circumferential wall stress under physiological pressures must be carried by approximately 1 percent of the wall material. The physiological range of extension ($\lambda = 1.4$ to 2.0) corresponds to wall stresses of 5×10^3 to 2×10^4 Nm^{-2} [Fig. 1a, (i)]. Inspection of Fig. 1 shows that, at similar extension ratios, the stress in isolated fibers is at least 100 times greater than the stress in the whole artery wall. In other words, the elastic fibers are present in sufficient quantity to account for the passive elasticity of the aorta under physiological pressures. At higher extensions the artery wall stiffens greatly, presumably a result of the recruitment of collagen fibers. Thus, the parallel arrangement of elastic fibers and collagen accounts for the mechanical properties of both octopus and vertebrate arteries.

Our study presents direct evidence of elasticity in the circulatory system of an invertebrate and describes a new rubber-like protein as the basis for this elasticity. Clearly, elegantly designed elastic arteries are not found only in vertebrates. Considering the functional benefits of elastic arteries and the widespread distribution of histologically identified "elastic fibers" (*4*), we believe that elastic blood vessels occur in many other invertebrates. Indeed, the presence of some sort of elastic reservoir may be a fundamental component of any circulatory system powered by a pulsatile pump. Of particular note is the extensive variation in amino acid composition among the protein rubbers (elastin, resilin, abductin, octopus elastic fibers), which suggests that each protein arose independently during evolution; and we suspect that other elastic tissues will contain as yet undescribed and distinct protein rubbers. Since there probably will not be enough "stretchy" names for all the protein rubbers, we propose a naming system based on organism and tissue type; therefore, the protein described here is called octopus arterial elastomer.

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8. This apparatus is modified from T. Weis-Fogh and W. B. Amos [*Nature (London)* **236**, 301 (1972)]. The sample is mounted with one end attached to a slender glass cantilever of uniform diameter and the other end attached to a movable glass plate (Fig. 2b). The sample is then stretched by sliding the plate (Fig. 2c). Tensile force in the sample is calculated from the deflection of the cantilever, while extension is determined by measuring changes in the spacing of surface features on the sample. All measurements are made with a Wild filar micrometer eyepiece on a Wild M21 polarizing microscope. We estimate the error to be within ± 5 percent. Since the fibers are rubbery only when hydrated, the samples are covered in distilled water at all times.
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13. Because the IE is insoluble and mechanically unaltered in formic acid we used this as a treatment to prepare chemically pure samples. This same method is used to isolate and purify elastin from vertebrate tissues [B. L. Rasmussen *et al.*, *Anal. Biochem.* **64**, 255 (1975)].
14. In this figure, the curve for the dog data is redrawn from R. H. Cox, *Am. J. Physiol.* **231**, 420 (1976).
15. The IE was shown in *O. vulgaris* by V. C. Barber and P. Graziadei [*Z. Zellforsch. Microsk. Anat.* **77**, 162 (1967)] but was described as a basement membrane. We found that the IE stained positively with aldehyde fuchsin [M. L. Cameron and J. E. Steele, *Stain Technol.* **34**, 265 (1959)], a technique that also stains elastin, and we confirmed other reports on cephalopod arteries that no elastic fibers were visualized in sections treated with conventional elastin stains (*3*, *4*).
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The Permeability of Plant Cell Walls as Measured by Gel Filtration Chromatography

Abstract. *The permeability of plant cell walls to macromolecules may limit the ability of enzymes to alter the biochemical and physical properties of the wall. Proteins of molecular weight up to 60,000 can permeate a substantial portion of the cell wall. Measurements of wall permeability in which cells are exposed to hypertonic solutions of macromolecules may seriously underestimate wall permeability.*

The permeability of the plant cell wall to macromolecules is of particular importance because it determines the ability of enzymes, extracellular glycoproteins, and polysaccharides to penetrate and alter the cell wall. Enzymes may play an important role in many cell wall processes such as cell wall biosynthesis (*1*), the dissolution of the cell wall by plant pathogens (*2*) or by the plant itself during fruit ripening (*3*) or leaf abscission (*4*), and growth-hormone-induced cell wall loosening (*5*).

Until recently, it was assumed that the cell wall is freely permeable to large macromolecules. In a recent report Carpita *et al.* (*6*) suggested that only small macromolecules (radius, 16 to 19 Å) could rapidly penetrate the cell wall of a variety of plant cell types. This size corresponds to a globular protein with a molecular weight of approximately 17,000. These results cast serious doubt on the possibility of enzyme involvement in cell wall biochemistry, since most enzymes are too large to penetrate pores of such small dimensions.

Carpita *et al.* (*6*) measured the ability of extremely high concentrations of macromolecules to penetrate through the cell wall. Using gel filtration chromatography, we have reexamined the permeability of the cell wall. With this technique it is possible to detect permeation of macromolecules into the cell wall matrix at much lower concentrations. These conditions should more closely imitate those to which plants are exposed in nature. Using proteins of known size, we calibrated a column packed with isolated cell wall fragments and determined the degree to which the proteins can penetrate the cell wall matrix. Our results show that proteins much larger (molecular weight, 40,000 to 60,000) than would be predicted from the work of Carpita *et al.* (*6*) can penetrate a substantial portion of the cell wall space. Using techniques analogous to those of Carpita *et al.* (*6*), we have established one reason why they might have underestimated the permeability of the cell walls they studied.

Cell walls were isolated from the hypocotyls of 7-day-old dark-grown bean (*Phaseolus vulgaris* L.) seedlings. Frozen hypocotyl segments were ground to a fine powder in a Sorvall Omnimixer cooled in a Dry Ice–ethanol bath. The powder was allowed to thaw, and attached cytoplasm was released by further disruption with a Blackstone ultrasonic probe (in 40-ml portions) for 30 seconds at 200 W. The slurry was diluted to 600 ml with deionized water and allowed to settle until two layers appeared

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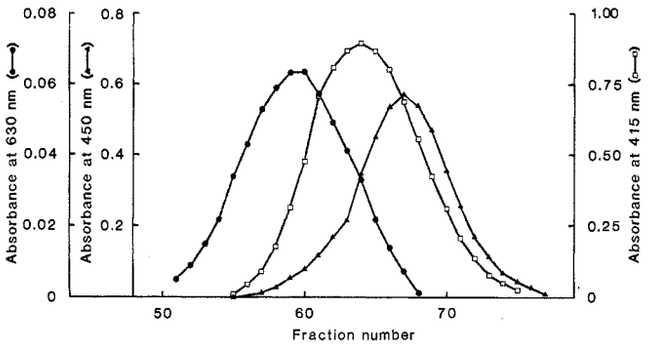

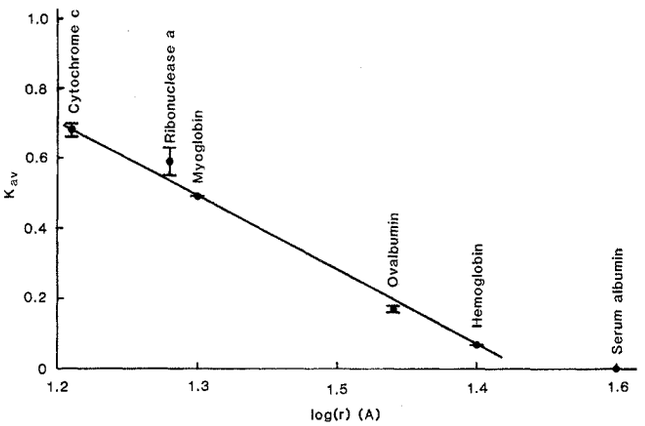

Fig. 1 (left). Elution profile of gel filtration chromatography on a bean cell wall column. A 50-μl sample containing 20 mg per milliliter of cytochrome c, blue dextran, and FMN was subject to chromatography on a column (0.5 by 28 cm) of bean cell wall fragments. The column was chromatographed in 10 m*M* sodium phosphate buffer. Two drop fractions were collected and diluted with 0.4 ml of deionized water. Absorbance was read at 630 nm for blue dextran (●), 450 nm for FMN (▲), and 415 nm for myoglobin (□). Fig. 2 (right). Elution coefficients (K_{av}) of proteins subjected to chromatography on a bean cell wall column with blue dextran as void volume (V_0) and FMN as total volume (V_t) markers; V_e is the elution volume. The K_{av} was calculated as $K_{av} = (V_e - V_0)/(V_t - V_0)$. The values of K_{av} shown are the means (± the standard errors) of three elutions. The molecular radii of the protein tested were taken from Laurent and Killander (*8*). A linear regression equation for all the data except serum albumin is as follows: $y = 3.419 - 2.243x$ ($r^2 = .975$). Similar results were obtained with Ficoll 400 and glucose as the V_0 and V_t markers, respectively.

(15 minutes). The upper layer (walls) was decanted and saved, whereas the lower layer was again diluted and allowed to settle. The upper layers from three decantations were pooled and centrifuged at 2000*g* for 15 minutes. The pelleted walls were resuspended in 500 ml of water and centrifuged twice more to remove residual cytoplasmic contamination. Walls were stored at −20°C. A slurry of the isolated cell walls was packed into a glass column (interior diameter, 5 mm) and equilibrated with 10 m*M* sodium phosphate buffer, *p*H 6.0, containing 3 m*M* NaN_3 and 0.5*M* NaCl. All experiments were conducted at 5°C. The relatively high salt concentration was required to neutralize ion-exchange interactions between the cell walls and the proteins tested. The salt did not affect the bed volume of the column and presumably did not cause either swelling or shrinkage of the wall fragments.

A typical elution profile (Fig. 1) of cytochrome c with blue dextran and FMN (void volume and total volume markers, respectively) shows that the resolving power of the cell wall column was rather low. This was probably due to a number of factors, including the large size and nonspherical shape of the particles, which resulted in a very loosely packed column (gel volume ≃ 20 percent of the bed volume). The low resolution made it necessary to elute the calibration proteins singly with the void volume and total volume markers. Nonetheless, the elution positions of the proteins were very reproducible.

The elution coefficients (K_{av}) of proteins with molecular weights ranging from 13,000 to 67,000 (radii, 17 to 35 Å) are shown in Fig. 2. Only the largest protein tested (bovine serum albumin, molecular weight, 67,000; radius, 35 Å) was completely excluded from the gel matrix. The K_{av} values of the other proteins correlated well with the logarithm of their molecular radii (regression coefficient $r^2 = .975$). This result confirmed that the cell wall column behaved as expected for a gel filtration column, and that nonspecific interactions did not have a noticeable effect on the elution position of the proteins.

These results show that large molecules can permeate a significant portion of the cell wall matrix. It is difficult to compare our results with those of Carpita *et al.* (*6*), since it is not clear what proportion of the wall matrix must be accessible to a given protein (as shown by our technique) in order for large amounts of it to penetrate completely through the cell wall.

Carpita *et al.* (*6*) reasoned that hypertonic solutions would cause plasmolysis if the solute was small enough to penetrate the cell wall, but that the cell wall would collapse with the protoplast (cytorrhysis) if the solute was too large to penetrate the cell wall. It is obvious that in this system water will diffuse out of the cell wall faster than a large solute molecule will diffuse inward, causing dehydration of the cell wall matrix. We predicted that wall dehydration would be accompanied by shrinkage. We examined fragments of isolated beet (*Beta vulgaris* L.) root cell wall under the microscope and observed significant shrinkage of the cell wall (4.9 ± 1.5 percent) in the presence of a 25 percent solution of polyethylene glycol (PEG) 6000. This concentration is considerably less than that used by Carpita *et al.* (*6*). We were unable to use concentrations similar to theirs because of the viscosity of the solutions. Presumably, at higher concentrations greater shrinkage would occur. Similar results were obtained with bovine serum albumin [50 percent (weight to volume)] and also with fragments of bean cell wall exposed to Ficoll 400. Equilibration of the bean cell wall column with 10 percent (weight to volume) Ficoll 400 caused considerable shrinkage of the column bed volume (12.3 percent), an indication that solutes that are excluded from the cell wall matrix cause wall shrinkage.

This finding raised the question of whether the observed osmotic shrinkage reduced the permeability of the cell wall matrix. We were unable to test this possibility with the bean cell wall column in 10 percent Ficoll 400; the elution behavior of the column was extremely poor, presumably because of the considerable viscosity of the Ficoll 400 solution and the compaction of the column due to shrinkage.

Instead, we modified the technique of Carpita *et al.* (*6*) to determine if osmotic shrinkage might have caused them to underestimate the permeability of the cell wall. We used living epidermal cells from the root tip of oat (*Avena sativa* L.) seedlings germinated on filter paper. As observed under the microscope, the cells shrank but did not plasmolyze in the presence of 0.20*M* sucrose, whereas 0.30*M* sucrose caused plasmolysis. The cells showed clear cytorrhysis in 50 percent (weight to volume) ovalbumin; up to 20 percent ovalbumin had no effect.

These results are similar to those obtained by Carpita *et al.* (*6*). When cells were exposed to a mixture of 0.2*M* sucrose plus 5.0 percent (weight to volume) ovalbumin, plasmolysis was observed. In this case, most of the osmotic potential required for plasmolysis was produced by a small solute (sucrose) which equilibrates rapidly through the cell wall and does not cause wall shrinkage. The presence of a small amount of the large solute (ovalbumin) should cause only minimal osmotic shrinkage. Under these conditions it appears that the ovalbumin was able to penetrate the cell wall and hence cause plasmolysis. We suggest that cell wall shrinkage in the presence of high concentrations of large solutes may have caused Carpita *et al.* (*6*) to underestimate significantly the size of molecule capable of penetrating the cell wall.

When Carpita *et al.* (*6*) exposed plant cells to extremely high concentrations of macromolecules, they found that only molecules whose diameter was less than 17 Å penetrated the cell wall in detectable amounts. They suggested that, over a longer period of time, somewhat larger molecules might have penetrated the cell wall. In our cell wall column experiments we have exposed cell walls to much lower concentrations of macromolecules; this should more closely reflect the conditions to which plant cells are exposed in nature (*7*). Under these conditions the cell wall of bean hypocotyl tissue is permeable to proteins of molecular weight up to 60,000. It is reasonable to expect that enzymes of moderate size could permeate enough of the cell wall matrix in vivo to play a significant role in cell wall biochemistry. Since we have shown that cell wall shrinkage can reduce the permeability of the wall matrix, it is possible that our results also underestimate the permeability of the wall in vivo, since the wall is considerably stretched by the turgor of the cells that it encloses.

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Antibiotic-Induced Inhibition of Pheromone Synthesis in a Bark Beetle

Abstract. *Ingestion of diet containing streptomycin inhibited the conversion of myrcene, a host plant terpene, to the male-specific pheromones ipsenol and ipsdienol in* Ips paraconfusus. *Synthesis of* cis*-verbenol, which is not a sex-specific pheromone, from the host plant terpene (−)-α-pinene and other metabolites from these two terpenes was not inhibited by the antibiotic.*

The bacterium *Bacillus cereus* isolated from hindguts of male and female *Ips paraconfusus* (Coleoptera: Scolytidae) produced the pheromone *cis*-verbenol when exposed in culture to vapors of α-pinene, a terpene found in the host, ponderosa pine (*1*). Exposure of male and female beetles to (−)-α-pinene vapor resulted in the production of *cis*-verbenol and myrtenol in the hindgut, indicating a relation between precursor and product (*2*). Another host plant chemical, myrcene, was shown to be converted to the pheromones ipsenol and ipsdienol, but only in male *I. paraconfusus* (*3–5*). Therefore, we added antibiotics to the diets of male *I. paraconfusus* and then exposed the insects to vapors of (−)-α-pinene and myrcene to determine if pheromone synthesis could be inhibited, thereby implicating symbiotic microorganisms.

Male *I. paraconfusus* were reared from naturally infested logging debris (Sierra and Eldorado National Forests, California) shortly before their use. The diets consisted of 62 ml of water, 34.5 g of powdered cellulose (Alpha-Cel, ICN Pharmaceuticals), 8 g of sucrose, and 22 g of freshly ground phloem (< 0.5 mm in diameter) from recently felled ponderosa pines. Streptomycin and penicillin G (1667 U/mg; both Sigma) were added to the diets at 10 mg each per milliliter of water in experiment 1 (17 November 1976) and each separately at 10 mg/ml in experiment 2 (16 November 1977). The diet ingredients were mixed and divided among three 10-cm petri dishes, and the mixture was tamped in each dish to 50 ml (air pockets < 1 mm in diameter) with a final concentration of each antibiotic in the diet estimated to be 4.1 μg/μl. Holes (3 mm in diameter by 8 mm deep) were made in the tamped diet mixes to promote rapid boring and feeding. In both experiments, 21 males were added to each dish of diet.

In the first experiment, 126 males were fed the diet that did not contain antibiotic, and 63 males were fed the antibiotic diet for 96 hours at 21°C under natural light. In the second experiment, 84 males were fed the nonantibiotic diet, 84 males were fed the penicillin diet, and 84 males were fed the streptomycin diet for 96 hours under similar conditions. At the end of the feeding period, groups of 20 males (experiment 1) or 10 males (experiment 2) were each placed in an aluminum screen cage inside a 70-ml glass jar containing 3 μl of (−)-α-pinene ($[\alpha]_D^{20}$ = −47.5°; Aldrich) purified by gas-liquid chromatography (GLC) and 3 μl of GLC-purified myrcene (Chem Samples) (each > 99.8 percent) for 18 hours at 21°C under natural light. Two groups of ten males in each experiment that had been fed the respective diets were not exposed to host terpene vapors to determine amounts of pheromone that were obtained from the ground phloem. In the second experiment, six groups of ten unfed males were exposed to host terpene vapors and compared to fed insects (*6*) (Table 1). The concentration of (−)-α-pinene and myrcene vapors in the exposure chamber were determined by withdrawing samples with a gastight syringe for GLC analysis [glass column, 1.8 m by 2 mm (inside diameter); 3 percent Apiezon L on 100/120 Gas-Chrom Q at 100°C; N_2 flow, 12 ml/min] (*4*) (Table 1).

After the exposure period, the mid- and hindguts were dissected out and extracted with diethyl ether (0.15 ml per ten males). Authentic samples of ipsenol, ipsdienol (each > 97 percent) and *cis*-verbenol (> 95 percent) (both Chem Samples), and myrtenol (> 99 percent; Aldrich) were compared to the gut extracts by GLC on the Apiezon L column. These compounds can be resolved by this GLC column. In previous studies unfed males were exposed to these terpenes by aeration. Metabolic products were identified in extracts of the hind gut by gas chromatography–mass spectrometry (*2, 4, 5*), nuclear magnetic resonance (*2, 5*), and by on-column hydrogenation (*4*). The percent mortality after feeding and after exposure to (−)-α-pi-

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