of atherosclerosis fine reticulin fibers stained with silver impregnation, which were considered to be type I collagen, were increased, and tissues of advanced sclerotic lesions were replaced mostly with thick bundles of fibers that stained blue by Masson's trichrome stain and that appeared to be type I collagen. It is likely that smooth muscle cells in an early atherosclerotic plaque produce a higher proportion of type III collagen and with progression of the lesion these cells are switched to synthesize predominantly type I collagen in response to functional requirements of the tissues.

The molecular composition of type V collagen has been a matter of controversy [that is, $\alpha A(\alpha B)_2$ (*7*) or $(\alpha A)_3$ and $(\alpha B)_3$ (*8, 15, 16*)]. I found that the type V collagen separated from whole aortas by the differential salt precipitation (*7*) was composed predominantly of αB chain. The ratio of αB to αA (7.6:1) was much larger than the reported ratio of 2:1 (*7*), suggesting that αA and αB are components of two separate collagen molecules (*8, 15, 16*). In the present study I noted that the αA chain comigrated with α1(I) on the SDS–polyacrylamide gel electrophoresis. Therefore, the ratio of α1(III) and αB calculated in terms of α1(I) are likely to be somewhat underestimated. Nevertheless, the marked increase in the proportion of αB is quite evident in the atherosclerotic plaque (Fig. 3B). Judging from the weights of lyophilized collagens separated by differential salt fractionation (*7*), pooled tissues from atherosclerotic plaques yielded a higher ratio of type V collagen to type I (0.41) compared to tissues from nonsclerotic intact medias (0.22).

It was observed by Stenn *et al.* (*17*) that cultured epithelial cells synthesize type V (AB_2) collagen. These workers suggested that the AB_2 collagen is necessary for the continual migration and movement of these epithelial cells (*16*). It is well known that in atherosclerosis, smooth muscle cells proliferating in the intimal layer are derived from the media through fenestrae of the internal elastic lamina (*18*). Type V collagen, therefore, may be related to such migration and movement of vascular smooth muscle cells. Finally, the increased proportion of αB or type V collagen provides at least a biochemical basis for transformation of smooth muscle cells proliferating in human atherosclerotic plaques.

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Bulk Solute Extrusion as a Mechanism Conferring Solute Uptake Specificity by Pinocytosis in *Amoeba proteus*

Abstract. *A variety of positively charged solutes induce pinocytosis in* Amoeba proteus, *ranging from metabolically useful material to solutes that may prove harmful, such as Alcian blue. Alcian blue is taken up by pinocytosis and then a fraction of the accumulated dye is expelled in bulk form through "extrusion channels." This response is not elicited by other solutes taken up by pinocytosis in the amoeba, implying that if any selectivity is associated with this process, it is by specific solute extrusion mechanisms in the cytoplasm.*

Pinocytosis, a mechanism by which cells can accumulate solute from the external medium, involves the uptake of bulk medium and membrane-bound solute by cell surface invagination and vesiculation. The initial step in pinocytosis in the amoeba is the binding of an inducer to the cell surface (*1*). This displaces a fraction of the surface calcium (*2*), which may in turn influence the permeability of the plasma membrane (*3*) and trigger the pinocytotic cycle. Inducers of pinocytosis in the amoeba, all cations, have been classified into three groups on the basis of how tightly they bind to the cell surface (*4*). The first group of inducers includes inorganic cations (such as Na^+) and amino acids that bind very loosely to the cell surface and which must be constantly present in the external medium to induce pinocytosis. The second category includes proteins that bind more tightly to the cell surface but can be removed from the cell by changes in the *p*H of the external medium. The third category includes basic dyes, such as Alcian blue, which bind irreversibly to the cell surface. A great variety of external solutes are taken up by pinocytosis in the amoeba, including solutes that may be beneficial to the cell as well as solutes that may prove harmful. This study is concerned with the fate of the three types of solute molecules ingested during pinocytosis in *Amoeba proteus* (*5*).

Under normal conditions, *A. proteus* streams about in a directed fashion along the substratum (Fig. 1a). When the cell is briefly exposed to 0.01 percent Alcian blue, the dye is bound to the cell surface in localized areas (Fig. 1b) and the cell ceases streaming and begins to withdraw its pseudopods. Five to ten minutes after the dye is added, pinocytotic channels can be observed in the cell (Fig. 1c) and membrane-bound solute (dye) uptake begins. After 20 to 30 minutes, the dye originally bound to the cell surface has been internalized and the pinocytotic channels have disappeared (Fig. 1d). The internalized dye, which presumably is still membrane-bound, appears to collect inside specific areas of the cell. During the next half hour, the cell begins to expel a portion of the dye, along with what appears to be some membrane and cytoplasm, through a distinct channel leading from the mass of dye in the cytoplasm to the cell surface (Fig. 1e and Fig. 2).

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Once this channel has been formed and opened to the exterior of the cell, the dye and associated material begin to move through the channel to the outside of the cell. The force responsible for the expulsion of material appears to be cytoplasmic contractions around the mass of dye. Although most cells form only one extrusion channel, two or more of these channels have occasionally been observed in a cell. After a portion of the dye has been eliminated from the cell, the cytoplasm around the tip of the channel contracts, pinching off the strand of membrane-bound dye and cytoplasm. The channel then disintegrates, often leaving only a small projection on the surface of the cell (Fig. 1f).

In a previous study of the excretion of indigestible residue in *A. proteus*, it was demonstrated that such material may be excreted by the selective extrusion of a vacuole (*6*). A former food vacuole with material to be eliminated from the cell comes in contact with the plasma membrane, generally in the functionally posterior region of the cell. Apparently the membrane of the vacuole fuses with the plasma membrane and the contents of the vacuole are expelled to the exterior; this is somewhat analogous to exocytosis in many other cell types. In *Chaos*, excretion of solid material also takes place in the posterior portion of the cell and again consists of expelling the contents of a vacuole to the exterior (*7*). The method of solute excretion observed in this study is quite different from that observed previously. Excretion in this case involves the development of a channel in the cytoplasm leading outward from the mass of ingested material in the central portion of the cell. Some of this accumulated dye, some cytoplasm, and, most likely, surface membrane to which the dye was originally bound is forced through the channel to the exterior of the cell. As is the case for pinocytosis, the motile force for this extrusion process probably resides in cytoplasmic filaments (*8*). Only a portion of the dye taken up is excreted in this manner.

Over the next several hours, many of the cells assume the classic rosette form (*4*) usually associated with the final phases of pinocytosis in the amoeba (Fig. 1g). Most then lose any surface projections they may have had. Also, the surface of the cell, which by now is generally spherical, becomes very smooth. The cell remains in this condition for the next several hours. By 5 hours after the initial exposure to Alcian blue, the remaining dye in the cell is distributed among a relatively large num-

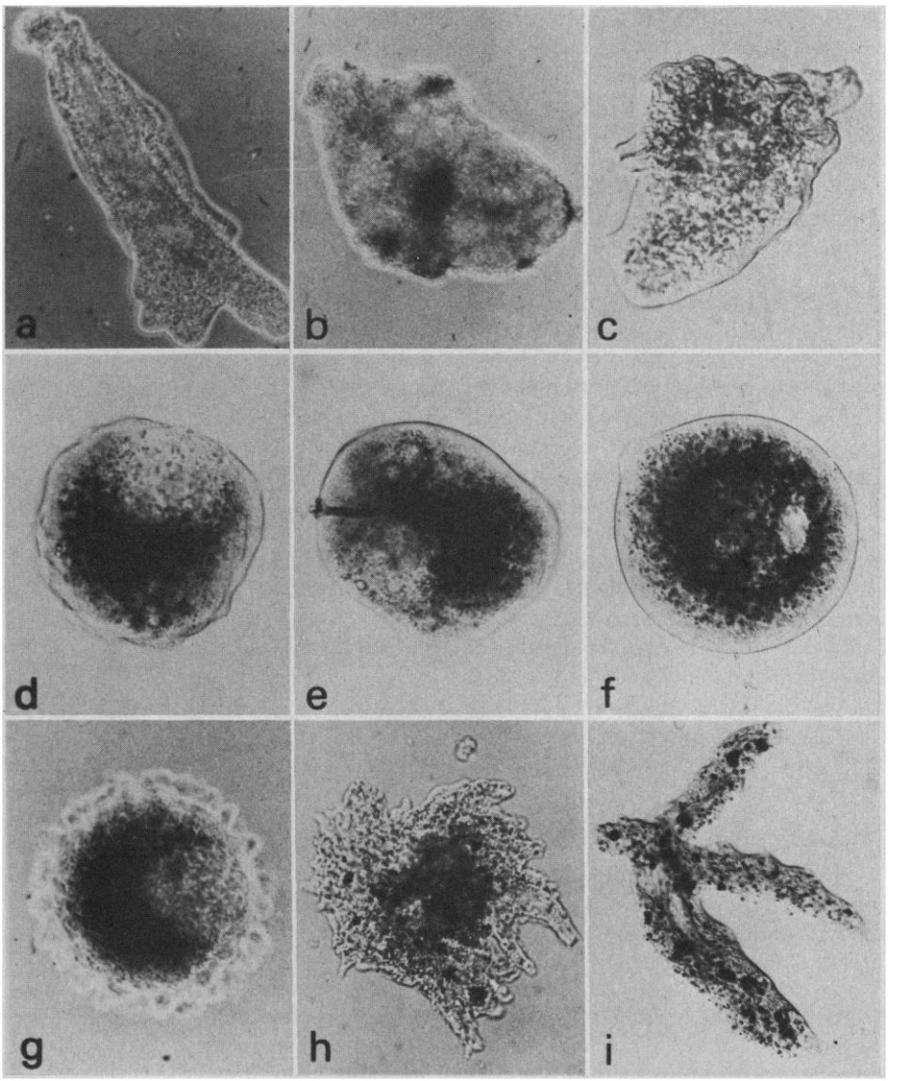
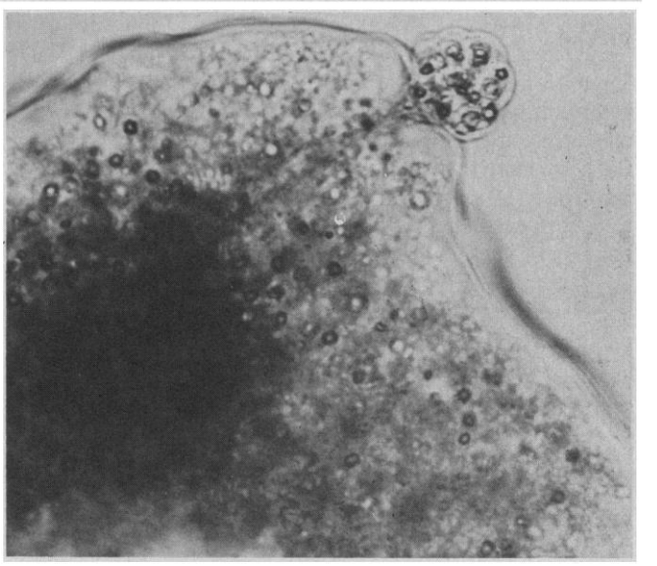


Fig. 1 (above). Pinocytosis cycle in *A. proteus*, induced with 0.01 percent Alcian blue. (a) Control cell (approximately 125 μm long). (b) Three minutes after exposure to Alcian blue, pseudopods have been withdrawn and the dye has accumulated in discrete areas on the amoeba's surface. (c) After 10 minutes pinocytotic channels have formed. (d) After 30 minutes the pinocytotic channels have disappeared and the dye has accumulated in the cytoplasm. (e) After 55 minutes an extrusion channel is established through which dye and probably some membrane and cytoplasm is forced to the surface. (f) After 75 minutes the extrusion channel disintegrates, often leaving a surface projection on the cell surface, and the remaining dye assumes a more uniform distribution in the cytoplasm. (g) After 90 minutes the cell has assumed rosette form. (h) After 5 hours the remaining dye has become segregated into relatively large clumps throughout the cytoplasm and the cell is beginning to send out pseudopods. (i) After 6 hours the cell is actively streaming. Blue residual bodies are distributed throughout the cytoplasm. Each photograph in the above sequence is of a different cell. Fig. 2 (right). Higher magnification of the solute extrusion phase. The extrusion channel, from the bulk of dye in the cytoplasm to the cell surface, is approximately 30 μm long.



ber of phagosomes (*9*) or blue residual bodies spread throughout the cytoplasm, and the cell begins to send out pseudopods (Fig. 1h). Within 6 to 8 hours, most of the cells are again actively streaming (Fig. 1i), still with a large number of blue residual bodies spread throughout the cytoplasm. Some of these bodies are still present 3 weeks later, gradually being eliminated by being pinched off at the posterior portion in the more usual manner described for the elimination of indigestible residue in the amoeba (*6, 7*).

If amoebas are treated in an identical fashion with 125 m*M* NaCl or 0.01 percent gelatin, they undergo an intense cycle of pinocytosis and then return to their normal condition within 1 or 2 hours after the induction of pinocytosis—without going through the extrusion process noted previously with Alcian blue. In amoebas, then, the fate of pinocytotically ingested material seems to vary based on the chemical nature of the solute (for example, how tightly it binds to the cell surface) and perhaps its nutritional value. In the case of gelatin, which is ingested by pinocytosis, extrusion is not necessary because this protein can be broken down and utilized by the cell. Induction of pinocytosis by a high level of Na^+ in the external medium also does not elicit the extrusion response noted for Alcian blue, even though a relatively large amount of Na^+ enters the cell under these conditions (*10*). *Amoeba proteus* is capable of regulating the cytoplasmic level of Na^+, and this may be associated with the activity of the contractile vacuole (*11*). Assuming that Na^+ does enter the cell during pinocytosis, it could be removed by activity of the contractile vacuole, restoring cytoplasmic Na^+ to the previous level.

When pinocytosis is induced with Alcian blue in *A. proteus*, the dye is taken up by the cell together with any solute that may be present in the external medium. For example, it was shown (*2*) that labeled sucrose in the external medium is taken into the amoeba when pinocytosis is induced with Alcian blue. Sucrose taken up in this manner reaches a maximum concentration 10 to 20 minutes after the induction of pinocytosis, and this concentration remains constant for the next hour or so. During this period, though, the cell begins to expel a portion of the accumulated dye through extrusion channels. This suggests that the cell is capable of discriminating between beneficial solute and potentially harmful substances taken up by pinocytosis, which are then treated in entirely different manners.

The only condition imposed on a successful inducer of pinocytosis in the amoeba is that it be a cation (*4*). Presumably these substances induce pinocytosis by binding to negative sites on the amoeba's surface. Thus the binding phase is nonselective. Any selectivity that may be associated with this mechanism of solute uptake must reside in the cytoplasm. Through a variety of extrusion mechanisms, including the quick elimination of a portion of a potentially harmful solute by bulk extrusion, the cell seems to be capable of coping with any type of solute it may encounter. This suggests that the bulk extrusion mechanism observed in the amoeba may reflect a primitive secretory capability shared by many other cell types.

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Sensitive and Rapid Diagnosis of Potato Spindle Tuber Viroid Disease by Nucleic Acid Hybridization

Abstract. *A sensitive and reliable new method for the detection of potato spindle tuber viroid in potato tubers has been developed. The method is based on hybridization of highly radioactive recombinant DNA to viroid RNA that has been attached to a solid support. The method can be automated and permits the rapid testing of large numbers of tubers.*

The potential of the potato for producing more well-balanced protein and calories per unit area, time, and water than any other major food crop is recognized in countries with low income, high population, and scarce food (*1*). Major efforts are in progress to adapt the potato to growth in subtropical and tropical climates, and already a lowland tropical potato cultivar has been developed (*1*).

Because potatoes are vegetatively propagated, virus diseases are a major problem in potato production, and practical means for the exclusion of viruses from "seed" potatoes are a necessity. A sensitive automated method for the simultaneous detection in potato tubers of several important viruses has been developed (*2*). This method, based on an enzyme-linked immunosorbent assay (ELISA), permits sampling of 300 tubers per hour.

The potato spindle tuber disease poses

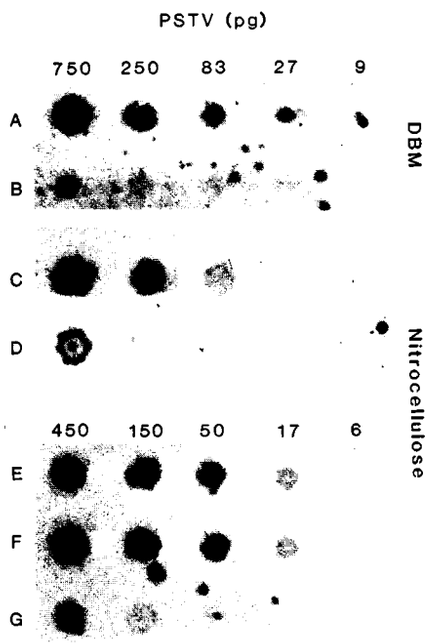

Fig. 1. Sensitivity of PSTV detection by hybridization and autoradiography after binding to DBM paper and nitrocellulose membranes. Low molecular weight RNA containing 0.4 to 0.5 percent PSTV was isolated from PSTV-infected tomato seedlings (*8*) and diluted with a mixture of 0.15*M* sodium acetate and 0.85*M* acetic acid (rows A and C), sap prepared from healthy Katahdin sprouts (rows B, D, and G), water (row E), or homogenization buffer (row F). Portions of successive threefold dilutions were then transferred to DBM paper (rows A and B) or nitrocellulose membranes (rows C to G).

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