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 image by colloidal lanthanum hydroxide which fills the extracellular space surrounding the individual subunits.

The tumors examined in this study resemble other cervical carcinomas described elsewhere (*13*). The tumor cells are somewhat pleomorphic but most closely resemble the intermediate layer cells in the normal epithelium. Regions of the plasma membranes not involved in junctions with adjacent carcinoma cells are typically separated by an enlarged extracellular space containing proteinaceous fluid. These spaces may be up to ½ $\mu$ in width. Where the cell surfaces are in closer apposition (less than 300 Å), microvilli and blunt cytoplasmic projections are attached to each other by desmosomes which usually have a laminar substructure identical to that seen in desmosomes in normal cervix. Poorly differentiated areas of tumor have fewer desmosomes than the normal cervical intermediate layer (*13*). Some desmosomes appear to be incompletely formed (Fig. 1C) and might possibly represent immature stages in desmosome formation.

Only rarely do adjacent tumor cell membranes come to within less than 100 Å of each other (Fig. 1C) to form nexuses. In two of the four tumors examined, no nexuses were detected after careful examination of many thin sections. In the other two tumors, an occasional area was found where a 20- to 30-Å "gap" separates the membranes of adjacent cells. In this gap, colloidal lanthanum is trapped and reveals that such tumor nexuses can have a closely packed array of subunits (Fig. 1D) identical to that seen at normal nexuses (Fig. 1B). In normal epithelium there may be as many as four nexuses connecting several interdigitating microvilli of adjacent cells (Fig. 1B) in the intermediate layer. Since the cells each have hundreds of microvilli, there may be many hundreds of nexuses per cell. Quantitation of nexuses in some well-differentiated regions of the two tumors in which nexuses could be demonstrated show that there can be enough nexuses to provide an average of up to four nexuses per cell in these areas although the exact distribution of nexuses has not been reconstructed from serial sections. It is quite possible that the small numbers of nexuses observed in well differentiated areas are clustered on a few cells and that many cells are totally free of nexuses. Thin sections of less well differentiated areas in these two tumors show very few or no nexuses.

We have thus demonstrated that nexuses are abundant in normal cervical epithelium but are deficient in invasive squamous carcinoma of the cervix. The use of colloidal lanthanum hydroxide in this study insured that all junctions present in sections were identified since nexuses cut obliquely as well as transversely can be readily identified when impregnated with this tracer. In preparations without added tracer, only transversely sectioned nexuses are recognized.

A deficiency of nexuses may explain Loewenstein's observations (*6*) that cells in invasive carcinomas lack electrical coupling. In many areas within a given tumor, carcinoma cells may be free of electrical coupling either because of inadequate numbers of nexuses or an incompetence of some of the nexuses that are present. Functional competence of junctions cannot be judged on the basis of their appearance in the electron microscope (*15*).

The deficiency of nexuses in squamous cell carcinoma does not appear to represent a simple recapitulation of the basal layer of the normal epithelium in which nexuses are less abundant than in the normal intermediate layer, since the majority of the squamous carcinoma cells closely resemble the cells of the intermediate layer. Rather, in invasive squamous carcinoma of the cervix there may be some abnormality in the control mechanism for forming nexuses, since, when formed in tumors, they can appear morphologically normal.

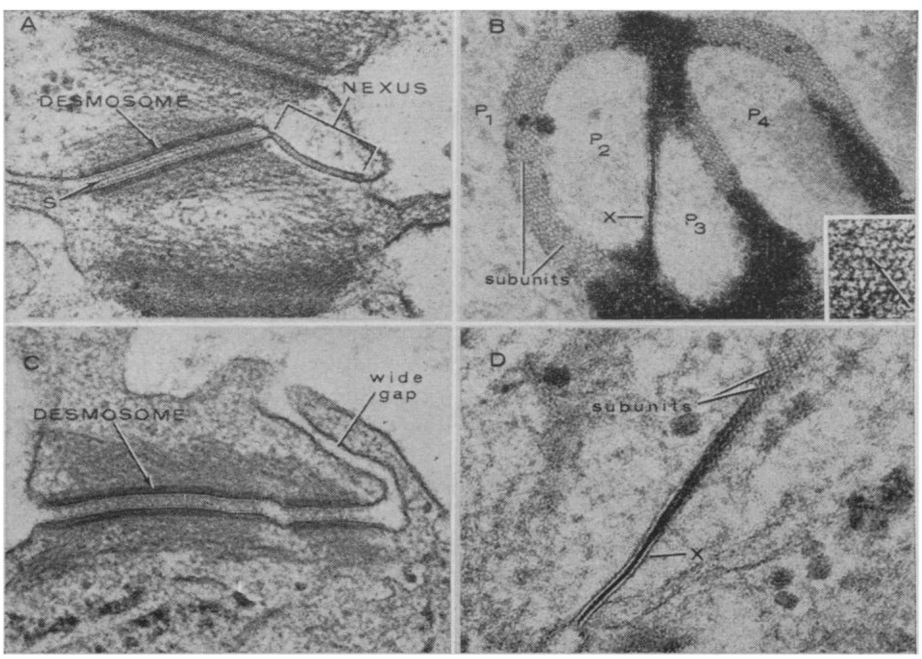


Fig. 1. Electron micrographs of regions of contact between adjacent cervical epithelial cells. (A) Normal cervical epithelium. Intermediate layer cells are connected to each other by attachment of their surface processes with desmosomes and nexuses. At a nexus, the cell membranes are very closely apposed. At a desmosome, the membranes are attached by dense proteinaceous material, often showing a distinct central stratum ($S$) (× 51,000). (B) Normal cervical epithelium. Four thin cell processes ($P_{1-4}$) are attached at nexuses. Opaque lanthanum hydroxide fills thin channels of extracellular space around subunits extending between the nexus membranes. Nexuses viewed *en face* show that the lanthanum outlines subunits in a closely packed array with 100 Å center to center spacing. In cross section, the subunit pattern is obscured owing to section thickness, and only a dark central 70 Å line is seen ($X$) (× 71,000). Inset: High magnification of a subunit array *en face* shows individual subunits (arrow) outlined by the lanthanum (× 168,000). (C) Squamous carcinoma of the cervix. Processes of adjacent cells may be attached at desmosomes but nexuses are infrequent, since the plasma membranes rarely approach each other closer than 100 Å. Some tumor desmosomes lack a central dense stratum. This possibly represents a stage in desmosome formation, since many tumor desmosomes have a normal laminar substructure (× 54,000). (D) Squamous carcinoma of the cervix. A rare nexus connects two tumor cells and has trapped the lanthanum, revealing the characteristic cross section ($X$) as well as a closely packed subunit array with a center-to-center spacing of 100 to 110 Å (× 87,000).

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## Hyperphagia in Rats with Cuts between the Ventromedial and Lateral Hypothalamus

Abstract. *Bilateral cuts between the ventromedial and lateral hypothalamus in female rats consistently produced hyperphagia. Hyperphagia occurred slightly less reliably when one of the cuts entered the ventromedial hypothalamus and only infrequently if one entered the lateral hypothalamus. The results are consistent with other evidence that suggests that fibers originating medially stop eating by inhibiting cells in the lateral hypothalamus.*

The lateral (LHA) and ventromedial (VMH) hypothalamic areas are generally thought to be the important neural regions governing hunger and satiety. The conceptual scheme that has emerged from the evidence is that when an animal is hungry, there is a high level of neural activity in the lateral hypothalamic "feeding center." As the animal becomes satiated, activity in the ventromedial "satiety" region increases and inhibits firing in the feeding center, and eating stops. There is substantial support for this conclusion. The evidence centers on observations that electrical (or chemical) stimulation of the lateral feeding center initiates eating in a sated animal while activation of the ventromedial satiety area arrests eating in a hungry animal (*1*). Conversely, lesions of the lateral area cause aphagia while removal of the medial satiety region gives hyperphagia (*2*).

The most widely accepted neural mechanism that accounts for these results is a direct inhibitory connection between the VMH and LHA. In support of this model, neural connections have been demonstrated histologically, and electrophysiological recordings have been obtained which show reciprocal patterns of neural activity between the VMH and LHA (*3*). However, this model has also been vulnerable to serious criticism. Electrical stimulation of the VMH will stop other ongoing activity as well as eating, and there is some evidence that the hyperphagia following lesions in the VMH may be due to irritation of the LHA by deposited heavy metal ions and scar tissue (*4*).

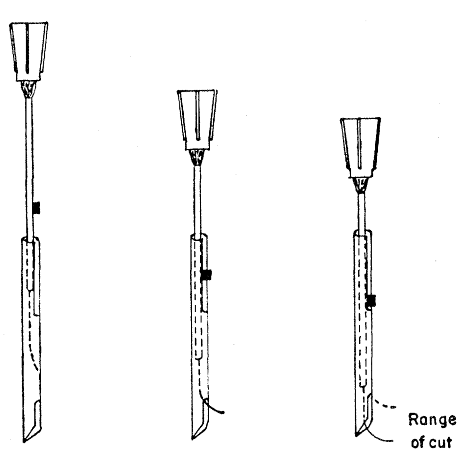


Fig. 1. Guide cannula and cutter used for making brain tissue cuts, showing cutter insert positions. The cutter is inserted into the guide cannula. The area cut begins where the blade emerges from the cannula.

The present experiment is intended to reexamine the functional interaction between the VMH and LHA. The method is to simply sever direct fiber connections by making a cut between these structures. If the VMH stops eating by inhibiting the LHA through direct fiber connections, cutting these pathways should result in an increase in food intake and a rapid weight gain similar to that occurring with VMH lesions.

Figure 1 shows the device used for making the cuts. The outer guide cannula (21-gauge stainless steel hypodermic tubing) was stereotaxically lowered into the brain of an anesthetized (sodium pentobarbital) female hooded rat (150 to 200 g). When the guide cannula was in place, a 26-gauge insert with a cutting blade made from stainless steel wire [diameter, 0.006 inch (0.015 cm)] was lowered into the cannula. When the cutting blade reached the lower slit, it extended and then cut through the neural tissue as it was lowered further. The cutting blade was removed and the guide cannula raised out of the brain. The size of the cutting blade and the distance the blade was pushed through the tissue were adjusted to give a cut of the desired dimensions (*5*). The cut used extended approximately 1 mm anterior and posterior to either end of the VMH. At the level of the VMH it extended ventrally to the base of the brain and dorsally about 0.0 to 1.0 mm above the fornix.

After surgery the animals were housed individually and maintained on a diet of wet mash, Purina lab chow, and water, which were freely available. Each animal was weighed daily for 20 days following the operation. The animals were then killed and their brains examined histologically.

On the basis of the histological examination the operated animals were divided into groups according to the placement of the cuts. For this classification, the hypothalamus was divided into three zones (Fig. 2). A VMH zone included cuts invading the VMH. A middle zone (M) was between the VMH and LHA and included cuts that did not extend medially into the VMH or further than 0.5 mm lateral to the fornix. Cuts entering the region further than 0.5 mm lateral to the fornix were in the LHA zone.