expected to form E-P). The specificity of the binding is the same in all cases, since inactive cardiac glycosides do not prevent or dilute the active binding. Even in the absence of added cations, anions, or nucleotides, some specific binding does occur on prolonged incubation. For example, in 3 minutes 1.5 pmole of H^3-digoxin was bound per milligram of protein; in 30 minutes 4.4 pmole, and in 120 minutes 6.2 pmole of H^3-digoxin per milligram of enzyme protein were bound. Na^+,K^+-adenosine triphosphatase activity is not destroyed under these prolonged incubation conditions. This is consistent with studies over protracted incubation periods in a system which continually removes ADP, maintains a constant concentration of ATP, and measures the oxidation of NADH (reduced form of nicotinamide adenine dinucleotide) as an indication of adenosine triphosphatase activity, so that the rate of ATP hydrolysis remains first-order for 1 hour (*8*). Under these conditions, addition of ouabain produces increasing inhibition with time.

These results do not disprove the presence or importance of E-P. They do, however, indicate that the conformational state of the enzyme is probably of primary significance in glycoside binding and hence in the mechanism of its action. The formation of E-P may be one of many ways in which the allosteric nature of the system is altered. Prolonged incubation or interaction with a variety of ions may also change the conformation or "expose" binding sites. The fact that stimulation or inhibition of H^3-digoxin binding by Na^+ can occur under conditions when E-P is maximum, minimum, or absent suggests that the binding site (or sites) becomes more or less accessible depending upon the particular structural state of the vesicle. These complex data are relevant to the allosteric pump concept of Jardetzky (*9*). Sodium ion stimulates hydrolysis of ATP at one site but inhibits at another (the K^+-stimulatory site) (*1, 2*). Incubation of the enzyme with specific substances may effect an "eversion" of the binding sites so that Na^+ now inhibits rather than stimulates glycoside binding. This suggests that digitalis drugs may interact, in intact systems, with both the "phospho" and "dephospho" form of the enzyme transport system (*2, 5*).

As suggested by Skou (*10*), ATP (and presumably other nucleotides) primarily causes an alteration in enzyme structure which somehow leads to the transport of ions. In fact, Skou has stated that E-P may not be of physiological relevance. With reference to phosphoglucomutase, a phosphoenzyme, Koshland has proposed the "induced-fit" or "flexible active site" concept; acyl phosphate formation (which does apparently occur in the Na^+,K^+-adenosine triphosphatase) and subsequent interaction with many cellular constituents may produce specific functional site alterations which are of importance in the activity of the particular enzyme (*11*). As suggested for hormones (*11*), active cardiac glycosides may affect transport systems by functioning as a "feedback-type" of inhibitor, specifically interacting with a regulatory or allosteric site on the transport enzyme system (*4, 12*). The present report supports the concept of an "allosteric type" (*9*) of pump.

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Problem of Sieve-Tube Slime

Abstract. *It is proposed that sieve tubes contain a stationary, living, filamentous reticulum that persists throughout their functioning life. The filaments are about 100 to 150 angstroms in diameter and often striated. By swelling they block the pores of old sieve plates after slime has been lost from the elements. In* Cucurbita, *slime may be loosely bound to the filaments; in many species it is hydrolyzed, swept out by the assimilate stream, and eventually metabolized.*

Recent work with the electron microscope indicates that the pores in the sieve plates of functioning phloem are normally open (*1*). Since open sieve plates should be capable of conducting the assimilate stream by a mass-flow mechanism, one of the principal objections to this theory of translocation has been eliminated. From such work (*1*), in which special methods of preparation avoided opening of the phloem to atmospheric pressure, it becomes evident that most of the impressions of slime- or protoplasm-filled pores were simply artifacts of the methods of preparation.

Probably the most serious problem remaining is the origin, nature, and function of sieve-tube slime. Esau (*2*) states: "The role of slime in this system [the phloem] continues to be an enigma." In reviewing the structure and function of phloem I have come to recognize a situation in phloem structure that seems to explain this enigma.

If one severs the end of a healthy squash stem, phloem exudes rapidly (*3*). Measurements prove that exudation starts with a velocity greater than 1000 cm/hour through the sieve tubes, but, within about 2 minutes, exudation slows and stops. If a 1-mm slice is then cut from the end, exudation resumes, only to stop again; this process may be carried on for hours, and the total volume of exudate collected is many was first hybridized in 0.6*M* NaCl and,

times the volume of the phloem of the stem that has been removed (*3*).

Microscopy of a section of the plugged phloem shows slime plugs on many sieve plates. The plugs vary in size and some plates have practically no slime; yet the pores are plugged, as indicated by stoppage of exudation and by electron micrographs (*4–7*). These are the plates of the old sieve tubes that have functioned for some time.

The velocity of movement of assimilates and tracers in sieve tubes has often been measured—about 100 cm/hour is considered average. Calculation of the rate of replacement of sap in individual sieve elements shows that each sieve element has its total liquid volume replaced many times hourly—100 times per minute is cited for sap flow in willow (*8*); for sieve elements 0.5 mm long, 100 cm/hour gives a replacement rate of 2000 times hourly.

I consider slime to be the dead decomposition residue from breakdown of slime bodies, nuclei, tonoplasts, dictyosomes, and other degraded cell constituents; probably it cannot remain very long in a functioning sieve tube. If it moves along with the assimilate stream as slime, it should pile up in the young sieve tubes of apical meristems, but in these one does not find it.

Examination of young and old sieve tubes in a section shows that young elements are full of plasmatic constituents, while old functioning sieve tubes are peculiarly void of such materials, having a thin parietal layer of cytoplasm, sieve-tube strands, and a few organelles (*9*). Much stainable, electron-dense material moves out of the sieve tubes as they attain their functioning state; I take this material to be slime, and, since it is not piled up in sieve tubes of meristems, apparently it has been reduced to the molecular state and resorbed by the surrounding companion and parenchyma cells. Since by acid hydrolysis it yields amino acids (*10*), it probably reenters the nitrogen pool and is used in growth of new cells.

How are sieve plates of mature functioning sieve tubes plugged within minutes when the phloem is cut open? Electron microscopy of plugged sieve-plate pores shows the plugs to be fibrillar in nature (*4–7*).

Examination of the fibrillar material often shows small filaments about 100 Å in diameter, often striated, and identifiable as part of a network of filamentous material that commonly occupies the lumen of the mature sieve tube. Northcote and Wooding (*5*) noted the presence of such a meshwork of 90- to 100-Å filaments contrasting with slime tubules 180 to 240 Å in diameter; they noted that this "cross banded fibrillar material seen at the orifices of the sieve-tube pores is continuous with the lumen fibrils." They considered the filaments to be of the same origin as the slime tubules, and showed the two types of fibrillar material within a single cell (*5*, fig. 8).

Behnke and Dörr (*6*) termed these finer units plasmatic filaments; they showed them magnified × 120,000 as a network (*6*, fig. 8). They stated that the filaments are 120 to 150 Å in diameter, but scaling of those shown by them indicates a range between 90 and 120 Å; they also showed a sieve plate of *Cucumis* magnified × 120,000 with fibrous material in the pores (*6*, fig. 12).

Cronshaw and Esau (*7*) distinguished between 231-Å tubules, which they termed P1-protein, and smaller striated 149-Å fibrils which they termed P2-protein; they showed both in close association in a single cell (*7*, fig. 8). They considered their P2-protein to derive from the P1-protein or slime.

I believe that slime is dead material that must break down to molecular form and disappear from functioning sieve-tube elements. My purpose is to point out that the fibrillar meshwork of Northcote and Wooding, the plasmatic filaments of Behnke and Dörr, and the striated P2-protein of Cronshaw and Esau are not slime. Such substances originate in the young sieve tube at the time of its origin from cambium; obscured by plasmatic contents they coexist with the slime during sieve-tube maturation; in functioning sieve tubes they are apparent by electron microscopy. I propose that they make up a stationary, living, filamentous reticulum that persists throughout the functioning life of the sieve element; they probably constitute the stainable contents of the sieve pores of *Tilia*, *Liriodendron*, and *Vitis* that survive winter dormancy and are instrumental in dissolution of definitive callose and in reestablishment of the function of translocation in the spring. It is these small striated elements that plug the pores of old sieve tubes long after the slime has been washed out by the assimilate stream. In normally functioning sieve tubes they apparently occupy only a nominal portion of conduit space.

Thus it seems that there are three distinct mechanisms that bring about sieve-plate plugging: (i) callose plugging by a wall material that, as it increases, constricts and eventually (as definitive callose) closes off the sieve pores; at early stages callose plugging may be reversible; (ii) slime plugging that occurs rapidly in young newly differentiated sieve tubes; it is attended by formation of slime plugs; and (iii) filament plugging, the mechanism responsible for closing the pores of older, functioning sieve plates with striated filamentous material; no slime plugs are formed.

There are three mechanisms by which the filament plugging may occur: (i) release of turgor upon cutting may cause a partial collapse of the sieve plate, decreasing the diameter of the pores; (ii) the shock of cutting may cause rapid increase in callose that constricts the pores (*11*); and (iii) possibly shock also triggers a mechanism bringing about agglutination of the filaments so that they hydrate and swell (*4–7*). It seems evident that the outrush of sap does not tear the meshwork free from the parietal cytoplasm, for there is no piling up of filamentous material in old sieve tubes, like the accumulations of slime found in younger elements.

Apparently the presence of the filamentous reticulum in young sieve tubes is obscured by the abundance of slime, nuclear material, dictyosomes, mitochondria, ribosomes, and other stainable inclusions. When these have broken down and left the cell the reticulum shows up as shown (*5*, fig. 28; *6*, figs. 7 and 8; *7*, figs. 10 and 13). The broken appearance of the networks results from thin sectioning of a three-dimensional structure; its absence from some preparations may be an artifact of fixation; Evert and Derr state that permanganate destroys strands in sieve tubes (*12*).

In carefully reexamining some old slides of *Cucurbita pepo* phloem I have found a reticulum occupying many mature sieve elements that is plainly visible under the light microscope. It is more in evidence in the center of longitudinal sections that lack slime plugs than at the ends where slime plugs abound. In this situation it seems possible that some slime may remain, lightly bound to the filamentous reticulum, after flow of the assimilate stream has become rapid. This lightly bound slime may make the reticulum visible; it may be washed free of the reticulum when the stem is cut, and

aggregated on the sieve plates in variable amounts; some emerges in the exudate. This finding may explain the high nitrogen content of phloem exudate of squash (*13*), the fact that the exudate coagulates, and the protein reaction of slime in microchemical tests. Broken-down membrane material may account for it being termed "lipoprotein" or "steroid."

Perhaps the old problem of strands in sieve tubes has finally been resolved (*12*, *14*). Since the individual filaments of the permanent network are far too small for resolution by the light microscope, the impression that strands existed in sieve tubes is understandable; under many conditions one could not see or photograph the strands. The coarser strands seen in some photographs probably represent aggregations of filaments into large fibrils of the type shown (*7*, fig. 17). Occasionally these strands can be seen stretched between sieve plates and extending through sieve pores (*12*, *14*, *15*). The distinction between slime and this filamentous reticulum is becoming increasingly apparent in many electron-microsope studies of phloem.

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Nerves in Cortical Bone

Abstract. *Modified electron-microscopic techniques permit the sectioning of fully mineralized compact bone. Cortical canals in human femur are extensively innervated. Most nerves are unmyelinated and range from 0.5 to 10 micrometers in diameter. I have found a few mixed nerves (myelinated and unmyelinated fibers), one of which was 130 micrometers in diameter.*

Despite its important implications, we know little about bone innervation. The technical difficulties with and limited scope of "special nerve stains" contribute to this lack of understanding.

Using modified electron-microscopic techniques, I have sectioned fully mineralized compact bone for ultrastructural studies (*1*). Nearly all haversian canals of adult dogs contain unmyelinated nerves 0.8 to 7 μm in diameter. The diameters of individual nerve fibers range from 0.25 to 0.6 μm. I have now examined fully mineralized cortex of the adult human femur. It is extensively innervated.

The cortical canals contain unmyelinated nerves 0.5 to 10 μm in diameter. They consist of fibers (axons) invaginated into recesses in the Schwann cell plasma membrane. The fibers contain mitochondria, 400- to 800-Å vesicles, and neurofilaments about 115 Å in diameter. A basement membrane 400 Å thick surrounds the nerve, and endoneurial collagen fibrils surround the basement membrane.

One canal contained a mixed nerve (myelinated and unmyelinated fibers) 130 μm in diameter (Fig. 1). A few other canals contained one myelinated fiber adjacent to one or more capillaries. The myelinated fibers are 5 to 9 μm in diameter.

Numerous investigators have demonstrated nerves in bone by gross dissection (*2*), routine histologic sections (*3*), and methylene blue and silver stains (*4*). Others suspected their presence as a result of clinical observations, especially during bone surgery with local anesthesia (*5*). Various investigators have disagreed about the exact distribution of these nerves. Most of them demonstrated periosteal nerves, nerves in trabecular bone, and fibers entering cortical canals, but technical limitations prevented them from determining much about the extent of the nerve supply in the haversian canal. Some thought nerves extended into bone matrix, and others thought fibers were closely related to bone cells. I have only seen the nerves in haversian canals and

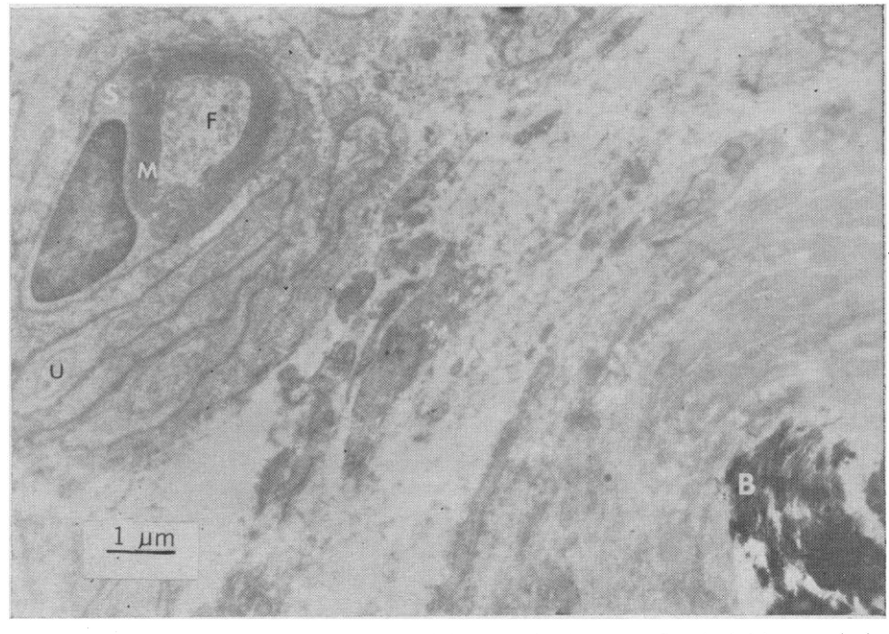

Fig. 1. Portion of a cortical canal containing a mixed nerve. Surgical biopsy of the distal femur of a 54-year-old woman. Note the mineralized bone matrix (*B*) which forms the canal wall. Unmyelinated fibers (*U*) are invaginated into recesses in the Schwann cell plasma membrane. The myelinated fiber consists of a central nerve fiber (*F*) containing neurofilaments surrounded by layers of myelin (*M*). This is surrounded by the Schwann cell (*S*) and a basement membrane.