2+} by the nonjunctional membrane itself as steps in one mode of alteration of junctional coupling (B. Rose and W. R. Loewenstein, *J. Membrane Biol.*, in press; G. M. Oliveira-Castro and W. R. Loewenstein, *ibid.*, in press). Systems that show prompt changes of junctional conductance upon polarization (*1*, *7*, *8*) are unlikely to involve mechanisms based on change in cytoplasmic composition, which depend on diffusion.
16. Interest centers on the earliest appearance of a reduced decay time since this bears on the possibility that uncoupling is mediated by a change in the nonjunctional membrane. We can rule out the possibility that the accelerated decay here reflects not a rise in nonjunctional conductance but rather electrical non-isopotentiality in the spatially extended cell system. [In such a system, the decay of potential near the current source after a fast pulse of polarization—a condition possibly satisfied by the brief secondary rise of E_I—can be much faster than decay at the end of a sustained polarization; see W. Rall, *Exp. Neurol.* **2**, 503 (1960).] Each of four trials showed that even at slow rates of depolarization occurring very early in the onset of uncoupling (≤ 20 mv/sec), cutoff of current is followed by E_I decay whose initial logarithmic rate is accelerated severalfold and that no comparable enhancement appears if the rise of E_I is duplicated in respect to rate and duration by applying a ramp current at a level of depolarization too small to initiate uncoupling.

17. Supported by grants from NIH and NSF. We thank Werner R. Loewenstein and Birgit Rose for assisting during some of the experiments as well, Michael V. L. Bennett for valuable comments, and Neil Pincus for indispensable electronic support. Preliminary results in this work were reported earlier [A. L. Politoff, S. J. Socolar, S. E. Straus, *Biophys. Soc. Annu. Meet. Abstr. 14th* (1970), p. 75a].

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