Novikoff (*12*) has suggested that their contraction could regulate blood flow in the vasa recta.

The occurrence of these unusual cylinders in dehydrated rats is consistent with the notion that ADH alters interstitial cell function. It cannot be stated, however, to what extent this represents a less specific reaction of the interstitial cells to the hypertonic papilla induced by ADH. It can nevertheless be pointed out that other cellular components of the medulla showed no such reaction.

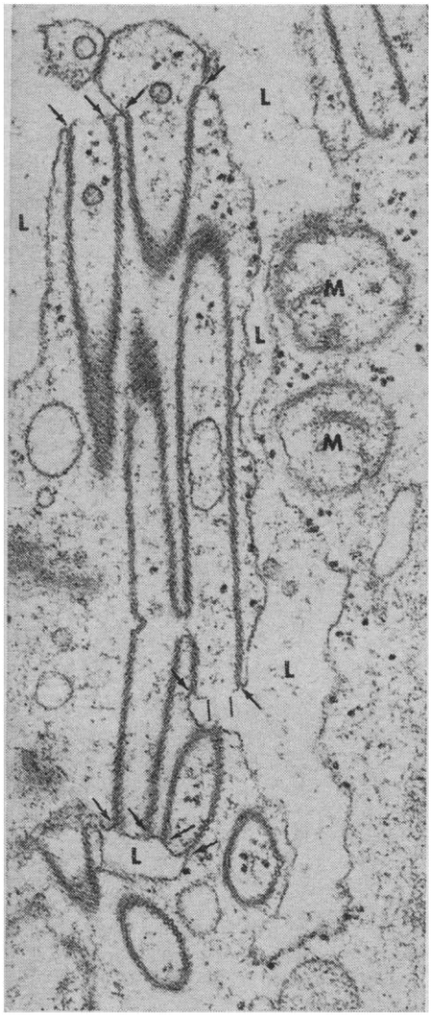

Fig. 3. Part of an interstitial cell, showing several cylinders cut in various planes. The lumens of several cisternae of endoplasmic reticulum are designated by *L*. The arrows indicate points at which the cisternal membranes become confluent with the cylinder walls. The lumens of the cisternae are obliterated at these points. These relationships are shown diagramatically in Fig. 2. Note that the lumen of the endoplasmic reticulum is not continuous with the lumen of the cylinder except when the bridging membrane at the cylinder base is artifactually broken (lines). The pointed ends on five of the cylinders result from oblique sectioning. *M*, mitochondrion (× 42,200).

The studies of Ginetzinsky (*13*) suggest that ADH produces an alteration in the mucopolysaccharide composition of the medulla. Though the cells responsible for the synthesis and maintenance of this material have not been identified, the interstitial cells are likely candidates, as they represent the principal connective tissue cells of this region.

As alterations in the structure of the granular endoplasmic reticulum have been correlated with defective protein synthesis in several other systems (*14*), the profound ergastoplasmic alterations described here may well reflect altered protein synthetic capabilities of renal interstitial cells in dehydrated rats that are producing hypertonic urine. If so, this would represent a unique response of interstitial cells, as other papillary components did not exhibit cylinders. As we have been unable to confirm the morphologic differences in other components of the papillae in various states of water balance, as previously reported (*15*), these alterations in the interstitial cells represent the most consistent change seen so far.

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Inositol Deficiency Resulting in Death: An Explanation of Its Occurrence in Neurospora crassa

Abstract. *The incorporation of radioactive inositol and choline into the cytoplasmic membranes of inositol- and choline-auxotrophic mutants of* Neurospora crassa *revealed that the membrane of particles which contain proteases is relatively poor in lecithin and rich in inositol-phospholipid. In mycelia of the mutant requiring inositol, grown in a suboptimum amount of exogenous inositol, the structural integrity of the protease particles is lost, and the bulk of intracellular protease activity is recovered in the soluble fraction of the cell. Death from this kind of inositol deficiency is interpreted as autolysis of the cytoplasm caused by free proteases.*

The mutants of *Neurospora crassa* requiring myoinositol grow abnormally in culture media containing insufficient amounts of inositol. The mycelia form tight colonies rather than spreading mats (*1*). Conidia of these mutants rapidly lose their viability when allowed to germinate in a minimal medium lacking inositol (*2, 3*). This phenomenon is the basis of a method for the efficient selection of auxotrophic mutants (*4*). In *Neurospora* (as in most organisms) inositol is incorporated into inositol-phospholipid, which is a structural constituent of the cytoplasmic membranes (*5*). The colonial growth, degeneration, and cell death resulting from culture in the absence of inositol has been explained by an imbalance between membrane synthesis and the formation of other cellular constituents (*6*). The striking differences between these mutants which degenerate in the absence

of inositol and other auxotrophic mutants which survive in the absence of the respective growth factors cannot be explained by such "unbalanced growth" (*3*).

Cell fractionation of *Neurospora* mycelia by density-gradient centrifugation has yielded particles containing proteolytic enzymes (*7*). These particles are constitutive structures present in the cytoplasm of all mutants tested so far. Functionally, they represent intracellular storage bags of proteases which are secreted into the growth medium if a protein (supplied as a nitrogen source) is to be digested extracellularly (*8*). Morphologically they are tiny spheres (diameters from 0.15 to 0.3 μ) with a single membrane envelope (*9*) whose phospholipid composition is greatly different from that of other cytoplasmic membranes. The incorporation of myo-inositol-C^{14} (*10*) and of choline-methyl-C^{14} (*11*) into mutants requiring inositol (*12*) or choline (*13*) revealed upon cell fractionation (*14*) and subsequent extraction and estimation of the labeled phospholipids (*15*) the intracellular distribution of the respective phospholipids (Fig. 1). Three fractions contain labeled cytoplasmic membranes: the protease particles at the interface of the sucrose gradient and the Urografin layer (*16*), the mitochondria (*17*), and a fraction with light membranes of yet unknown origin. Obviously the membranes of the protease particles contain radioactive lecithin at a relatively low concentration (about 3 to 4 percent of the total amount present in the homogenate) in comparison with a much higher percentage of the total inositol-phospholipid (about 14 percent). As shown by the distribution curve of the acid-protease activity, this enzyme is concentrated in the protease particles; only little activity is recovered in the soluble fraction at the top of the gradient system.

If the inositol-deficient strain is cultured at a suboptimum concentration of inositol (0.5 μg/ml), where it grows in colonies, all the inositol-phospholipid in the cytoplasm is reduced to about one-fourth of that in normally supplied cells. At the same time, the intracellular distribution of the inositol-phospholipid and the localization of the protease activity have greatly changed (Fig. 2). The highest percentage of the lipid radioactivity appears in the mitochondrial fraction; the light membranes contain much less inositol-phospholipid, and the peak indicating the position of the protease particles has disappeared almost completely. However, the most striking change is the location of protease activity, about 75 percent of which is in the soluble fraction. Only a small fraction of the total proteolytic activity is located in the protease particles. At a concentration of 5 μg of inositol per milliliter of culture medium an intermediary state of the two strains mentioned above can be observed: more than one-half of the protease activity is present in the protease particles, and about 30 percent is soluble. The distribution of lipid radioactivity resembles closely that of cells cultured in excess of inositol though the percentage of the label present in the protease particles is somewhat lower.

These data support the hypothesis that the phenomena associated with culture in scant amounts of inositol are results of a specific change in the membrane composition of the protease particles. Since inositol-phospholipid is a much more important constituent of these than of other cystoplasmic membranes, its limited synthesis causes defective protease particles. Consequently the membranes no longer fulfill their function of separating the proteases from the cytoplasm. The release of proteases as indicated by their appearance in the soluble cell fraction initiates the damage of the cytoplasm. It can be demonstrated that this event is a cause rather than a consequence of cell death. If decreasing amounts of inositol are added to the culture media at a concentration of 5 μg per milliliter the release of proteases can first be observed; down to a concentration of 2 μg/ml increasing activities of protease are liberated from the protease particles, but the integrity of mitochondria and light cytoplasmic membranes is maintained as indicated by normal amounts of protein in the respective fractions. But at still lower concentrations of inositol most of the protease activity is soluble and the cytoplasmic structures rapidly degenerate. The lytic effect of the free proteases is probably delayed by the presence of specific inhibitors in the soluble cytoplasm (*8*).

Conidia as well as hyphae contain

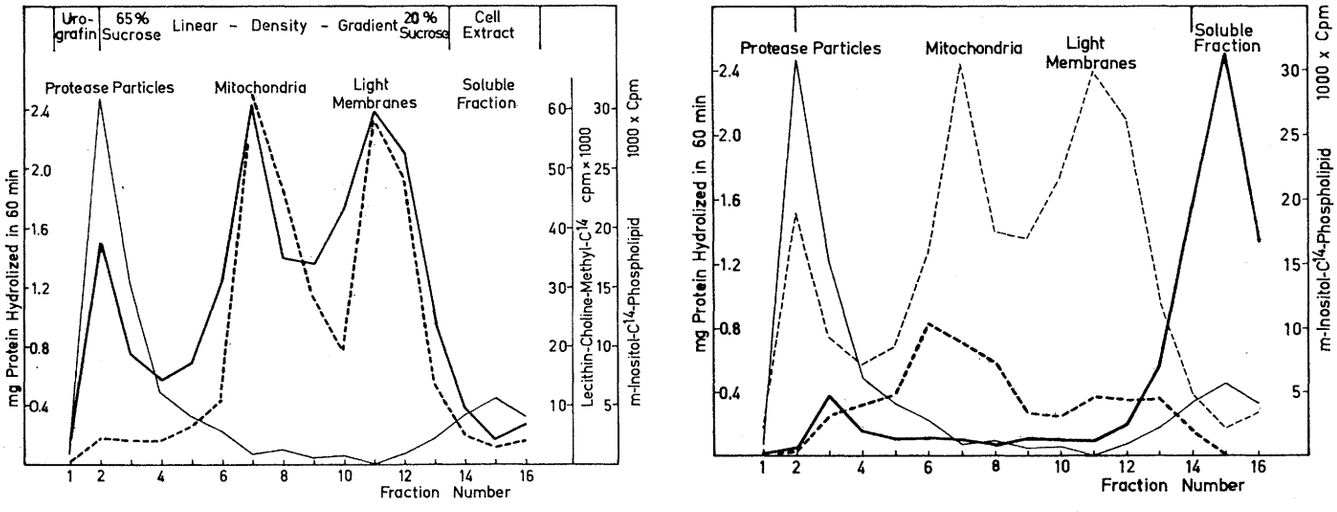

Fig. 1 (left). Intracellular distribution of lecithin-choline-methyl-C^{14} (broken thick line), myoinositol-phospholipid (solid thick line), and acid protease (solid thin line) in mycelia of choline- and inositol-requiring mutants of *Neurospora crassa*. The culture media contained 20 μg of choline or 50 μg of inositol per milliliter. Fig. 2 (right). Comparison of the intracellular distributions of myoinositol-phospholipid (broken lines) and acid protease (solid lines) in mycelia of inositol-requiring *Neurospora crassa* cultured at high (50 μg/ml; thin lines) or low (0.5 μg/ml; thick lines) concentrations of exogenous inositol.

proteases (*8*); therefore the death of conidia germinating in the absence of inositol can be interpreted as a consequence of cell autolysis caused by the release of protease from incompletely formed protease particles. Autolysis may be complete in the case of germinating conidia because the germ tubes have not yet formed septa. At low concentrations of inositol a mycelium will grow until the exogenous inositol is exhausted. At this moment septa have already been formed, and the beginning autolysis will affect mainly the cells which have been formed last after exhaustion of the inositol. Most probably this event starts in the cells at the tip of the hyphae. The autolysis of *Neurospora* cells results in the liberation of free inositol (*5*) which may support a further limited growth of the surviving part of the hyphae. It seems very likely that this path leads to the formation of small colonies consisting of a manifold branched mycelium (*6*). According to this hypothesis there are always some cells of such a colony undergoing autolysis; in fact electron micrographs obtained from colonies showed cell damage of varying degree (*6*).

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10. Radiochemical Centre, Amersham, England; 50 μg/ml, 1 μc/100 ml of culture medium.
11. New England Nuclear Corp., Boston; 20 μg/ml; 1 μc/100 ml of culture medium.
12. Strain No. 89601, FGSC No. 546.
13. Strain No. 34486, FGSC No. 485.
14. Cultures in liquid minimal medium containing 1 percent of sucrose, grown in an oscillating incubator. Mycelia harvested 16 hours after inoculation. See (*8*).
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16. Homogenization and low-speed centrifugation as described (*8*). Sucrose linear-density gradients (3,8 ml) ranging from 65 to 20 percent sucrose (weight-volume) were built on 0.5 ml of Urografin (methyl-glucamine salt of 3,5 diacetyldiamino-2,4,6-triiodobenzoic acid) with a density of 1.26 g cm^{-3}. Urografin is manufactured by Schering GmbH, Vienna, Austria; 1.0 ml of centrifuged homogenate containing 7 mg of protein were layered on top of the sucrose gradient. Centrifugation was carried out at 39,000 rev/min in a SW-39 swinging bucket rotor of a Spinco ultracentrifuge for 3 hours. After centrifugation the bottom of the tubes were punched, and the outflowing content was divided into 16 fractions.
17. For the identification of the mitochondria see (*15*).

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Uncoupling of a Nerve Cell Membrane Junction by Calcium-Ion Removal

Abstract. *Calcium ion participates in maintaining electrical connections between the nerve cells of Retzius* (Hirudo medicinalis). *The conductance across the junction between these cells decreases with decreasing concentration of free, extracellular* Ca^{++}. *At a certain level of* Ca^{++} *withdrawal from the cell system, junctional conductance reaches a critical low point at which the cells become functionally disconnected: the nerve impulses which are normally discharged in synchrony by the cells become asynchronous. These effects of* Ca^{++} *on junctional connection are irreversible, in contrast to those on nonjunctional surface membrane permeability.*

Close membrane complexes at cell junctions are now known to exist in a wide variety of tissues (*1*). For some tissues these junctional complexes clearly function as pathways for ionic communication between cells (*2*). We have now made an attempt at interrupting this communication by removing Ca^{++} from the tissue, a technique long used by biologists for mechanical separation of cells. The question was whether Ca^{++}, which has a well-known role in cell adhesion, also plays a role in cell-to-cell communication.

In the experimental approach to this question, we were fortunate in that cell communication is much more sensitive to Ca^{++} withdrawal than is cell adhesion. Thus, interruption of ionic communication (hereafter referred to as *uncoupling*) could be achieved with relatively mild procedures. Since Ca^{++} removal is also known to affect permeability of cell surface membranes, it was clear from the outset that uncoupling could only be demonstrated if the processes affecting junctional coupling and surface membrane permeability had different sensitivities or different rates. It turned out that the processes differed in both respects, particularly in their rates of reversal. While the processes pertaining to surface permeability were reversible within minutes, those pertaining to junctional coupling were not reversible at all. The processes could therefore be readily separated; this report deals only with Ca^{++} effects on junctional coupling obtained after reversal of the effects on surface membrane permeability.

We used the nerve "cells of Retzius" of the ganglion of the leech *Hirudo medicinalis*. The junctional coupling between these cells provides a means for synaptic transmission (*3*) of the electrical kind now known to occur in a variety of nerve cells (*4*). The cells were suitable for our purpose because of their large size (60 to 80 μ) and good visibility, and because of the rapid exchange of ions between cells and bathing fluid (*5*).

The ganglion was isolated in physiological saline (*6*); its connective tissue capsule was opened so as to expose the nerve cells and their glia; and three or four microelectrodes were inserted into the two nerve cells arranged to measure attenuation of surface-membrane voltage or junctional resistance. For measurements of voltage attenuation, current was passed between the inside of one cell and the cell exterior, and the resulting resistive voltage drops were recorded simultaneously across the surface membranes of this cell and the adjacent one, as illustrated in Fig. 1 (inset). In an alternate arrangement, all electrodes including the ground electrode were intracellular, and the cell exterior was effectively by-passed as a current path by placing the preparation in isotonic sucrose, a medium of high resistivity. Current flowed then directly from one cell interior to the other, and junctional resistance was directly measurable. In both arrangements, the microelectrodes were left inside the cells throughout all changes of test solutions. In the saline, the cells could be kept up to 3 hours with little change in their electrical properties.

An example of partial uncoupling is illustrated in Fig. 1*A*. The preparation was bathed first in normal saline; then in Ca^{++}-free saline for 17 minutes (*7*); and finally in normal saline again. Figure 1*A* (right) shows the effects