side G_{M1}) (*12*). It has been demonstrated also that liposomes that become associated with cells via glycolipid-lectin interactions may be taken up by fusion with the cells. Fusion of liposomes with HeLa cells in tissue culture was markedly enhanced by galactosyl or lactosyl ceramide in the liposomes (*13*).

From a therapeutic standpoint, under the conditions we used, successful treatment of sporozoite-induced malaria with liposomal glycolipids was an all-or-none phenomenon. Of 74 animals that did not develop patent infections at 10 days, all remained alive and apparently free of "clinical" malaria after 40 to 46 days. These data suggested that the liposome-injected animals had been cured (*14*).

In the few animals (7 to 15 percent) that did develop patent infections despite treatment with liposomes containing appropriate glycolipids, the average prepatent period was not significantly different from that of untreated animals, or that of animals that were treated with liposomes containing ineffective glycolipids (Table 1). The reason for the apparent complete lack of efficacy of liposomal glycolipids against the infection in 7 to 15 percent of the animals is not clear.

Treatment with liposomes containing glycolipids is particularly interesting because extremely high dilutions of liposomes were therapeutically effective, and there was an apparent lack of acute toxicity (that is, lethality) of undiluted liposomes (*15*).

In chemotherapy of leishmaniasis, the liposomal phospholipid and surface charge had strong influences on efficacy of treatment (*2*). Further experiments may reveal the influence, if any, of liposome composition on suppression of sporozoite-induced malaria.

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14. To test further for viable parasites in the livers of mice that did not develop patent infections, minced livers or collagenase-treated livers from liposome-treated mice that were free of patent infections 42 to 45 days after sporozoite injection were injected into fresh uninfected mice [D. A. Foley, J. Kennard, J. P. Vanderberg, *Exp. Parasitol.* **46**, 179 (1978)]. Minced liver from four mice or collagenase-treated liver from two mice free of patent infections were injected intraperitoneally into a total of 40 uninfected mice (24 mice received 12 to 30 mg of minced tissue; 16 mice received 7×10^5 to 9.5×10^6 collagenase-treated cells). None of the mice injected with "infected" liver had a patent infection 21 days after injection. This assay also tested for any small numbers of viable parasites that might have been present in the blood that was in the injected liver.
15. In animals treated with 0.15 ml of liposomes containing galactosyl ceramide, a dose-response analysis showed that a 1:1,000,000 dilution of liposomes still had half the effectiveness of undiluted liposomes. There was no mortality among 42 uninfected animals injected with 0.1 to 0.25 ml of liposomes containing normal saline (24 animals were injected with liposomes with galactosyl ceramide, and 18 animals were injected with liposomes lacking glycolipid). None of the injected animals showed any signs of distress within 24 hours after injection.
16. The skillful technical assistance of Cynthia Skelton and John Shaker is gratefully acknowledged.

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Determination of the Pore Size of Cell Walls of Living Plant Cells

Abstract. *The limiting diameter of pores in the walls of living plant cells through which molecules can freely pass has been determined by a solute exclusion technique to be 35 to 38 angstroms for hair cells of* Raphanus sativus *roots and fibers of* Gossypium hirsutum, *38 to 40 angstroms for cultured cells of* Acer pseudoplatanus, *and 45 to 52 angstroms for isolated palisade parenchyma cells of the leaves of* Xanthium strumarium *and* Commelina communis. *These results indicate that molecules with diameters larger than these pores would be restricted in their ability to penetrate such a cell wall, and that such a wall may represent a more significant barrier to cellular communication than has been previously assumed.*

The mechanical strength and contiguous nature of plant cell walls imparts structural rigidity to the entire plant. It also creates a biological problem, since plant cells must sense their environment through this large, porous network. Thus, the diameter of the pores, or capillaries, in the cell wall must ultimately impose a restriction on the size of molecules (nutrients, toxins, herbicides, elicitors, genetic elements, and viruses and other pathogens) with which the cells can interact. Despite the importance of the size of these pores, very little information is available concerning the capillary diameters in the cell walls of higher plants. The pores easily allow the passage of salts, sugars, and amino acids, since these substances can move freely in the plant (*1*). In addition, phytohormones can be translocated from cell to cell through the cell wall space (*2*). The

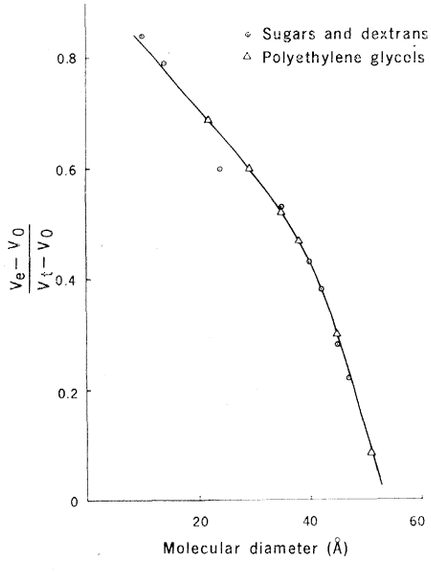

Fig. 1. Relationship between molecular radius and elution behavior during gel chromatography. A Bio-Gel P-10 column (200 to 400 mesh; 1 by 115 cm) was equilibrated at 23°C, with water as the solvent. Radii of sucrose, stachyose, and dextrans were calculated from known molecular weights or were taken from the literature; the points for the various PEG polymers were positioned on this graph on the basis of their elution behavior, and radii were obtained thereby. V_e is eluted volume, V_0 is void volume, and V_t is total volume.

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pores, therefore, must be large enough to accommodate such molecules. Our goal was to determine the upper limit to the size of molecules that freely permeate the cell wall and thus make cell-cell and cell-environment interactions possible.

To achieve this goal, we devised a method to determine the capillary diameters of the walls of living plant cells. The technique requires only a phase-contrast microscope and nonionic solutes of various molecular weights. The theory for the determination of capillary diameters is based on the following considerations. When cells are placed in solutions of a solute concentration higher than that within the cells, a net movement of water from within the cell to the external solution occurs, with a concomitant reduction in cell volume. If the external solute is able to penetrate the cell wall capillaries and thus permeate to the plasma membrane, plasmolysis, or the pulling away of the plasma membrane from the cell wall, occurs. However, if solutes are used that are high in molecular weight (and thus unable to penetrate the cell wall pores), then water movement out of the cell results in cytorrhysis (*3*), or the collapse of the cell wall around the shrinking protoplasm. Therefore, if one adds solutes of various molecular sizes to a solution containing the cells of interest and observes whether plasmolysis or cytorrhysis occurs, the maximum pore diameter should be equivalent to the diameter of the largest molecule capable of causing plasmolysis.

Solutes used to define the pore size range were mannitol and polyethylene glycol (PEG) 400, 600, 1000, 1540, 4000, and 6000, all obtained commercially. In addition, dextran fractions of various molecular radii were prepared by chromatography of a commercial preparation of dextran T10 (Pharmacia) on a column of Bio-Gel P-10. By this procedure, fractions of more defined molecular weights, ranging from 2000 to 10,500, were collected, and the mean molecular weight of each fraction was determined from its dry weight and reducing sugar content. From these molecular weight values, the radii of gyration (r) of the dextrans were determined by using the Einstein-Stokes equation

$$r = \frac{RT}{6\pi\eta DN_0}$$

where R is the gas constant, T is temperature, η is the viscosity of water (= 1), and N_0 is Avogadro's number. Diffusivity (D) values were interpolated from values reported by Granath (*4*) for a similar group of dextrans. The relationship between molecular radius and elution volume on a Bio-Gel P-10 column was determined according to Ackers (*5*). Standards used for this column included our own set of dextrans as well as sucrose and stachyose; radii for the latter two compounds were taken from Longsworth (*6*). Using this calibrated column, we determined the radii of the various PEG polymers from their elution volumes (Fig. 1).

The solutes of defined radii were first used to test for their ability to cause plasmolysis or cytorrhysis of living cells obtained from a suspension culture of *Acer pseudoplatanus* (*7*). Table 1 summarizes the results of these experiments, and Fig. 2 shows representative examples of plasmolysis and cytorrhysis. The transition from immediate and obvious plasmolysis to collapse occurred with solutes having a molecular radius of 38 to 40 Å. (The plasmolysis and cytorrhysis phenomena were reversible by diluting the solutes with water.) In terms of the solutes used, this corresponds to a molecular weight of 1300 to 1600 for PEG and about 6500 for a dextran. For comparison, a globular protein of this radius would have a molecular weight of about 17,000.

The low-molecular-weight sugars, mannitol, and PEG's were then used to determine the pore sizes of three types of differentiated plant cells (*8*). Table 1 summarizes the results of these tests. The transition from plasmolysis to collapse varied depending on cell type; the pores of hair cells from *Raphanus sativus* (radish) roots and from *Gossypium hirsutum* (cotton) fibers were somewhat smaller than those of the cultured sycamore maple cells (35 to 38 Å), whereas

Table 1. Comparison of molecular weights and sizes of various solutes with their ability to cause plasmolysis or cytorrhysis of several cell types. Either whole plant tissue or about 0.1 ml of a cell suspension was mounted on a microscope slide with a cover slip. The hypertonic solution to be tested was added dropwise to one side of the cover slip, and the test solution was drawn across the cells by absorbent toweling placed at the opposite side. As the osmoticum flowed over the cells, plasmolysis or cytorrhysis was observed by phase-contrast microscopy.

Solute	Molecular weight	Molecular diameter (Å)	Effect				
			Acer pseudoplatanus (liquid culture)	*Xanthium strumarium* (palisade parenchyma)	*Commelina communis* (palisade parenchyma)	*Raphanus sativus* (root hairs)	*Gossypium hirsutum* (cotton fibers)
Mannitol	182	8	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis
Sucrose	342	10	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis
Stachyose	666	14	Plasmolysis				
PEG 400	380–420	22	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis
PEG 600	570–630	29	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis	Plasmolysis
PEG 1000	950–1050	35	Momentary cytorrhysis, then plasmolysis	Momentary cytorrhysis, then plasmolysis	Momentary cytorrhysis, then plasmolysis	Cytorrhysis and plasmolysis	Cytorrhysis and plasmolysis
PEG 1540	1,300–1,600	38	Cytorrhysis and plasmolysis	Momentary cytorrhysis, then plasmolysis	Momentary cytorrhysis, then plasmolysis	Cytorrhysis	Cytorrhysis
PEG 4000	3,000–3,700	45	Cytorrhysis	Cytorrhysis and plasmolysis	Cytorrhysis and plasmolysis	Cytorrhysis	Cytorrhysis
PEG 6000	6,000–7,500	52		Cytorrhysis	Cytorrhysis	Cytorrhysis	
Dextran	2,010*	24	Plasmolysis				
Dextran	4,050*	35	Plasmolysis				
Dextran	6,560*	40	Cytorrhysis and plasmolysis				
Dextran	7,050*	42	Cytorrhysis				
Dextran	9,600*	45	Cytorrhysis				
Dextran	10,500*	47	Cytorrhysis				

*Mean molecular weight; range not determined.

the pores of palisade parenchyma cells of *Xanthium strumarium* (cocklebur) and *Commelina communis* were somewhat larger (45 to 52 Å) (*9*).

We have not uncovered any existing reports on pore size of cell walls of living higher plants. There are, however, reports concerning the pore size of cell walls of dried or rehydrated woody tissues. Frey-Wyssling and Mitrakos (*10*) made electron microscopic observations of metal deposition within the pores of the cell walls of ramie fibers and found that the pore diameters were generally less than 100 Å. More recent measurements, typically by a liquid N_2 adsorption technique, have been made by a number of investigators (*11*), whose estimates indicate that the average pore diameter is about 38 Å. Stone and Scallan (*12*) used a solute exclusion technique similar to ours to determine the pore widths of lignified and delignified wood. Most of the pore diameters were between 10 and 50 Å. The bulk of these reports, then, indicate close agreement with our data for the pore diameters of the living cells we tested. However, we were surprised that living, undifferentiated cells (such as those of the sycamore maple), which have only a thin (about 0.1 μm) primary wall, possess pore widths similar to those in the cell walls of highly differentiated wood fibers, which have thick secondary walls in addition to the primary wall. Despite the differences in composition and structure of the cell walls of various plant tissues, the limiting pore diameters may be similar; the primary cell wall in differentiated cells may parallel the fine-mesh sieve in a series of sieves. The technique used here should be applicable for testing this concept in a variety of cell types, including those with secondary walls. In this respect, it is of interest that the pore diameters determined by us are also similar to those estimated for organisms with quite different wall composition (a bacterium, a yeast, and fungal mycelia) (*13*).

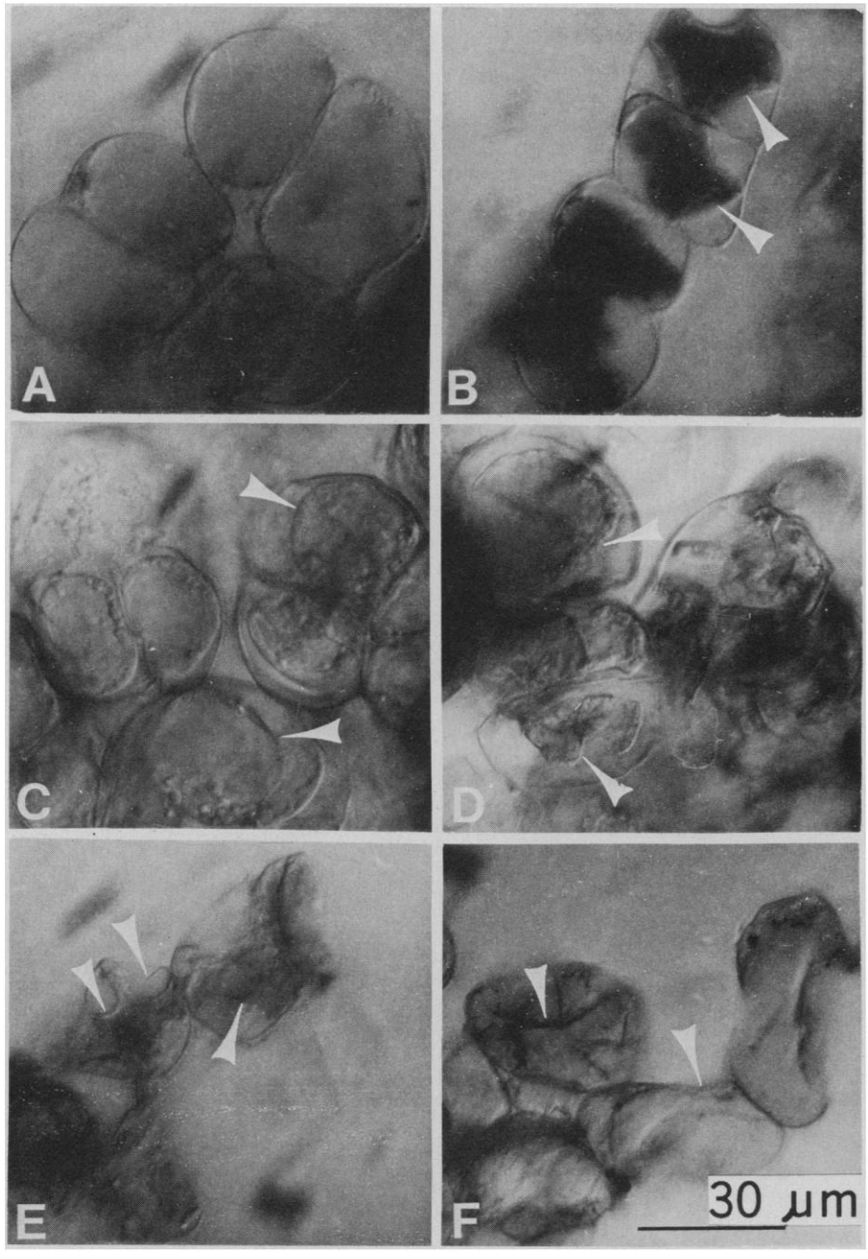

Fig. 2. Representative examples of observation of plasmolysis or cytorrhysis of sycamore maple cells by solutes of various molecular radii. Cells were stained with neutral red to enhance the visibility of plasmolysis and observations were made with a Zeiss standard microscope. (A) Cells before treatment, showing neither plasmolysis nor cytorrhysis. (B, C, and D) Plasmolyzed cells in mannitol, PEG 600, and PEG 1000, respectively. (E) Cells in PEG 1540, demonstrating both plasmolysis and cytorrhysis. (F) Cells showing cytorrhysis only in PEG 4000.

Although a pore size limit is clearly indicated by our results, this size limit would seem to contradict some reports that substances of much larger molecular diameter are capable of passing through plant cell walls. For example, the sycamore maple cells we used secrete large (molecular weight > 100,000) extracellular polysaccharides (SEP) into the incubation medium (*14*). Furthermore, a number of polysaccharide hydrolases are secreted by or washed from intact cells in liquid culture (*15*). A small number of larger pores could serve as channels of secretion, but it is not known whether such pores would allow free exchange of substances or whether the channels would be blocked by the secreted molecules themselves. There is evidence that SEP can inhibit the secretion of newly synthesized polysaccharide, possibly by binding to the secretion channels (*16*). Root-cap cells also secrete a large polysaccharide that lubricates the root as it advances through the soil (*17*); however, in this case, the cell wall appears to be a barrier to the secretion of this polysaccharide, and channels of secretion result from enzymatic hydrolysis of cell wall polysaccharides (*18*).

Thus, it is possible that some cell types possess specialized channels of secretion that may be blocked to the entry of external large solutes by the ongoing process of secretion, or that a small number of larger accessible pores exist that would allow very slow rates of permeation of large solutes. Since we observed plasmolysis in a few seconds after the introduction of the low-molecular-weight solutes whereas the cytorrhysis by the high-molecular-weight solutes was still evident after 1 hour, such large

pores could constitute only a very small percentage of the total cross-sectional area of the cell walls we examined.

Assuming that the pore diameters we measured represent the maximum size for free exchange between the plasma membrane and the environment, this value becomes an important consideration for many physiological functions as well as scientific manipulations. To cite a few examples, pore size may limit the effective size of toxins that function through binding to the plasma membrane (*19*) and of elicitors of phytoalexin synthesis (*20*), and pore size probably plays a role in a variety of other host-pathogen interactions, including recognition phenomena involving lectins (*21*). A limiting pore diameter must also be considered by researchers in their design of experiments involving the uptake of molecules (nucleic acids, proteins, oligosaccharides) by intact plant cells.

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8. Seeds of *R. sativus* were germinated on water-saturated filter paper. The radicle tips containing extending epidermal hair cells were excised and stained with neutral red to enhance the visibility of plasmolysis. Unfertilized ovules of *G. hirsutum* were cultured in vitro according to C. A. Beasley and I. P. Ting [*Am. J. Bot.* **60**, 130 (1973)]. Intact extending fibers were used after 8 days of culture and also were stained with neutral red. Fibers at this age possess only a primary wall. Palisade parenchyma cells of *X. strumarium* were isolated according to T. D. Sharkey and R. Raschke (*Plant Physiol.*, in press), and those of *C. communis* were obtained by peeling away the lower epidermis and scraping the cells gently into water with a fine dissecting needle. The palisade parenchyma cells were obtained from fully expanded leaves.
9. It should be noted that of the cell types examined here, only the leaf cells were obtained by mechanical disruption of the plant tissue; thus, we cannot exclude the possibilty that the larger pore size observed by us may have resulted from the isolation procedure.
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[^{3}H]GABA Binding in Brains from Huntington's Chorea Patients: Altered Regulation by Phospholipids?

Abstract. *Binding sites for tritium-labeled γ-aminobutyric acid (GABA) in cerebellar cortex of Huntington's chorea patients have an increased affinity but unaltered maximum capacity as compared to binding sites in tissue from control patients. A similar binding pattern is produced in control membranes by treatment with Triton X-100, phospholipase C, or glycerophosphoethanolamine. Thus, it is likely that phospholipids or their metabolites regulate the accessibility of the GABA binding site and that this regulation is abnormal in Huntington's chorea.*

In the striatum and some other brain areas from Huntington's chorea patients, there is a large loss of neurons that contain γ-aminobutyric acid (GABA) and its synthesizing enzyme L-glutamic acid decarboxylase (*1*). Pharmacological attempts at GABA replacement therapy in Huntington's chorea have been mainly inconclusive (*2*), although some success in treatment of recently diagnosed patients with a GABA-mimetic agent has been reported (*3*). The potential success of GABA replacement therapy is dependent on the integrity of GABA receptors. Initial reports on GABA receptors (as estimated by [^{3}H]GABA binding) in Huntington's disease indicated that these receptors are largely intact (*4*). However, subsequent studies have demonstrated that in the striatum there is a severe loss of GABA receptors in this disease (*5, 6*). Despite this large loss of striatal GABA receptors, GABA replacement therapy may still be efficacious (i) if GABA receptors outside the striatum are relevant to treatment of the disease or (ii) if the remaining GABA receptors are supersensitive. The present report focuses on the second possibility.

The cerebellar cortex was chosen for study because (i) a large amount of homogeneous tissue is available, whereas the degeneration and gliosis occurring in the striatum often do not leave enough tissue for detailed study; (ii) clinical signs of cerebellar dysfunction are often present in Huntington's disease (*7*); and (iii) several of the neurochemical changes typical in Huntington's disease occur in the cerebellum, although they are less marked than in the striatum (*1, 8*). Despite the neurochemical and clinical observations of cerebellar dysfunction in Huntington's disease, the tissue appears normal. However, in the juvenile form of the disease, severe cerebellar degeneration may occur (*9*). These results suggest that in the adult form changes occur in the cerebellum which are not detected in light microscopic examination.

The kinetics of GABA receptors were studied by the [^{3}H]GABA binding technique as adapted in our laboratory (*10*). The materials from control and Huntington's patients were matched for age and postmortem time. Tissue from the same brains was used for all experiments. The clinical diagnosis of Huntington's disease was verified by histopathological examination in all cases. Concentrations of GABA for the binding assay (*10*) ranged from 5×10^{-9} to $5 \times 10^{-7} M$, and binding affinity was estimated from the dose-response, Scatchard, or Lineweaver-Burk plots. As all methods of data analysis yielded very similar results, only the kinetic parameters from the Scatchard analyses are reported. For all patients, only one slope and intercept was observed in the Scatchard plot.

The affinity of [^{3}H]GABA binding to cerebellar membranes (Table 1) is increased (decreased K_d) threefold in membranes prepared from Huntington's cerebellum as compared to controls; the tendency toward an increased number of binding sites is not statistically signifi-

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