for neuroleptic inhibition of stimulated [^{3}H]dopamine release, a presynaptic site of neuroleptic action might explain many of the neuroleptic effects in brain. The neuroleptic inhibition of impulse-secretion coupling in the dopamine preterminals may possibly elicit the neuronal feedback activation of nigral cell firing (*3*) as a compensatory mechanism; the increased firing might activate tyrosine hydroxylase (*20*) and thus possibly lead to the observed increase in brain dopamine turnover (*21*) as an overcompensation.

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Golgi Complex—Endoplasmic Reticulum Transition Region Has Rings of Beads

Abstract. *The smooth surface of the rough endoplasmic reticulum that makes the forming face of the Golgi complex has beadlike structures arranged in rings at the base of transition vesicles. The beads can only be seen easily after staining in bismuth salts. They are 10 to 12 nanometers in diameter and occur in a variety of cell types and organisms.*

Gomori-type reactions involving the precipitation of lead phosphate after the enzymatic formation of free phosphate give a questionable localization of energy-releasing phosphatases (*1*). However, if phosphates are part of a cell structure, either naturally or through fixation, it may be possible to show their presence by an increased electron opacity after reaction with bismuth salts. Bismuth oxynitrate has been successfully used to stain DNA because of its reaction with phosphate (*2*).

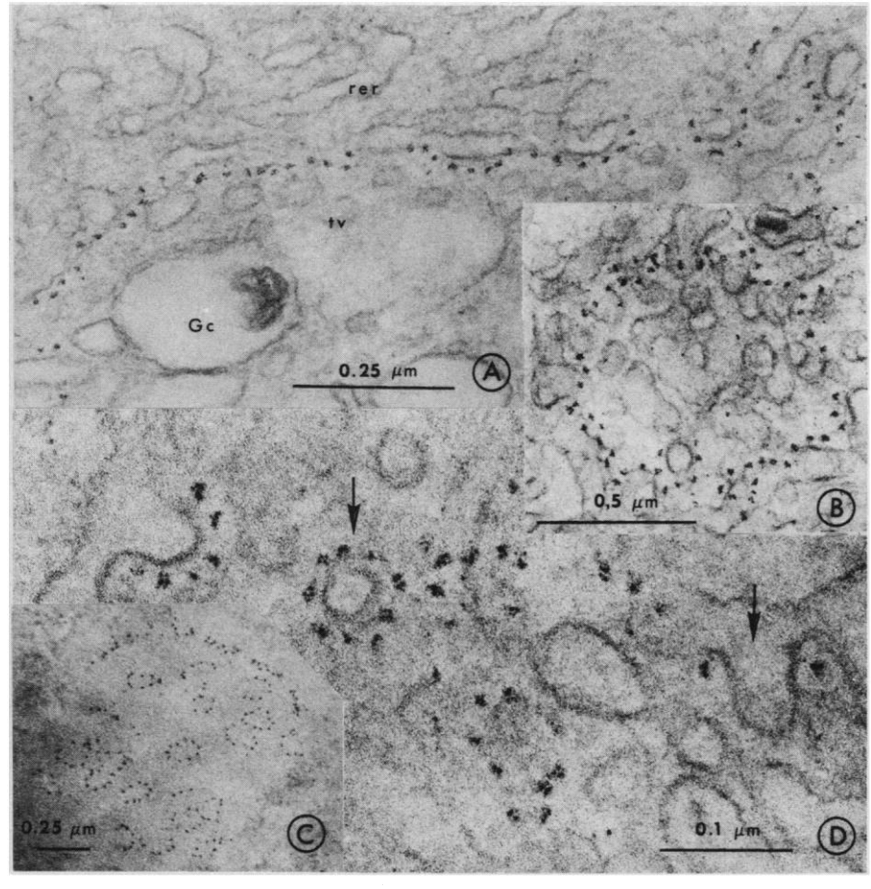

Fig. 1. (A) Beads at the smooth surface of the cisterna of rough endoplasmic reticulum on the forming face of the Golgi complex from the silk gland of *Calpodes*. Abbreviations: *rer*, rough endoplasmic reticulum; *tv*, transition vesicles; *Gc*, saccular region of the Golgi complex. (B) Beads on the smooth tubular endoplasmic reticulum where transition vesicles arise in the small Golgi complexes of oenocytes. (C) Pattern of rings formed by beads in a 0.25-μm section of the Golgi complex from an epidermal cell viewed at 100 kv; this section was not fixed with osmium tetroxide. (D) Beads form rings at the base of transition vesicles. Arrows mark the two views interpreted in Fig. 2.

Using this rationale, we have treated glutaraldehyde-fixed insect tissues with bismuth salts and looked for a localization of bismuth in the transition vesicle region of the Golgi complex [since vesicle movement is known to be energy-dependent (*3*)] and at the plasma membrane plaques at the tips of microvilli, where newly secreted cuticle first appears [since one might also expect the transmembrane transport or assembly of cuticle precursors to be energy-dependent (*4, 5*)]. Although we have yet to verify the rationale behind this approach, we have obtained an easily repeatable specific staining of components in the Golgi complex and in plaques, as well as in nuclei and centrioles. In this report we describe the procedure for localizing the reaction in a hitherto undescribed component of the Golgi complex.

Tissues of fifth-stage larvae of *Calpodes* were fixed overnight at room temperature in a solution consisting of 5 percent glutaraldehyde and 2 percent sucrose in 0.05*M* sodium cacodylate buffer at *p*H 7.4. The tissues were rinsed in 0.1*M* triethanolamine buffer at *p*H 7.8 and allowed to react for 1 hour in the bismuth solution made up in the same buffer. The reaction mixture was prepared by dissolving 200 mg of bismuth oxynitrate in 10 ml of 1*N* NaOH containing 400 mg of sodium tartrate (*6*). One part of this solution was added to one to two parts of 0.2*M* triethanolamine hydrochloride buffer at *p*H 7.5, and the *p*H was adjusted with HCl to 7.5 to 8.0. The final mixture was clear and stable for 2 to 3 hours; it contained about 20 m*M* bismuth oxynitrate and 60 m*M* sodium tartrate. Tissue was postfixed in 1 percent osmium tetroxide, embedded in Araldite, sectioned, and observed at 80 kv on a Philips EM-300 electron microscope without further enhancement of contrast (*7*).

The bismuth staining showed a similar specificity in all tissues examined (epidermis, fat body, oenocytes, pericardial cells, nerve cells, muscle, silk gland, glial cells, tracheal epidermis, and hemocytes). There was little or no nonspecific background. All the density attributed to the bismuth was in a particular pattern within nuclei, in plasma membrane plaques and centrioles, and in one region of the Golgi complex. The forming face of the Golgi complex had beadlike deposits of bismuth arranged in subequatorial rings at the base of transition vesicles, where they arise from the smooth-surfaced membrane of the rough endoplasmic reticulum (Fig. 1, A and D). The beads were 10 to 12 nm in diameter and about 14 nm from the membrane surface. A careful search of other membranes throughout the cell showed that beads were present only on this endoplasmic reticulum surface. The cisternae in this transition vesicle region are readily identified by the presence of ribosomes on their outer faces, and beads and transition vesicles on their inner faces. Although transition vesicles may shuttle back and forth (*8*), beads were absent from the saccular membrane. Sections 0.25 μm thick tangential to the forming surface usually showed rings of nine beads with a spacing between centers of 27 nm (Fig. 1C). Conventionally prepared material showed no easily resolvable structure corresponding to these beads. However, tissue stained in block with uranyl acetate (*7*) showed faint structures in the positions expected for the bismuth-stained beads. The Markham rotation technique with favorably oriented preparations gave images with repeating patterns having the dimensions seen on images obtained after bismuth staining. Figure 2 shows an interpretation of this arrangement. We conclude that most beads are in rings at the base of transition vesicles. Very rarely there may be open-ended strings, but there are few or no single beads.

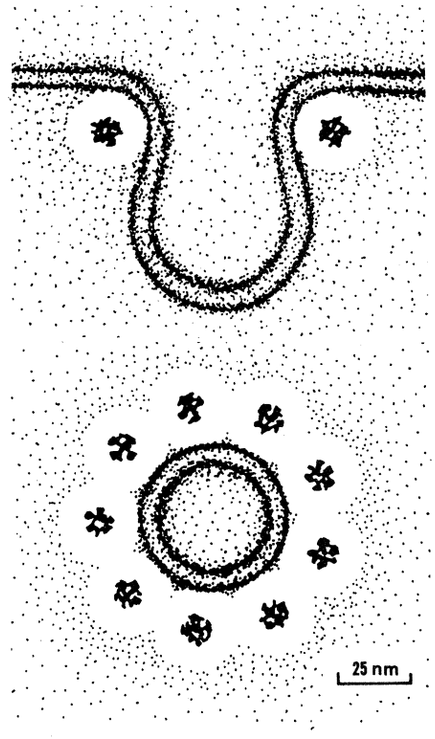

Fig. 2. Scale diagrams of the beads and their arrangements near the transition vesicles attached to the membrane of the endoplasmic reticulum.

Beads were found in all endoplasmic reticulum–Golgi complex transition regions from all cell types, but were not found elsewhere in the cell. The ubiquity of the beads in *Calpodes* Golgi complexes suggested that they might have an even wider distribution. We therefore examined epidermis and fat body from mealworm larvae (*Tenebrio,* Coleoptera) and adult locust (*Locusta,* Orthoptera), and digestive gland from a crayfish (*Orconectes,* Crustacea). In all these organisms the beadlike structures were found on the rough endoplasmic reticulum surface making up the forming face of the Golgi complex. We therefore believe that the beads may be generally important in the organization of Golgi complexes, and hope that other workers will look for them in their material.

The pattern of distribution of the beads in *Calpodes* was remarkably consistent in all cell types, varying only in proportion to the size and nature of the Golgi complex. The Golgi complexes in the oenocytes were of particular interest in this regard. The oenocytes have very few bound ribosomes. Nearly all of the cell is filled with smooth tubular endoplasmic reticulum (*9*). The Golgi complexes are diminutive and consist of transition vesicles, a saccule, and a very few secretory vesicles all embedded in the smooth tubular endoplasmic reticulum. However, these small Golgi complexes were all clearly outlined by rings of beads where the transition vesicles break free from the smooth endoplasmic reticulum (Fig. 1B). Thus, the beads are related to the forming face of the Golgi complex, whether it be rough or smooth endoplasmic reticulum.

The distribution of beads in rings in association with the formation of microvesicles at only one location in the cell must be meaningful. The fat body Golgi complex is involved sequentially in synthesis of blood proteins (*10*), formation of the isolation membranes concerned with autophagy (*11*), and formation of primary lysosomes (*12*). Transition vesicle beads were present without modification at times when the Golgi complex was primarily involved with each of these functions. The beads must therefore be related to some more basic function of the Golgi complex.

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7. Several variations of the procedure were tested. Osmium tetroxide is not necessary for the stability of the bismuth deposits. Sections (0.25 μm) of tissue not fixed in osmium tetroxide were viewed at 100 kv to show the arrangement of beads in the depth of the section (Fig. 1C). The deposits occurred in tissue fixed in osmium tetroxide alone or glutaraldehyde alone and in tissue fixed in glutaraldehyde and osmium tetroxide before the reac-

tion. The reaction was not influenced markedly by the length of fixation or by *p*H over the range 7.5 to 9. Nor did heating the reaction mixture increase the density of the deposits, although it increases staining by uranyl s[illegible] [M. Locke, N. Krishnan, J. T. McMahon, *J. Cell Biol.* **50**, 540 (1971)]. We conclude that our procedure leads to saturation of the binding sites and that their small size reflects a correspondingly small structure. Staining on the section rather than in the tissue gave only a specificity. The nature of the bismuth binding site remains to be determined, but the reaction is not mimicked by lead or calcium, and it is therefore not due to labile phosphate groups. We have not excluded the possibility that the reaction shows exposed phosphate on DNA. If the bismuth binds to DNA (*2*), then the beads could be informational D[illegible] [E. Bell, C. Merrill, C. B. Lawrence, *Eur. J. Biochem.* **29**, 444 (1972)] and the plaques could be membrane-bound D[illegible] [W. Meinke, M. R. Hall, D. A. Goldstein, D. E. Kohne, R. A. Lerner, *J. Mol. Biol.* **78**, 43 (1973)], both concerned with local specific protein synthesis.

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Origin of Horizon A: Clarification of a Viewpoint

As shown in earlier studies (*1*), Weaver and Wise (*2*) report that siliceous microfossils (diatoms, sponge spicules, radiolarians) occur in some of the high-purity Tertiary opaline deposits of the Atlantic and Gulf Coastal Plain. They conclude therefore that these deposits as well as horizon A must be biogenic in origin. We wish to comment on this as it regards their interpretation of our earlier report (*3*) on this subject.

As a result of the JOIDES (Joint Oceanographic Institutions for Deep Earth Sampling) drilling program, a prominent and widespread oceanic seismic reflector known as horizon A has been shown to consist of hard, siliceous beds containing diatoms and radiolarians and to have a narrowly defined age from late Early to early Middle Eocene (*4*). A principal problem has been to explain the origin of this geographically widespread and nonlinear siliceous horizon and not the other scattered and discontinuous siliceous deposits situated stratigraphically higher or lower.

Impressed by the presence of siliceous microfossils, Dietz and Holden (*5*), Berggren and Phillips (*6*), and Ramsay (*7*) have offered explanations based on several oceanic circulation models to explain horizon A as an entirely biogenic deposit. A major biogenic role in the formation of horizon A is undeniable. But no oceanic circulation model *alone* can explain the occurrences of smectites and zeolites that are found associated with almost all of the sediments correlating with this unusual horizon and found not only in the Atlantic and Gulf Coastal Plain but also on the shelf and in the Caribbean. Accordingly, Gibson and Towe (*3*), supported by Mattson and Pessagno (*8*), considered that a combined volcanic and biogenic explanation for the time-equivalent deposits was more consistent with *all* of the facts than a strictly biogenic explanation. The widespread distribution of horizon A in the western North Atlantic and the composition of the deposits themselves led us to a dual and partially sequential cause: direct volcanic contributions to help explain the presence of smectite and zeolite but with accompanying increased nutrients (phosphorus, iron, and silica from dissolved fine pyroclastics) which would increase the productivity of siliceous organisms above normal background levels, providing increased contributions to the sediments. We wish to clarify that we did not state that the entire source of the relevant deposits was altered volcanic ash. We did not extend our conclusions to other siliceous deposits of different ages in the Atlantic and Gulf Coastal Plain nor did we extend them to siliceous deposits in other oceans, as Weaver and Wise have implied (*2*).

Silica is constantly being mobilized and deposited by diatoms in the world oceans, and few will argue about this biogenic contribution. But for horizon A, an oceanwide "chert" deposit, some mechanism is needed to raise the siliceous productivity and the deposition and preservation above the normal background level over a wide area in the Atlantic region. Changes in sediment dilution or in oceanic circulation patterns can be invoked to explain only part of the deposit, since such changes do not normally also provide a mechanism for zeolitic and smectite clays. However, wind and ocean currents can distribute soluble, *fine* pyroclastics and thus add potential planktonic nutrients that would contribute to the formation of the varied deposits observed. The relationship between siliceous organisms and volcanism has been noted from the time of Lyell (*9*) up to the present (*10*). In support of this concept, Lisitsyn (*11*) has presented consistent evidence for the *indirect* influences of volcanism in the active Bering Sea region on such nutrients as iron and phosphorus and the importance of these elements to plankton, notably diatoms (*12*). More recently, Huang *et al.* (*13*) have provided still further support for this viewpoint.

We noted for horizon A the consistent occurrence of correlative deposits indicative of both volcanic and biogenic activity (*3*), although the degree of influence of one aspect or the other varies from place to place as might be expected. We believe this to be a noncoincidental cause-and-effect relationship, and we may be wrong; but, be that as it may, in order that any alternative explanation be correct, it must be based on all the relevant data rather than the misleading and highly selected data chosen by Weaver and Wise (*2*).

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12. Lisitsyn (*11*) is, however, careful to deny any *direct* chemogenic relationship between major siliceous deposits and volcanism, a viewpoint popular in some earlier geologic literature.
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We are pleased that Gibson and Towe (*1*) accept our documentation (*2*) of biogenic opaline silica deposits within the Southeastern Coastal Plain but are mystified that they consider our data "misleading and highly selected" since these data cover a far broader range of evidence than they themselves are willing to consider. We have demonstrated (*2*, *3*) a historic pattern of intermittent biogenous silica deposition in coastal plain sediments ranging from Paleocene to Eocene in age [it should be noted that, in South Carolina and elsewhere, these opaline lithologies are found well into the Miocene (for example, Coosawhatchie clay of the Hawthorne Formation)]. Until recently, practically all of these deposits have been variously classi-