Table 1. Specific adsorption of neutralizing antibody by fragments of bacteriophage T2 antigens in RNA extracted from T2-infected macrophages. One-tenth milliliter of a solution of RNA (250 μg/ml) from T2-infected macrophages was made 0.3*M* in KOH and incubated at 37°C for 20 hours. After neutralization, the sample volume was adjusted to 0.2 ml, and 0.5 ml of a 10^{-4} dilution of a standard antiserum to T2 serum was added. Adsorption was carried out for 2 hours at 37°C. The reaction mixture was then assayed for residual antibody activity by scoring for the ability of the reaction mixture to inactivate a standard number of bacteriophage as described (*10*). An equal amount of this RNA was treated with Pronase prior to alkali digestion and carried through in an identical manner, as were 25-μg samples of RNA from macrophages not exposed to T2 bacteriophage and of yeast RNA. The samples of RNA's were tested in two separate experiments against antiserum to T2 with phage T2 and, in one experiment, against antiserum to R17 with phage R17. Plaque input refers to the standard number of plaque-forming units of each bacteriophage used in the assay.

Fragment treatment	Phage counts		
	T2		
	Experiment a	Experiment b	R17
Plaque number input	285	312	320
Adsorbed with alkali-digested RNA from T2-treated macrophages	141	186	48
Adsorbed with alkali-digested RNA from macrophages not exposed to T2 bacteriophage	84	107	52
Adsorbed with alkali-digested yeast RNA	87	117	59
Adsorbed with Pronase-treated and alkali-digested RNA from T2-infected macrophages	58	121	47
Unadsorbed antiserum	56	116	49

structure of these antigens nor the RNA carrier of the RNP complex is required for recognition of specific neutralizing antibody by the tail fiber antigens of T2 bacteriophage.

If increasing amounts of RNA from T2-infected macrophages were used to adsorb the standardized neutralizing antiserum to T2, all of the total neutralizing antibody could be removed by this RNA (Fig. 1). In contrast to the result with RNA from T2-infected cells, the use of larger amounts of yeast RNA or RNA from noninfected macrophages did not result in significant loss of neutralizing antibody from the standardized antiserum. Thus, all of the neutralizing antibody formed by immunization of rats with intact T2 bacteriophage can be adsorbed by fragments of T2 bacteriophage antigens in the RNA derived from macrophages previously exposed to T2 bacteriophage. The fragments of antigen in this RNA are capable of inducing neutralizing antibody against the intact bacteriophage (*1*). The precise role of the antigenic fragments linked to macrophage RNA in the antibody response in vivo is not clear. The data are compatible with the suggestion that the processing of T2 antigens by macrophages is a pathway (perhaps the principal pathway) by which T2 antigens are presented to the cell destined to form antibody.

Furthermore, the labeled antigenic fragments in RNA derived from macrophages infected with ^{125}I-labeled T2 are distributed in the RNP complex (Fig. 2). Nearly all of the labeled antigen is found to have the same R_F on 10 percent polyacrylamide gels as that of RNP which has been purified by chromatography on tetraethylaminoethyl (TEAE)-cellulose (*2*). This result is similar to that obtained with a soluble immunogenic synthetic copolymer poly L-(Glu^{50}Ala^{40}Tyr^{10}), in which the distribution of labeled antigen in the whole RNA extracted from macrophages exposed to this polymer is exclusively resident in the RNP fraction (*10*). Examination of cesium sulfate density gradients of macrophage RNA derived from macrophages infected with ^{125}I-labeled T2 phage reveals a distribution of small polypeptide fragments along the gradient (*2*). In view of the distribution of antigen shown in Fig. 2, it would appear likely that these fragments are released from the RNP complex in solutions of high ionic strength. Thus, the antigens are not randomly distributed in the RNA population.

The presence in RNP of T2 antigenic fragments which specifically react with neutralizing antibody indicates that these fragments represent small fractions of the whole T2 proteins and that the preservation of extensive tertiary structure of these antigens is not necessary for recognition of specific antibody. These results are in agreement with the finding of complement-fixing T2 antigens in RNA from T2-infected macrophages by Friedman *et al.* (*6*) and with our previous studies which suggested that the T2 antigens present in macrophage RNA represented digested fragments of the original T2 proteins. The data do not rule out the possibility that some of the T2 antigens present in the RNP may not have their antigenic specificity preserved. However, the demonstrated immunogenicity of the T2-RNP (*1*) must be attributable to those antigenic fragments whose specificity is preserved. Whether the processing of T2 bacteriophage antigenic fragments by macrophages is an obligatory step in the formation of antibodies against T2 bacteriophage remains to be shown.

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Temperature-Dependence of Resistance at an Electrotonic Synapse

Abstract. *The junctional resistance at septa of the crayfish lateral giant axon is inversely related to temperature with a* Q_{10} *of about 3 over the range from 5° to 20°C. Nonjunctional axonal membrane is much less affected. Resistance changes occur rapidly with temperature changes. No correlates in ultrastructure of the synapses have been found.*

Transmission of impulses across a septum of the lateral giant axon of crayfish (*Procambarus*) is electrically mediated, the septum behaving like a fixed resistance inserted in the path of current along the axonal core (*1*). Each segment of the fiber is a separate axon with its own cell body (*2*); electrotonic synapses connecting successive axons are located in the septa. Morphological studies indicate

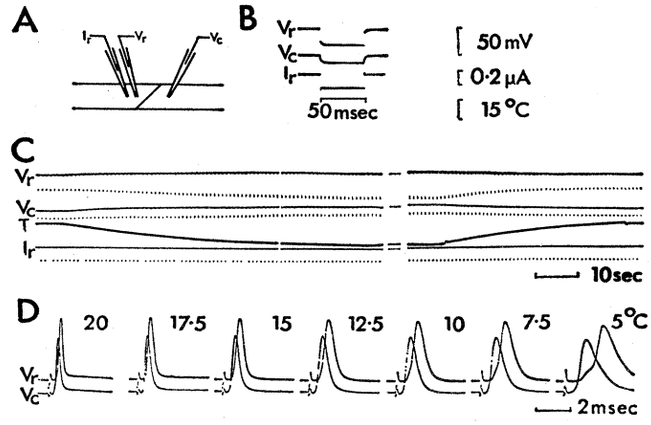


Fig. 1 (right). (A) Arrangement of recording electrodes (V_r and V_c) and current-passing electrode (I_r) at the septum. (B) Example of voltages on rostral (V_r) and caudal (V_c) sides of the septum when current (I_r) was passed on the rostral side. (C) Record during cooling and rewarming of voltages due to current pulses applied one per 0.8 second on the rostral side (I_r). About 30 seconds of the record is omitted between cooling and rewarming. The temperature (T) ranged from 20° to 5°C. During cooling V_r increased while V_c remained nearly constant. (D) Transmission of impulses at different temperatures. Action potentials were recorded on both sides of the septum following an external stimulus caudal to the recording sites.

that only a small part of the septum is involved in electrotonic coupling of adjacent axons (*3*, *4*). Over most of the septal region, the axons are separated by Schwann cell processes and a layer of fibrillar material. Scattered throughout the septum are small windows in this intervening material no more than a few microns in diameter. At these sites short processes of the adjacent axons come together and their plasma membranes form a "tight junction," that is, a region where the two cell membranes are so closely apposed as to occlude most or all of the extracellular space (*4*). There are both experimental and comparative data indicating that junctions of this kind are sites where current passes between cells (*4–7*). The calculated resistance of the membrane is very low in these regions, less than 1 ohm cm^2 (*4*), whereas that of the nonjunctional membrane of the axon is 1000 to 3000 ohm cm^2 (*1*).

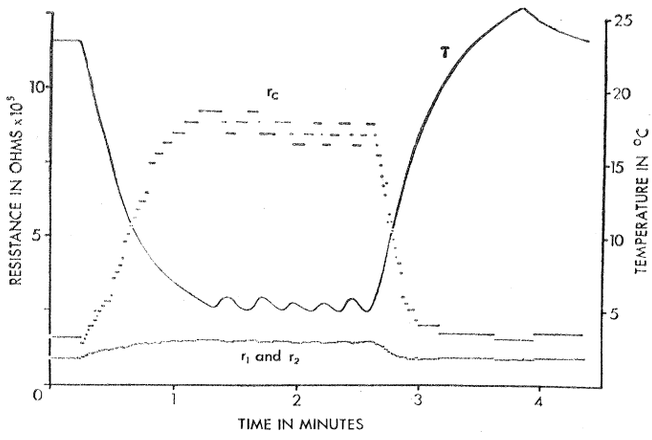
Substitution of propionate for Cl^- in the bathing media causes an increase in junctional resistance of 3- to 12-fold (*4*). These increases are reversed upon return of the preparation to physiological saline. Injury of one axon can also cause an increase in junctional resistance, but these changes are not reversible. Electron microscopy of specimens fixed when junctional resistance is increased show that the increase in resistance is associated with separation by several microns of the axonal processes forming the junctions and movement of glial processes into the intervening space. Return to normal resistance is associated with reestablishment of normal morphological relations at the junctions. Calculations indicate that when junctional membranes separate their resistance increases markedly.

The complex morphological changes suggested that active cell movements were involved in the changes in junctional resistance. We therefore wondered whether at low temperatures, where active cell movements ought to be blocked, one could still obtain the same effects. Temperature changes alone were found to cause alterations in junctional resistance that were both large and rapid. We think the most likely explanation of this finding is that temperature changes affect membrane structure.

Experimental procedures and the electrical model for this junction have been described (*1*, *6*). The septal region can be represented as the junctional resistance, r_c, connecting the interiors of the two axons; the nonjunctional resistances, r_1 and r_2, are the input resistances of the axons excluding current crossing the septum. A voltage recording electrode was placed in each axonal segment close to the septum; a third electrode was placed on either the rostral or caudal side for passing current (Fig. 1A). Rectangular hyperpolarizing current pulses were passed and the resulting membrane potentials were recorded on the polarized and unpolarized side of the septum. The changes were the same whether current was passed on the rostral or caudal side of the septum, and the resistances, r_1 and r_2, were approximately equal. Thus one can calculate changing values of r_c, r_1, and r_2 by assuming that r_1 and r_2 are equal.

A record obtained when current was passed on one side of the septum is shown in Fig. 1B. For determining the time course of the resistance changes during relatively rapid temperature changes, we applied a rectangular current pulse 50 msec long at a frequency of about one per second on one side of the septum and recorded the resulting potentials and the output of the thermistor bridge monitoring the temperature on moving film. Cooling was by means of a thermoelectric element to which a bath of Silastic (Dow Corning) had been attached. As the temperature dropped, the potentials produced in the polarized (rostral) axonal segment increased markedly. The potentials produced in the other segment remained more or less constant. On rewarming, the potentials returned to their original values (Fig. 1C). The calculated values of r_1, r_2, and r_c from a similar experiment are shown in Fig. 2. On cooling there was a rapid increase in junctional resistance; on rewarming the resistance returned to its original level. When cooling from 20° to 5°C the mean increase of junctional resistance in 13 experiments was 5.6 times (range, 2.8 to 13.0 times) which gives a Q_{10} of 3.1 (range, 2.0 to 5.5). A definite but smaller increase in nonjunctional resistance was also observed (mean 1.6 times, range 1.3 to 1.9; $1.2 \leq Q_{10} \leq 1.5$). In seven of these experiments the time taken to lower or raise the temperature between 20° and 5°C was about 1 minute. In the other experiments the temperature changes took about 15 minutes. No significant delay in these changes of resistance could be detected at the fastest rate of cooling employed. The changes in junctional resistance were sensitive enough to reflect the small variations of temperature that resulted from on-off operation of the thermostat controlling the cooling unit. These variations are seen in Fig. 2 as small temperature oscillations just under 1°C in amplitude associated with changes of r_c of about 5 percent.

In the experiment of Fig. 2, the junctional resistance was about the same whether the preparation was being cooled or warmed; that is, there was little hysteresis (Fig. 3). A degree of hysteresis was observed in some experiments in which the temperature changes were rapid. This effect may have been due to different rates of temperature change at the thermistor and the prep-



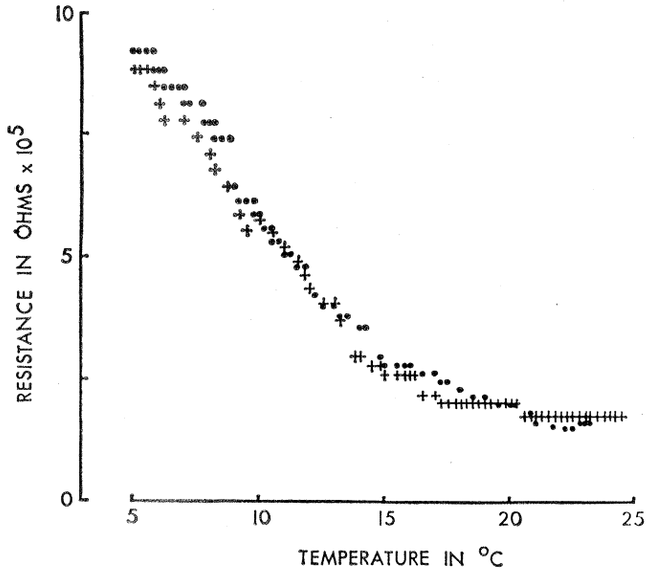


Fig. 2 (left). Changes in junctional resistance, r_c, and nonjunctional resistances, r_1 and r_2, in response to temperature changes (T). This experiment was similar to that of Fig. 1C. Fig. 3 (right). Relation of junctional resistance to temperature during cooling (circles) and rewarming (plus signs). Data are the same as in Fig. 2.

aration. Longer periods at low temperatures, up to 30 minutes, did not lead to appreciable changes in junctional resistance after the immediate ones.

At low temperatures, stimulation of the lateral giant fibers by external electrodes continued to initiate spike responses in both rostral and caudal cells, although the time course of these spikes was prolonged (Fig. 1D). Also at low temperatures the postsynaptic potential preceding spike initiation in the postseptal cell rose more slowly, primarily because of the increased junctional resistance. The point at which the spike arose from the postsynaptic potential could then be seen more clearly. There was no consistent change in firing level measured by intracellular stimulation and recording; it was sometimes increased slightly and sometimes decreased slightly. In a few experiments only a postsynaptic potential was seen at 5°C in response to the presynaptic spike. Apparently, in these cases the increase in junctional resistance was adequate to block transmission (both axons remained excitable in these latter experiments).

Resting potentials on the order of 80 mv were recorded initially on penetration of the axons. During cooling the recorded potentials shifted in the positive direction by a small amount, never more than 10 mv. The potentials returned to initial levels on rewarming. Alterations in tip or junctional potentials rather than membrane potentials may have been responsible for these observations, as changes of about the same amplitude could be observed when the electrodes were outside the cells.

The degree of electrotonic spread along the axon was little affected by temperature changes as determined in three experiments. Since in a uniform axon the space constant, λ, is given by

$$\lambda = (r_m/r_i)^{1/2}$$

[where r_m is the resistance of the membrane of a unit length of axon and r_i is the (longitudinal) resistance of a unit length of axonal cytoplasm] the temperature changes would have to alter r_m and r_i equally for λ to remain constant. For a long uniform axon the input resistance, R_{in}, corresponding to r_1 and r_2, is given by $R_{in} = (r_m r_i)^{1/2}$. Thus the average of 1.6-fold increase in r_1 along with the relative stability of the space constant indicates that both r_m and r_i increased by about 1.6-fold.

Preparations that had been held at 5°C for about 2 minutes were fixed at 5°C with 2.5 percent glutaraldehyde in cacodylate buffer at *p*H 7.2. The morphology of these preparations was compared with that of others that had been maintained and fixed at 20°C. In each type of preparation, the tight junctions appeared the same; that is, no morphological change was seen which could correlate with the change that cooling must have produced. Other preparations were fixed after actual measurements of increase in resistance due to cooling. In these cases also there was no obvious change in junctional relations. These results contrast with the marked morphological changes observed in association with the increases in junctional resistance due to replacement of chloride ions or injury (*4*).

The effect of temperature on junctional resistance in this preparation differs from that observed in *Chironomus* salivary gland cells, where uncoupling has a slow onset and is associated with considerable depolarization of the cells (*8*). The morphological basis of electrotonic coupling is also very different in the two cases (*6*, *7*). The relatively rapid time course in the septate axon indicates that there is a direct effect on the junctions themselves rather than an indirect action through a reduction of metabolic pumping, as postulated for the *Chironomus* cells.

The changes in resistance of nonjunctional membrane and axoplasm, as well as the changes in resting and action potentials, are consistent with those observed in other axonal membranes (*9*). The changes in axoplasmic resistance are very close to the changes in viscosity of water and can be ascribed to simple slowing of ion movement. No greater increase was observed in what was thought to be cytoplasmic resistance of cardiac Purkinje fibers (*10*). Since these fibers are made up of many cells in series coupled by tight junctions (*11*), the smallness of the change indicates that cooling produces little change in resistance at these junctions.

The resistance changes in junctional membrane observed in this study are significantly greater that those in nonjunctional membrane. One possible explanation of this difference is that ion movement across the junctions is a temperature-dependent process involving a pump or carrier system. This possibility seems ruled out by the nonspecific na-

ture of the junctional permeability to small ions (*12*) and the constant resistance of the membrane in the face of large transmembrane potentials of either polarity (*1*). A more likely possibility is that the membrane structure is affected by temperature. There is substructure at many tight junctions (*5*, *7*, *13*) including the septal tight junctions (*4*). Since specific membrane resistivity is very low at the septal junctions, the membrane must differ in some way from adjacent nonjunctional membrane. Conceivably at low temperatures the membrane tends to return to the "ordinary" high resistance structure. However, no ultrastructural correlates of the changes in membrane resistivity have yet been found. Nonetheless, our finding seems to provide an important clue to the modifications of membrane at electrotonic synapses.

Note added in proof: From further morphological studies in which fixation in the presence of lanthenum hydroxide was used, it is now clear that the septal junctions are "gap junctions" as described in other tissues by Revel and Karnovsky (*13*) and Brightman and Reese (*14*).

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Carcinoma of the Cervix: Deficiency of Nexus Intercellular Junctions

Abstract. *Intercellar junctions where cell membranes are in intimate contact (nexuses) are very abundant in the epithelium of normal human cervix. Squamous carcinoma cells are deficient in nexuses although a rare nexus is seen. Nexuses may be involved in normal growth regulation, while a deficiency of nexuses may be related to the invasive property of malignant growth.*

Abnormalities at the surface of cancer cells may account for their ability to invade surrounding normal tissues aggressively. Since abnormal cell movements are difficult to study in vivo, interactions of carcinoma cells have been examined in tissue culture. Normal cells in tissue culture do not move in the direction in which they are in contact with other cells, a property which is called contact inhibition of motility (*1*). In sharp contrast, carcinoma cells do move over one another, revealing a loss of contact inhibition (*2*).

Ultrastructural studies of tissue culture cells exhibiting contact inhibition show that adjacent cell plasma membranes are separated by at least 200 Å of extracellular space except in small regions (nexuses) where the membranes come into contact (*3*). Nexuses form sites of low-resistance electrical coupling between cells (*4–6*) indicating that small ions such as potassium can pass freely from the cytoplasm of one cell to the cytoplasm of another cell without significant leakage into the extracellular space. Nexuses may also allow passage of larger molecules (*4*, *6*, *7*), some of which could have growth regulatory activity. The ultrastructure of nexuses has not previously been studied in malignant tumors in vivo.

Although Potter *et al.* (*4*) have demonstrated electrical coupling between tumor cells in culture, Loewenstein *et al.* (*6*) have reported an absence of electrical coupling between carcinoma cells in vivo compared to a very high degree of electrical coupling between normal epithelial cells or benign tumor cells. They have suggested that this absence of electrical coupling reflects an inability of carcinoma cells to intercommunicate. However, neither Potter nor Loewenstein relate their findings to alterations in carcinoma cell membrane ultrastructure. We now report a striking deficiency of nexuses between human cervical carcinoma cells and describe the ultrastructure of the infrequent nexuses present in these tumors.

Three-millimeter punch biopsies were obtained from cervices of five normal human females, including one pregnant patient, and from four patients with invasive squamous cell carcinoma of the cervix (*8*), previously diagnosed by tissue biopsy. All biopsies were obtained prior to surgical, hormone, or radiation therapy. Each biopsy was hemisected. One half was fixed in a mixture of 2 percent paraformaldehyde and 2 percent glutaraldehyde (900 milliosmoles) (*9*) and the other half was similarly fixed but also impregnated with colloidal lanthanum hydroxide to aid in identifying nexuses in thin sections (*10*). The specimens were then post-fixed in osmic acid and embedded in Epon 812 (*11*). Examination of thin sections in the electron microscope showed that the preservation of ultrastructure was excellent in the controls and the tumors.

Normal human exocervix is covered with nonkeratinized, stratified, squamous epithelium that is subdivided into three layers: basal, intermediate, and superficial (*12*, *13*). The surfaces of the epithelial cells have many microvilli which are attached to the microvilli from neighboring cells. The two types of specialized membrane structures for cell-to-cell attachment are desmosomes and nexuses, which are most abundant in the intermediate layer (Fig. 1, A and B). At desmosomes, the cell membranes of adjacent cells are 250 to 300 Å apart but are attached by dense proteinaceous material within the extracellular space (*14*). At nexuses, the adjacent cell membranes come into such intimate contact that their outer portions are commonly thought to have fused (*5*, *12*), although special preparative techniques reveal a 20-Å space between the closely apposed cell membranes (*10*). Nexus membranes have a modified structure with small subunits spanning the 20-Å extracellular space to form small regions of contact between adjacent cell membranes. In the plane of the nexus, the subunits appear to be closely packed and are outlined in negative