plucked for examination. The hair was immediately fixed in buffered 1-percent osmium tetroxide and embedded in Epon 812. The sections were stained with uranyl acetate and studied with an RCA EMU 3G electron microscope. By this technique melanocytes can be readily located in the hair bulb.

The melanocytes were large and contained a well-developed Golgi complex. Surrounding the latter were precursors of melanosomes, melanosomes, and melanin granules. The granules were large and numerous. Each granule was limited by a single membrane and contained a number of strands which made up the granule matrix. The melanosome granules were 2 to 3 times as thick and approximately twice as long as normal melanosomes. The large granules contained approximately twice as many strands as are normally found within a matrix of pigment granules, and in some instances the strands appeared to be more irregular than those found in the typical matrix of melanosomes. Melanin was present, although its relative concentration on the larger melanosomes may have been less than it is in fully developed, normal melanosomes (Figs. 2, 3, and 4).

Thus the morphologic basis of the pigmentary anomaly in this disease is almost certainly based on a defect similar to that responsible for the morphologic abnormalities of the leukocytes. The clinical appearance of the pigmentation defect may be a function of less total quantity of melanin, but the peculiar quality of the color can probably be most easily explained by invoking one of the known properties of melanin: when highly aggregated (that is, on large granules in this instance) it is less perceivable as color.

Melanocytes originate in the neural crest, and melanosomes have been characterized as the products of a cellular secretory process (*7*). Polymorphonuclear cells are mesothelial in origin, and their granules are, by all current criteria, lysosomes (*11*). The common morphogenesis which this work suggests for the organelles of these two diverse cell lines is most intriguing. Possibly the genetic defect is an abnormality of granule formation, including lysosome formation, which may be manifest in most or all cell lines. It is reasonable to postulate that further study may reveal abnormally large organelles having limiting membranes in other tissues, a possibility already suggested by the work of Page *et al.* (*5*) and Kritzler *et al.* (*3*). It then might be useful to look on the disease as a genetic disease of the membranes themselves.

An attractive alternative hypothesis, in view of the known role of some hormones in melanin formation (*12*) and the apparent association of lysosomal structures with hormonally controlled tissue regressions (*13*), is that these giant granules reflect an inherited abnormality of the functioning of a more central process which controls formation of certain membrane-bound structures within the cells.

If this is the case, patients with Chediak-Higashi syndrome may be revealing an important control mechanism or cellular function which plays a telling role in resistance to infection as well as to malignancy of the lymphoid system.

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References and Notes

1. M. Chediak, *Rev. Haematol.* **7**, 362 (1952); O. Higashi, *Tohoku J. Exptl. Med.* **59**, 315 (1954).
2. Pierini's case as described by O. C. Stegmaier and L. A. Schneider, *Arch. Dermatol.* **91**, 1 (1965); also Higashi's case.
3. R. A. Kritzler, J. Y. Terner, J. Lindenbaum, J. Magidson, R. Williams, R. Preisig, G. B. Phillips, *Am. J. Med.* **36**, 583 (1964).
4. W. L. Donohue and H. W. Bain, *Pediatrics* **20**, 416 (1957); P. Efrati and W. Jonas, *Blood* **13**, 1063 (1958); L. A. Schneider and S. R. McCreadie, *Proc. 7th Int. Congr. Int. Soc. Haematol.* (Grune and Stratton, New York, 1958), p. 1554.
5. A. R. Page, H. Berendes, J. Warner, R. A. Good, *Blood* **20**, 330 (1962).
6. G. A. Padgett, R. W. Leader, J. R. Gorham, C. C. O'Mary, *Genetics* **49**, 505 (1964).
7. M. S. C. Birbeck, E. H. Mercer, N. A. Barnicott, *Exptl. Cell Res.* **10**, 505 (1956); M. Seiji, T. B. Fitzpatrick, M. S. C. Birbeck, *J. Invest. Dermatol.* **36**, 243 (1961); A. S. Zelickson, *Electron Microscopy of Skin and Mucous Membrane* (Thomas, Springfield, Ill., 1963), p. 108; S. R. Wellings and B. V. Siegel, *Ann. N.Y. Acad. Sci.* **100**, 548 (1963).
8. M. Bessis, J. Bernard, M. Seligman, *Nouv. Rev. Franc. Hematol.* **1**, 422 (1961); C. Mauri and V. Silingardi, *Acta Haematol.* **32**, 114 (1964).
9. J. G. White, personal communication.
10. T. B. Fitzpatrick, *Metabolic Bases of Inherited Disease* (Blakiston, New York, ed. 2, 1960), p. 428.
11. Z. A. Cohn and J. G. Hirsch, *J. Exptl. Med.* **112**, 983, 1015 (1960); J. G. Hirsch and Z. A. Cohn, *ibid.*, p. 1005.
12. Various authors, *Ann. N.Y. Acad. Sci.* **100**, 622–719 (1963).
13. A. B. Novikoff, in *The Cell* (Academic Press, New York, 1961), p. 2473.
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Endoplasmic Reticulum in Rat Renal Interstitial Cells: Molecular Rearrangement after Water Deprivation

Abstract. *Cylindrical bodies in renal interstitial cells of dehydrated rats are confluent with membranes of endoplasmic reticulum. The cylinder walls, composed of helically arranged pentagonal tubules, may represent a molecular rearrangement of the membrane structure. The cylinders may represent a morphologic expression of altered ergastoplasmic function possibly related to the production of concentrated urine.*

The renal papilla is important in the process of urine concentration (*1*). We have been studying the morphology of the rat renal papilla during dehydration and water diuresis in order to determine if ultrastructural data can contribute to an understanding of the mechanisms involved in urine concentration.

The rat papilla is composed of large collecting ducts, capillaries, thin limbs of Henle's loop, and an abundant interstitium containing elongated interstitial cells. These cells are oriented perpendicular to the long axis of the tubules and vessels like the rungs of a ladder, and have long, branching cytoplasmic processes that seem to encircle tubules and vessels. The cytoplasm of the interstitial cells is characterized by abundant rough-surfaced endoplasmic reticulum, lipid droplets, and many fine filaments. The cells are partially surrounded by basement membrane material.

Animals were studied in various states of water balance. In one experiment rats were deprived of drinking water for 1 to 13 days. In another, rats with exteriorized urinary bladders were given 8 ml of water or 12.5-percent ethyl alcohol per 100 g of body weight. The presence of a water diuresis was confirmed by a urine output

of 0.1 ml/min or greater, or by an osmolarity less than 300 milliosmols per liter if alcohol had not been administered. Kidneys were fixed by injection into the renal pelvis, immersion, or vascular perfusion of either 2-percent OsO_4 buffered with *s*-collidine (*2*) or 6.25-percent glutaraldehyde buffered with 0.1*M* cacodylate and post-osmicated (*3*).

In the rats deprived of water for 1 to 13 days, many interstitial cells contained unusual cylindrical bodies (Fig. 1, part 1). Though the actual number observed was not directly proportional to the duration of water deprivation, numerous cylinders were seen in many dehydrated animals after all types of fixation utilized. They were identical in appearance in tissue fixed in glutaraldehyde with post-osmication. In several hundred micrographs of papillae from rats undergoing water diuresis only two cylinders were seen.

The bodies were generally cylindrical, though the walls occasionally showed indentations, protrusions, and inflections. The cylinders were 0.1 to 0.2 μ in diameter and have been measured at up to 4 μ in length.

The walls of the cylinders appeared to be composed of a helical array of tubules with a helix angle of 35°. In cross-sectional views of the tubules comprising the wall, a staggered row of profiles was seen. The profiles were approximately 170 Å in diameter. The tubular profiles appeared pentagonal in cross section but their small size made it difficult to exclude other geometric forms (Fig. 1, parts 6 and 7).

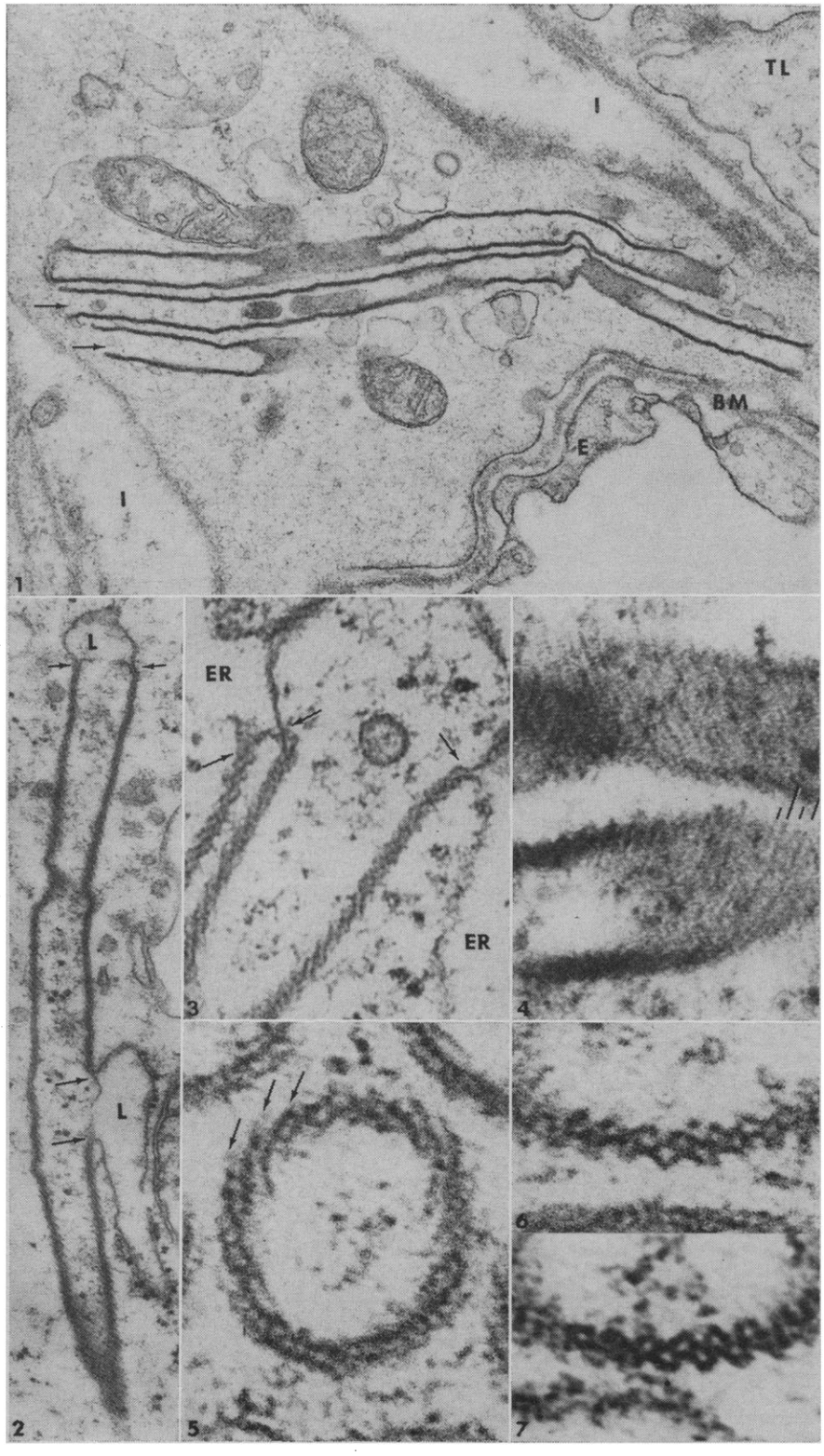

Fig. 1. (Part 1) An interstitial cell from the renal papilla of a dehydrated rat, showing longitudinal sections of three cylindrical bodies. Note that in at least two of the bodies continuity between the cylinder interior and the cytoplasmic ground substance can be seen (arrows). Only a basement membrane (*BM*) separates the interstitial cell from the capillary endothelium (*E*). Thin limb, *TL*; interstitium, *I* (× 31,300). (Part 2) A longitudinal section of a cylinder showing continuities (arrows) between the cylinder walls and the membranes of the endoplasmic reticulum. The lumen (*L*) of the endoplasmic reticulum is obliterated at the point of membrane continuity. Thus the lumen of the endoplasmic reticulum does not communicate with the lumen of the cylinder (see Fig. 2) (× 46,600). (Part 3) Higher magnification picture showing continuity of cylinder wall with membranes of endoplasmic reticulum (*ER*). Note the transition of the membranes into the complex mural structure (arrows) (× 101,900). (Part 4) A tangential section of a cylinder surface showing a pattern of dense lines (long lines) and less dense lines (short lines) separated by electron-lucent areas. Although the spacing of the dense lines corresponds to that of alternate pentagon peaks, the identity of the dense and less dense lines is not certain (× 112,600). (Part 5) Body cut almost normal to its axis, showing a nearly circular profile. Because the section is partially oblique a pattern of dense lines can be seen (arrows) (× 214,800). (Parts 6 and 7) Oblique sections of a cylindrical body showing what are interpreted as cross sections of the tubular elements comprising the wall (× 203,800).

Each pentagon shared lateral walls with two adjacent pentagons. The apices of adjoining pentagons alternately extended toward and away from the cylinder axis (Fig. 2). The centers of adjacent pentagons were consequently offset, forming the staggered pattern. The diameter of the unit and the angle and lead of the helix indicate that approximately 11 to 22 tubules form the wall. In oblique sections of the wall, dense lines formed a pattern (pattern 1) with an ~170-Å periodicity, although considerable variation could be seen on different micrographs (Fig. 1, parts 3 and 5). This corresponded to the approximate diameter of the cross-sectional profiles of the mural tubules when these two intervals were measured on the same micrograph. In fortuitous sections, the walls of the tubular profiles appeared continuous with these dense lines.

In more tangential sections of the wall, an alternating pattern of dense and less dense lines, separated by electron-lucent areas, was seen (Fig. 1, part 4). The repeat period of this second pattern (pattern 2) was ~200 Å. Although considerable variation was seen on different micrographs, when the repeat period and distance between projecting peaks of alternate pentagons were measured on the same micrograph they were found to be of approximately the same length. Pattern 1 is probably formed by the walls of adjacent pentagons. While the identity of pattern 2 cannot be stated with certainty, its repeat period appears to correspond with the spacing of alternate pentagon peaks.

In longitudinal sections the tubular unit appears to have a beaded structure. This appearance would be consistent with either a repeating subunit such as pentagonal prisms in the pentagonal tubule or with a secondary helical structure in each tubule.

The lumen of the cylinder contained flocculent material identical in appearance with the cytoplasmic ground substance. Circular and ellipsoidal membranous profiles were often seen within the lumen of the cylinder. In favorable sections (Fig. 1), the lumen of a cylinder communicated directly with the cytoplasmic ground substance.

Continuities were frequently seen between the membranes of the endoplasmic reticulum and the walls of the cylinders (Fig. 1, parts 2 and 3; Figs. 2 and 3). The most frequent site of continuity occurred at the end of a cylinder, although regions did exist where confluence with the cylinder wall occurred along the lateral surface (Fig. 1, part 2). When these continuities occurred, the lumen of the endoplasmic reticulum disappeared and, in a profile view, both membranes of the endoplasmic reticulum seemed to merge into the cylinder wall (Fig. 1, part 3; Fig. 2). The membranes of the endoplasmic reticulum fused with the structure of the wall and it seemed likely that a molecular reorganization might occur at the point where the membrane sheet became part of the helically arranged tubules.

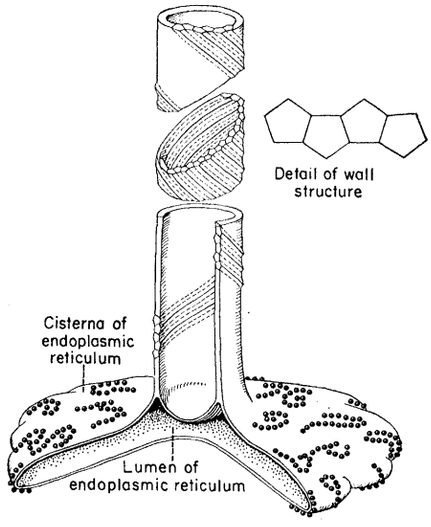

Fig. 2. Diagrammatic representation of a possible three-dimensional interpretation of a cylinder, showing its relationships with the endoplasmic reticulum and the detailed structure of the wall. The cross sections of the tubular units of the cylindrical wall in this drawing are represented as pentagons, although their small size made it difficult to exclude other geometric forms such as circles or hexagons.

A possible interpretation of these relationships in three dimensions is shown in the diagram (Fig. 2). Observe the relationships between the membranes of the cisterna of endoplasmic reticulum and the wall of the cylinder. Note that the lumen of the endoplasmic reticulum is obliterated at the point of membrane continuity between these two structures and does not communicate with the lumen of the cylinder. The cross section of the tubular units making up the cylinder wall is represented as pentagonal, an interpretation favored by micrographs such as parts 6 and 7 of Fig. 1. It should be noted however, that the small size of these units makes it impossible at the present time to exclude other geometric forms. The bases of alternate pentagons are indicated by broken lines to help visualize the presumed three-dimensional relationships. Corresponding lines are not seen in electron micrographs.

The cylinders demonstrate a repeating substructure which may result from a reorganization of ergastoplasmic membrane components. Examples of membrane substructure have been identified [see review by Kavanau (*4*)] that differ from each other and from the structure reported here. From observation of electron micrographs, Robertson (*5*) first proposed the "unit membrane" hypothesis, and many investigators concur with his interpretations. Other investigators, however, have reported examples of alternative geometrical configurations within membranes.

Granular or disc-like subunits were seen in red-blood-cell membranes with a variety of techniques (*6*). Hexagonal patterns of ~180-Å spacing have been seen with freeze-etching of yeast cells (*7*). A globular substructure has been seen in certain cell membranes by Sjöstrand (*8*) with $KMnO_4$ fixation or freeze substitution. Using permanganate fixation, Robertson (*9*) identified a honeycomb pattern (repeat period of 95 Å), consisting mainly of hexagons, in synaptic discs of retinal Mauthner cells. He postulated that this pattern was localized mainly in the outside lipoprotein or lipopolysaccharide-protein layer of the "unit membrane." A similar pattern with a center-to-center spacing of 80 to 90 Å was seen in plasma membranes of rat livers negatively stained at 37°C (*10*), whereas other regions of these membranes showed globular units. Although a few pentagonal units were seen in the surface patterns of Robertson (*9*) and Benedetti and Emmelot (*10*), and constitute the basic structure of certain viral capsomers (*11*), membrane substructure largely involving pentagonal units appears to be unique to the cylindrical bodies described here.

The precise role of the interstitial cells in medullary function is not known. Previous workers have noted their similarity to smooth muscle or pericapillary cells, their intimate relationship with collecting ducts, their well-developed ergastoplasm, and their numerous lipid droplets (*12*). Sternberg *et al.* (*12*) have speculated that they represent target organs for action of antidiuretic hormone (ADH), and

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.

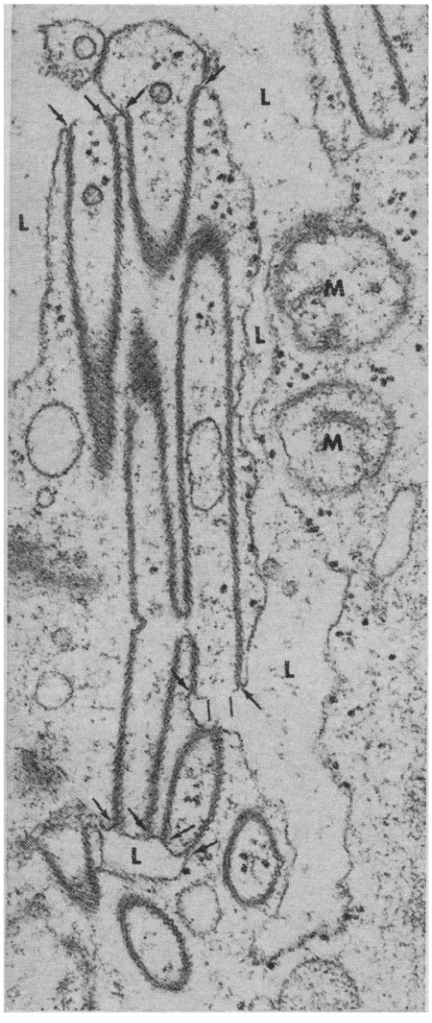

Fig. 3. Part of an interstitial cell, showing several cylinders cut in various planes. The lumens of several cisternae of endoplasmic reticulum are designated by *L*. The arrows indicate points at which the cisternal membranes become confluent with the cylinder walls. The lumens of the cisternae are 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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References and Notes

1. H. Wirz, B. Hargitay, W. Kuhn, *Helv. Physiol. Pharmacol. Acta* **9**, 196 (1951).
2. H. S. Bennett and J. H. Luft, *J. Biophys. Biochem. Cytol.* **6**, 113 (1959).
3. D. D. Sabatini, K. Bensch, R. J. Barrnett, *J. Cell Biol.* **17**, 19 (1963).
4. J. L. Kavanau, *Structure and Function in Biological Membranes* (Holden-Day, San Francisco, 1965), vol. 2.
5. J. D. Robertson, *Symp. Biochem. Soc.* **16**, 3 (1959).
6. J. Hillier and J. F. Hoffman, *J. Cellular Comp. Physiol.* **42**, 203 (1953); D. R. Coman and T. F. Anderson, *Cancer Res.* **15**, 541 (1955); J. F. Hoffman, *J. Cellular Comp. Physiol.* **47**, 261 (1956); H. Fernández-Morán, *Circulation* **26**, 1039 (1962); H. Fernández-Morán, in *The Interpretation of Ultrastructure*, R. J. C. Harris, Ed. (Academic Press, New York, 1962), vol. 1, p. 411.
7. H. Moor and K. Mühlethaler, *J. Cell Biol.* **17**, 609 (1963).
8. F. S. Sjöstrand, in *The Interpretation of Ultrastructure*, R. J. C. Harris, Ed. (Academic Press, New York, 1962), vol. 1, p. 47; F. S. Sjöstrand and L. G. Elfvin, *J. Ultrastruct. Res.* **10**, 263 (1964).
9. J. D. Robertson, *J. Cell Biol.* **19**, 201 (1963).
10. E. L. Benedetti and P. Emmelot, *ibid.* **26**, 299 (1965).
11. D. L. D. Caspar and A. Klug, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 1 (1962).
12. W. v. Möllendorff, *Handbuch der Mikroskopischen Anatomie des Menschen. Harn und Geschlechtsapparat* (Springer, Berlin, 1930), vol. 7; B. J. Vimtrup and B. Schmidt-Nielsen, *Anat. Record* **114**, 515 (1952); W. H. Sternberg, E. Farber, C. E. Dunlap, *J. Histochem. Cytochem.* **4**, 266 (1956); A. B. Novikoff, in *Biology of Pyelonephritis*, E. L. Quinn and E. H. Kass, Eds. (Little, Brown, Boston, 1960) pp. 113–144.
13. A. G. Ginetzinsky, *Nature* **182**, 1218 (1958).
14. E. A. Smuckler, O. A. Iseri, E. P. Benditt, *J. Exptl. Med.* **116**, 55 (1962).
15. J. S. Robson, in *Memoirs of the Society for Endocrinology, No. 13, Hormones and the Kidney*, P. C. Williams, Ed. (Academic Press, London, 1963), pp. 105–119; M. S. Sabour, M. K. MacDonald, A. T. Lambie, J. S. Robson, *Quart. J. Exptl. Physiol.* **49**, 162 (1964); R. C. Muehrcke, S. Rosen, F. I. Volini, in *Progress in Pyelonephritis*, E. H. Kass, Ed. (Davis, Philadelphia, 1965), p. 422.

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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