(*18*), then it must be postulated that the toxin can pass retrograde across the synapse from motor neurons to inhibitory nerve terminals. This study demonstrates that [^{125}I]tetanospasmin is carried retrograde within axons, providing direct evidence for axonal transport of a macromolecule that interferes with synaptic transmission in the CNS.

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References and Notes

1. S. I. Zacks and M. F. Sheff, in *Neuropoisons*, L. Simpson, Ed. (Plenum, New York, 1971), vol. 1, pp. 225–262.
2. L. Weinstein, *Curr. Conc. Med. Intell.* **289**, 1923 (1973); C. Lamanna and C. J. Carr, *Clin. Pharmacol. Ther.* **8**, 286 (1967); E. B. Adams, in *Neuropoisons*, L. Simpson, Ed. (Plenum, New York, 1971), vol. 1, pp. 213–224.
3. V. B. Brooks, D. R. Curtis, J. C. Eccles, *J. Physiol. (Lond.)* **135**, 655 (1957); D. R. Curtis, in *Neuropoisons*, L. Simpson, Ed. (Plenum, New York, 1971), vol. 1, pp. 263–281.
4. S. I. Zacks and M. F. Sheff, in *Neurosciences Research*, S. Ehrenpreis and O. C. Solmitzky, Eds. (Academic Press, New York, 1970), vol. 3, pp. 210–287; G. P. Wright, *Proc. R. Soc. Med.* **46**, 319 (1953); *Pharmacol. Rev.* **7**, 413 (1955); V. Morax and A. Marie, *Ann. Inst. Pasteur* **17**, 335 (1903).
5. The toxin was obtained via P. Cuatrecasas (Department of Pharmacology, Johns Hopkins Medical School) from R. O. Thomas (Wellcome Laboratories). The toxin was iodinated by a modification [P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1264 (1971)] of the method described by W. M. Hunter [in *Handbook of Experimental Immunology*, D. M. Weir, Ed. (Blackwell, Oxford, England, 1973), pp. 17.1–17.36].
6. The nerves were fixed with 3 percent glutaraldehyde in 0.1*M* cacodylate buffer with 3 percent sucrose, washed in the same buffer with 10 percent sucrose, postfixed in OsO_4, dehydrated in alcohol, and embedded in Epon 812. For light autoradiography, 1.5-μm sections were dipped in Kodak NTB-2, developed in Kodak D-19, stained with toluidine blue, and examined in a Zeiss photomicroscope II.
7. Although nerve crush ultimately results in Wallerian degeneration of the distal stump, previous studies have shown that retrograde transport persists for hours following axotomy [L. Lubinska and S. Niemierko, *Brain Res.* **27**, 329 (1970)]. Dark-staining axoplasm is commonly present in proximity to the crush; ultrastructural study shows that the axoplasm consists of mitochondria and membranous and vesicular organelles. The central light-stained portion represents a core of neurofilaments [J. Zelena, L. Lubinska, E. Gutmann, *Z. Zellforsch. Mikrosk. Anat.* **91**, 200 (1968)].
8. Initial studies with dye tracers showed that this type of injection resulted in spread of tracer through the endoneurial space without producing histological changes in the nerves.
9. U. C. Seib, B. Hensel, H. Wiegnad, H. H. Wellhoner, *Naunyn-Schmiedebergs Arch. Pharmakol.* **276**, 403 (1973).
10. J. H. Baylis, J. Mackintosh, R. S. Morgan, G. P. Wright, *J. Pathol. Bacteriol.* **64**, 33 (1952).
11. S. I. Zacks and M. F. Sheff, *J. Neuropathol. Exp. Neurol.* **25**, 422 (1966); *Science* **159**, 643 (1968).
12. A. A. Fedinec, in *Principles on Tetanus*, L. Eckmann, Ed. (Huser, Bern, Switzerland, 1967), pp. 169–176.
13. K. Kristensson, *Acta Neuropathol.* **16**, 293 (1970); ——— and Y. Olsson, *Brain Res.* **29**, 363 (1971); *Progr. Neurobiol.* **1**, 87 (1973); J. H. LaVail and M. M. LaVail, *Science* **176**, 1416 (1972).
14. S. I. Zacks and A. Saito, *J. Histochem. Cytochem.* **17**, 161 (1969).
15. H. A. Hansson, *Exp. Eye Res.* **16**, 377 (1973); J. H. LaVail and M. M. LaVail, *J. Comp. Neurol.* **157**, 303 (1974).
16. D. R. Curtis and W. C. deGroat, *Brain Res.* **10**, 208 (1968).
17. A. Arregui, W. J. Logan, J. P. Bennett, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3485 (1972); R. Werman, *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **50**, 145 (1972); A. Ljungdal and T. Hokfelt, *Brain Res.* **62**, 587 (1973); A. I. Matus and M. E. Dennison, *J. Neurocytol.* **1**, 27 (1972); L. L. Iversen and F. E. Bloom, *Brain Res.* **41**, 131 (1972).
18. G. A. R. Johnston, W. C. deGroat, D. R. Curtis, *J. Neurochem.* **16**, 797 (1969); R. H. Osborne, H. F. Bradford, D. G. Jones, *ibid.* **21**, 407 (1973); R. H. Osborne and H. F. Bradford, *Nat. New Biol.* **244**, 157 (1973).
19. We thank G. McKhann, S. Snyder, D. Bodian, and J. Bennett for helpful discussions. This work was supported by NIH grants NS 10580-01A1 and NS 19020-02.

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Cell Surface Differences in Ducts from Cancerous and Noncancerous Human Breasts

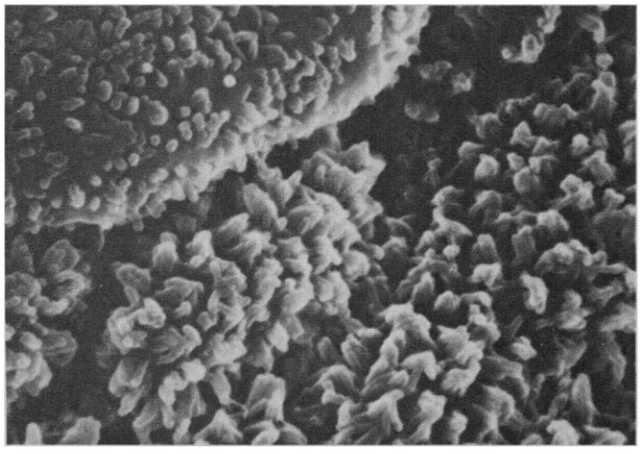
Abstract. *The scanning electron microscope reveals structural differences between the apical microvilli of duct cells from cancerous and noncancerous human breasts. The alterations in the microvilli from carcinomatous breasts appear to be highly specific, to extend throughout the affected breast, and may be pathognomonic for this condition.*

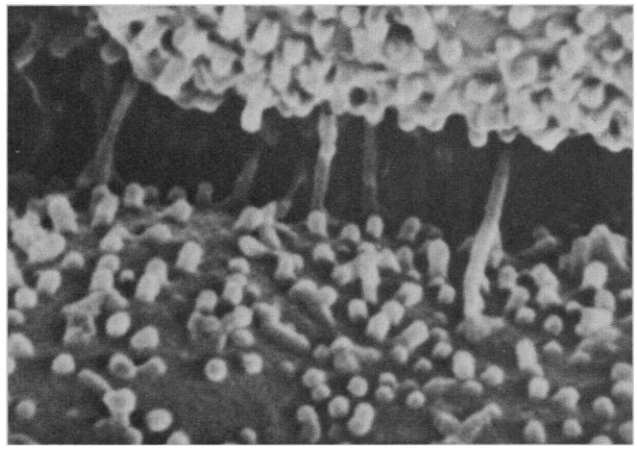
The structure and composition of the plasma membrane seem to vary among different cell types (*1*) and with the functional activities of any given cell (*2*). In addition, it is commonly suspected that cancer cells may have unique or highly specialized plasma membranes which give rise to or facilitate their loss of contact inhibition, accelerated growth, tumorigenicity, and metastatic spread throughout the body (*3*). Recently, Porter *et al.* and others (*4, 5*), using the scanning electron microscope, found several differences between the surface morphology of normal and transformed cells in vitro, while others described differences in the binding of plant lectins to the outer surface of the plasma membranes from normal and cancer cells (*6*).

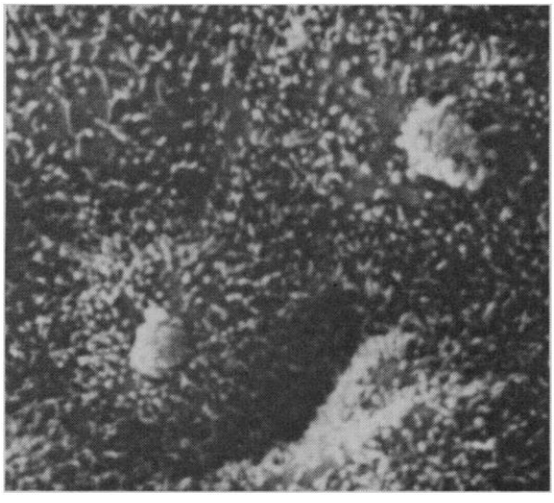
Since last year we have been studying the surface topography of human breast tissues with the scanning electron microscope. We have observed material obtained at biopsy or mastectomy from 32 women with the following breast abnormalities: 11 dysplasias, 4 fibroadenomas, 2 lobular carcinomas in situ, and 15 infiltrating duct carcinomas. The results to date strongly suggest that the duct epithelium within dysplastic breasts or those containing benign neoplasms is distinctly different from the duct epithelium within carcinomatous breasts. Thus, the purpose of this report is to draw attention to previously undetected differences in the surface morphologies of ducts from cancerous and noncancerous breasts which may be of potential diagnostic or prognostic importance. The surface morphology of mammary carcinoma cells will be described in detail elsewhere (*7*).

The surgical specimens were rinsed briefly in 0.1*M* cacodylate buffer, *p*H 7.4, fixed for 4 days to 2 weeks in a modified Karnovsky's fixative (*8*), dehydrated in alcohol, and frozen in liquid Freon. The frozen tissue was cracked with a precooled scalpel (*9*) and critical-point-dried in liquid CO_2 (*10*). Then the dried tissue was viewed under a dissecting microscope and the upper halves of ducts close to the surface of the specimen were teased away. As a result, the observer obtains an unimpeded view of essentially all cell surfaces bordering one half of the duct lumen. The specimens were mounted on stubs with silver conducting paint, coated with gold about 200 Å thick on a rotating stage in a vacuum evaporator, and viewed at 20 kv in a Kent-Cambridge S_4 scanning electron microscope.

Usually, the apical surface of the glandular epithelial cells within both normal and hyperplastic ducts from noncancerous breasts is covered with minute projections—the microvilli. The diameter of the microvilli is fairly constant (about 0.1 μm). The average microvillus is 1.5 to 2 μm long; however, there is moderate variation in the length of the microvilli on individual cells as well as among adjacent cells. Sometimes the surface contains only small spherical knobs, but it is more common to find knobs intermingled with microvilli. Since the diameter of these knobs is about 0.1 μm and we found no new surface structures on these cells with the transmission electron microscope, they may be rudimentary microvilli or surface blebs which are disappearing from or forming on the surface of the cells during specific portions of the cell cycle or in response to other alterations in their environment [see (*4*) for discussion]. Cells undergoing apocrine







Figs. 1–3. The apical surface features of duct epithelial cells thought to be specific for carcinoma are shown in these scanning electron micrographs. Fig. 1 (top left). Clumped microvilli are seen on several cells as well as rudimentary microvilli or knobs on the surface of a giant cell in the upper left corner of the figure (× 9,600). Fig. 2 (top right). Intercellular microvillus contacts between two cells are shown at higher magnification (× 19,100). Fig. 3 (left). Two adjacent cells, each with a central clump of thickened, irregular microvilli (× 7,000).

metaplasia generally have few microvilli; however, their positive identification depends upon their characteristic size and shape rather than their microvilli or surface topology.

The apical surface of the duct epithelium from cancerous breasts displays some or all of the characteristics of ducts from noncancerous breasts. However, duct cells from cancerous breasts show greater variation in size and shape and in the number, length, and arrangement of microvilli. Moreover, there is a decided tendency for these cells to have fewer microvilli and more knobs or rudimentary microvilli than duct cells in noncancerous breasts. Because it is possible with our mode of specimen preparation to view thousands of cells at one time within a given duct, the observer immediately sees these general surface features even at low magnification (about × 2000 or less). In our experience, when these general alterations are prominent and fairly extensively distributed along the duct there is good reason to suspect that the specimen came from a breast with carcinoma. However, alterations which appear to be specific for carcinomatous breasts are the following: (i) the partitioning of the surface microvilli into small groups or clusters composed of three or more microvilli clumped together at their tips (Fig. 1); (ii) the presence of intercellular microvillus contacts, that is, microvilli from adjacent cells touching at their apices (Fig. 2); and (iii) a prominent clump of thickened, irregular microvillus-like projections in the center of the apical surface (Fig. 3).

Tissues from the last 19 women in our series were processed and analyzed without knowledge of their clinical diagnosis. In *every* case there was perfect agreement between the pathological diagnosis and the one we made with the scanning electron microscope. Hence, it appears that the distribution and arrangement of the apical microvilli on mammary duct epithelium may be a pathognomonic sign of carcinoma. In other words, we did not have to find infiltrating duct carcinoma cells within the stroma of the gland in order to predict whether the tissue came from a cancerous or noncancerous breast: the surface features of the duct epithelial cells alone were sufficient morphological evidence for distinguishing the two conditions. In addition, in seven out of seven mastectomy patients, ducts from so-called normal areas and at graded distances [that is, up to 7 inches (18 cm)] from the primary lesion or biopsy site also showed these structural characteristics. Therefore, the unusual surface features seem to extend throughout the affected breast. We have no idea when and how these structural alterations arise, what relationship they may have to either the multicentric or focal origin of carcinoma, and whether they are a preneoplastic lesion or the response of noncancerous cells to some influence exerted by the malignant breast cells or to systemic factors from the patient. We also do not yet know how useful the scanning electron microscope will be for distinguishing certain very confusing benign conditions such as plasma cell mastitis or florid sclerosing adenosis from carcinoma. Nevertheless, in view of the reasonably large number of patients studied, the reproducibility of the observations, and the ease with which the scanning electron microscope could be used by pathologists, it seemed justifiable to report these findings at this time.

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References and Notes

→ W. Stoeckenius and D. M. Engelman, *J. Cell Biol.* **42**, 613 (1969); D. F. H. Wallach, *Biochim. Biophys. Acta* **265**, 61 (1972); E. L. Benedetti, I. Dunia, A. Diawara, *Eur. J. Cancer* **9**, 263 (1973).

2. P. K. Crane, in *Intracellular Transport*, K. B. Warren, Ed. (Academic Press, New York, 1966), p. 71; A. L. Jones and E. Spring-Mills, in *A Guide to Molecular Pharmacology-Toxicology*, R. M. Featherstone, Ed. (Dekker, New York, 1973), p. → E. Spring-Mills and Y. J. Topper, *J. Cell Biol.* **44**, 310 (1970).

3. H. Rubin, in *Major Problems in Developmental Biology*, M. Locke. Ed. (Academic Press, New York, 1966), p. . → M. Abercrombie, J. Heaysman, H. Karthauser, *Exp. Cell Res.* **13**, 276 (1957);

M. Abercrombie and E. J. Ambrose, *Cancer Res.* **22**, 525 (19 → R. Sheinin and K. Onodera, *Biochim. Biophys. Acta* **274**, 49 (1972).
→ K. R. Porter, G. T. Todaro, V. Fonte, *J. Cell Biol.* **59**, 633 (1973).
5. K. R. Porter, V. Fonte, G. Weiss, *Cancer Res.* **34**, 1385 (19 → L. P. Everhart, Jr., and R. W. Rubin, *J. Cell Biol.* **60**, 434, 442 (1974).
6. M. J. Cline and D. C. Livingston, *Nat. New Biol.* **232**, 155 (1971); K. D. Noonan and M. M. Burger, *J. Biol. Chem.* **248**, 4286 (1973); G. L. Nicolsen, *Nat. New Biol.* **223**, 244 (1971); *ibid.* **243**, 218 (1973).
7. E. Spring-Mills and J. J. Elias, in preparation.
8. M. J. Karnovsky, *J. Cell Biol.* **27**, 137A (1965).
9. W. J. Humphreys, B. O. Spurlock, J. S. Johnson, *ibid.* **59**, 153A (1973).
10. T. F. Anderson, *Trans. N.Y. Acad. Sci.* **13**, 130 (1951).
11. The research upon which this report is based was performed pursuant to contract No. NIH-NCL-G-72-3861 with NIH, Department of Health, Education, and Welfare. We thank Ms. Maria Maglio for expert technical assistance with scanning microscopy and Dr. Rosa Armstrong, Dr. Albert Jones, Ms. Margaret Williamson, and the Department of Pathology, University of California, San Francisco, for their help and advice.

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Retinal Degeneration Associated with Taurine Deficiency in the Cat

Abstract. *A degeneration of the retinal photoreceptor cells develops in cats when casein is the source of dietary protein. Amino acid profiles indicate that the degeneration is associated with a selective decrease in plasma and retinal taurine concentrations. A sulfur amino acid deficit in the casein diet combined with specific amino acid requirements of the cat appear related to this unique expression of taurine deficiency.*

Kittens or adult cats fed casein as the source of dietary protein develop a retinal degeneration within 3 months that is visible with the ophthalmoscope (*1*). The initial fundus change is a hyperreflective granular zone in the area centralis similar in appearance to that reported for feline central retinal degeneration (*2*). A gradual decrease in the amplitudes of the cone and rod components of the electroretinogram and a time delay in the electrical response of the cone have been demonstrated coincident with degeneration of the photoreceptor cells. Ultrastructurally, vesiculation, disorientation, and disintegration of the photoreceptor outer segments occur in the earliest stages in the area centralis (Fig. 1), and are followed by the subsequent degeneration of the entire photoreceptor population (*1*).

Casein appears to be related to the retinal degeneration, since the retinopathy can be prevented or reversed by substituting lactalbumin or egg albumin for the dietary casein (*1*). Since casein is low in sulfur amino acids and because the retinas of several mammalian species have been shown to contain high levels of taurine, an amino sulfonic acid, the concentration of free amino acids in plasma and retina were investigated to determine whether sulfur amino acids were affected in this specific photoreceptor degeneration.

Eleven kittens and seven adult domestic cats were fed a semipurified diet containing casein (16 to 27 percent of the calories as protein) or a commercial chow as previously described (*1*) for periods of 12 to 52 weeks, at which time plasma amino acids were analyzed (*3*). In a subsequent study eight kittens were fed a casein diet (27 percent of the calories as protein) for 4 to 24 weeks, when retinal samples were assayed for amino acid and DNA concentration (*3*).

In the first study all the cats fed the casein diets had evidence of retinal degeneration after 3 to 12 months (*1*). Their plasma amino acid profiles (Table 1) revealed an essential absence of plasma taurine, whereas the concentrations of the other amino acids, including methionine and cystine (taurine precursors), were comparable to control values. As in other species, taurine was the principal free amino acid in the normal cat retina, and the other measured amino acids were also within reported limits (*4*). The retinas of casein-fed kittens revealed a progressive and selective decrease in taurine content beginning with the initial assay at 4 weeks. This decrease in taurine preceded any change in DNA concentration, indicating that taurine depletion preceded cell death. After 24 weeks of the casein diet, retinal taurine was reduced by 80 percent, and a 13 percent decrease in total retinal DNA was observed (Table 2). The DNA decrease correlates with more advanced degeneration where progressive loss of the outer nuclear layer and outer plexiform layer predominates in the area centralis and extends into the peripheral retina (*1*).

Table 1. Plasma amino acid concentrations (in nanomoles per milliliter) in cats fed control (chow) or casein diets (nine animals per group) for three or more months. The results are expressed as the mean ± standard deviation.

Amino acid	Control	Test
Isoleucine	76± 27	60± 14
Leucine	137± 49	108± 34
Lysine	112± 26	119± 42
Methionine	75± 34	92± 35
Phenylalanine	106± 53	71± 9
Threonine	147± 52	113± 36
Valine	161± 55	172± 73
Alanine	472±173	495±171
Arginine	154± 64	118± 43
Asparagine	66± 26	51± 17
Aspartic acid	38± 13	42± 21
Half-cystine	23± 7	28± 9
Cysteic acid	34± 12	23± 11
Glutamic acid	186± 55	211± 84
Glutamine	484±298	468±279
Glycine	385±120	322± 93
Histidine	138± 34	161± 32
Proline	154± 62	194± 86
Serine	255± 84	216± 95
Taurine	54± 24	1± 0
Tyrosine	70± 21	66± 23

The decrease in plasma and retinal taurine is thought to be associated with the retinal degeneration, since this sulfur-containing amino acid is normally present in high concentrations in the retina as well as in muscle and brain (*4*), where it may function directly as a neurotransmitter or indirectly as a regulator of calcium flux influencing membrane potential and the excitability of nerves and muscles. It has also been shown that intravitreal injection of taurine in the chicken depressed the *b*-wave of the electroretinogram and that light stimulation caused the release of taurine in vitro (*5*).

The disappearance of plasma taurine in cats fed casein may have resulted from a combination of factors affecting sulfur amino acid metabolism in cats. Kittens rank among the fastest growing mammals and require approximately 29 percent of their calories from protein for maximum growth. This is also reflected by the high protein content of cat milk (*6*). Adult cats also have high protein requirements, and when protein (from fish and liver origin) comprised less than 21 percent of the diet on a dry weight basis, taurine disappeared from the urine. By contrast, urinary felinine, an isopentanol derivative of cysteine peculiar to feline species (*7*), continued to be excreted during this period of inadequate protein consumption (*8*), suggesting that the biosynthesis of felinine takes precedence over that of taurine when protein is limiting.

Casein is low in total sulfur amino acids, particularly cystine, the precursor for taurine synthesis via oxidation and decarboxylation reactions involving cysteine sulfinic acid, cysteic acid, and hypotaurine (*4*). Comparison of the amino acid composition of casein with the two proteins found to prevent the degeneration—lactalbumin and egg albumin—indicated that these proteins contained 168 and 181 percent more sulfur amino acids than casein.

The cat liver normally contains high levels of taurine, but in comparison to the rat or dog, cannot decarboxylate appreciable