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Transport of Auxin (Indoleacetic acid) through Lipid Bilayer Membranes

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Summary. Diffusion of auxin (indole-3-acetic acid) through planar lipid bilayer membranes was studied as a function of pH and auxin concentration. Membranes were made of egg or soybean lecithin or phosphatidyl serine in *n*-decane (25–35 mg/ml). Tracer and electrical techniques were used to estimate the permeabilities to nonionized (HA) and ionized (A^-) auxin. The auxin tracer flux is unstirred layer limited at low pH and membrane limited at high pH, i.e., when $[A^-] \gg [HA]$. The tracer flux is not affected by the transmembrane voltage and is much higher than the flux predicted from the membrane conductance. Thus, only nonionized auxin crosses the membrane at a significant rate. Auxin transport shows saturation kinetics, but this is due entirely to unstirred layer effects rather than to the existence of an auxin "carrier" in the membrane. A rapid interconversion of A^- and HA at the membrane surface allows A^- to "facilitate" the auxin flux through the unstirred layer. Thus, the total flux is higher than that expected for the simple diffusion of HA alone. The relation between flux (J_A), concentrations and permeabilities is: $1/J_A = 1/P^{UL}([A^-]+[HA]) + 1/P^M_{HA}[HA]$. By fitting this equation to our data we find that $P^{UL} = 6.9 \times 10^{-4}$ cm/sec and $P^M_{HA} = 3.3 \times 10^{-3}$ cm/sec for egg lecithin-decane bilayers. Similar membrane permeabilities were observed with phosphatidyl serine or soybean lipids. Thus, auxin permeability is not affected by a net surface charge on the membrane. Our model describing diffusion and reaction in the unstirred layers can explain the "anomolous" relationship between pH and weak acid (or weak base) uptake observed in many plant cells.

The transport of auxin (indole-3-acetic acid) plays a major regulatory role in the growth and development of plants (Goldsmith, 1977). Closely related indole derivatives, pesticides and their metabolites are important in both plant and animal physiology and toxicology (Rubery & Sheldrake, 1973, 1974; Hammond, Carlson & Breeze, 1978; Pritchard, 1979). Thus, the mechanisms of transport of indoleacetic acid and related compounds are being studied in a number of laboratories.

Auxin is a moderately lipophilic weak acid ($pK \simeq 4.7$) which penetrates cell membranes primarily in the nonionic form at $pH < 5$ (Albaum, Kaiser & Nestler, 1937; Rubery & Sheldrake, 1973). Raven (1975) has estimated that the permeability of an algal cell membrane to nonionized auxin (HA) is about 10^{-3} cm sec^{-1}, about a thousand times higher than the permeability to the ionic form (A^-). In addition to simple, nonionic diffusion of HA, a carrier mediated transport of A^- has been proposed, based on auxin uptake kinetics, effects of competitive inhibitors, etc. (e.g., Rubery, 1978; *reviewed by* Goldsmith, 1977).

One aspect of auxin transport which has not been investigated is the role of chemical reactions in the aqueous unstirred layers adjacent to the cell membrane. In plant cells the unstirred layer includes the space within the cell wall. Although the membrane permeability to A^- is relatively low, chemical reactions between HA, A^- and H^+ may allow A^- to "facilitate" the diffusion of auxin through the unstirred layer. In this report we show how this type of facilitated diffusion can produce saturation kinetics similar to those expected for carrier-mediated transport. Our model can also explain a number of "anomolous" observations on the relation between pH and the uptake of weak acids and weak bases by plant cells.

Theory

The simplest way to measure the membrane permeability to a permeant weak acid is to place identi-

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cal solutions on both sides of the membrane and then add a small amount of tracer to one side only. Under such conditions the net flux of acid is zero, and no pH or buffer gradients exist in the unstirred layers. Tracer equilibrates rapidly between HA and A^- so that the specific activities of the two forms are similar throughout the unstirred layers, i.e., $[^*A^-]/[A^-] = [H^*A]/[HA]$. This means that when $pH > pK$, most of the tracer flux through the unstirred layers will be in the form of $^*A^-$, even though $^*A^-$ does not cross the membrane at a significant rate.

If only HA crosses the membrane and if the rate of tracer equilibration between HA and A^- is fast compared to diffusion through the membrane and unstirred layers, then the total one-way flux will be determined by three permeabilities as shown in Eq. (1) (Gutknecht & Tosteson, 1973).

$$\frac{1}{J_A} = \frac{1}{P_{HA}^{UL}[HA] + P_{A^-}^{UL}[A^-]} + \frac{1}{P_{HA}^{M}[HA]} \tag{1}$$

where J_A is the total one-way flux across the system, P_{HA}^{UL} and $P_{A^-}^{UL}$ are the unstirred layer permeabilities to HA and A^-, and P_{HA}^{M} is the membrane permeability to HA.

Equation (1) can be converted into a linear form by assuming that $P_{HA}^{UL} = P_{A^-}^{UL} \equiv P^{UL}$ and multiplying both sides by $([HA]+[A^-])$ to give:

$$\frac{[HA]+[A^-]}{J_A} = \frac{[HA]+[A^-]}{P_{HA}^{M}[HA]} + \frac{1}{P^{UL}}. \tag{2}$$

Thus, a graph of $([HA]+[A^-])/J_A$ *vs.* $([HA]+[A^-])/[HA]$ yields a straight line with a slope of $1/P_{HA}^{M}$ and an intercept of $1/P^{UL}$. This allows a statistical estimation of P_{HA}^{M} and eliminates the need to work at very high pH where tracer fluxes are lowest and errors due to background radiation and radiochemical impurities are greatest.

To get the best statistical estimate of P^{UL}, Eq. (1) can be rearranged to give:

$$\frac{[HA]}{J_A} = \frac{[HA]}{P^{UL}([HA]+[A^-])} + \frac{1}{P_{HA}^{M}}. \tag{3}$$

Thus, a graph of $[HA]/J_A$ *vs.* $[HA]/([HA]+[A^-])$ yields a straight line with a slope of $1/P^{UL}$ and an intercept of $1/P_{HA}^{M}$.

Materials and Methods

Lipid bilayer (optically black) membranes were made by the brush technique of Mueller and Rudin (1969). Unless otherwise specified, the membranes were formed from a mixture of egg lecithin (25–35 mg/ml) in *n*-decane. Membranes were formed on a 1.6-mm diameter hole in a polyethylene partition which separated two magnetically stirred solutions of 1.1 ml each. The temperature was 22–25 °C.

The aqueous solutions usually contained NaCl (90 mM), pH buffer (5–10 mM) and auxin (0.01–2.0 mM). In order to vary the $[A^-]$ at constant [HA], we varied the pH as described by the Henderson-Hasselbalch equation. Experiments were conducted over a pH range of 2.7 to 7.2, and solutions were buffered with HCl (pH 2.7), citrate (pH 4.1–5.4), MES (pH 5.7–6.7), BIS-Tris (pH 6.2–7.0), and phosphate (pH 6.9–7.2).

After a stable membrane was formed, 0.5–1.0 μCi of ^{14}C-auxin was injected into the rear compartment which was covered with a Teflon plug. The rate of appearance of radioactivity in the front compartment was measured by continuous perfusion (1–2 ml/min) and collection of samples at 3-min intervals. The samples were collected by aspiration into a vacuum trap. During the flux experiment the rear compartment was sampled with a microsyringe. The samples were counted in a liquid scintillation counter.

The one-way flux of auxin was calculated by the equation:

$$J_A = \frac{^{14}C^F}{t A S A^R} \tag{4}$$

where J_A is the flux (mol cm^{-2} sec^{-1}), $^{14}C^F$ is the total amount of tracer (cpm) entering the front compartment during the time interval t (sec), A is the surface area of the membrane (cm^2) and SA^R is the specific activity of tracer in the rear compartment (cpm/mol).

We measured the membrane resistance at approximately 3-min intervals by applying a known voltage pulse across the membrane in series with a known resistance (voltage divider circuit). The membrane potential was recorded as the potential difference between two calomel-KCl electrodes which made contact with the front and rear solutions.

The partition coefficient for auxin between hydrocarbon and water was measured by the method of Finkelstein (1976), modified slightly to increase the speed of equilibration. About 0.4 ml of aqueous solution containing about 1 μCi of ^{14}C-auxin was placed in a small glass vial which contained a magnetic stirring bar. About 400 μl of decane was carefully layered on top of the aqueous phase. The vial was filled with argon and sealed with a screw cap. The aqueous phase was stirred slowly (about 60 rpm). Under these conditions the half-time for equilibration was about 6 min. Both the aqueous and hydrocarbon phases were sampled periodically with a microsyringe. The partition coefficient (K_p) was calculated as the ratio of cpm (ml hydrocarbon)$^{-1}$/cpm (ml water)$^{-1}$.

The ^{14}C-auxin from both New England Nuclear Corp. and Amersham Corp. contained small amounts of lipophilic impurity. The impurity was detected in partition coefficient measurements which gave spuriously high values for the first two equilibration periods. By replacing the hydrocarbon phase five times, we were able to obtain a constant value of K_p.

The amount of lipophilic impurity in two shipments of auxin from New England Nuclear was high enough (3–7%) to cause significant errors in the tracer flux measurements at high pH, i.e., when $[HA]/[A^-] < 0.01$. The amount of lipophilic impurity in two shipments from Amersham was much lower (0.7%), and this material was used for most of our experiments. In the Amersham tracer the carboxyl group was labeled with ^{14}C, i.e., 3-indolyl[1-^{14}C] acetic acid. In the New England Nuclear tracer the labeled carbon was adjacent to the carboxyl group.

Egg lecithin and phosphatidyl serine (bovine) were obtained from Lipid Products (Surrey, England). Soybean lipids (Type II S) were obtained from Sigma Chemical Company (St. Louis, Mo.). Decane (99+%) was obtained from Aldrich Chemical Company (Milwaukee, Wisc.).

Results

The Auxin Flux is Nonionic

Figure 1 shows the auxin flux through a thick (colored) and thin (optically black) lipid bilayer made of phosphatidyl serine in decane (25 mg/ml) at pH 6.7 ($[A^-]/[HA]=100$). In this membrane thinning occurred quite rapidly 21–25 min after membrane formation. Thus, the fourfold increase in flux was clearly associated with a decrease in thickness. Figure 1 also shows that the auxin flux is not affected by a membrane potential of -60 mV, despite the fact that 99% of the auxin is in the ionic form. Similar noneffects of membrane voltage were observed with membranes made of egg lecithin and soybean lipids (data not shown). These results suggest that only nonionic auxin (HA) crosses the membrane at a significant rate.

Another way to demonstrate the nonconductive nature of the auxin flux is to compare the observed flux (J_A) with the A^- flux (J_{A^-}) expected from the membrane conductance (G_m) and transference number for A^- (t_{A^-}), i.e.,

$$J_{A^-}=\frac{R\,T\,t_{A^-}\,G_m}{z_{A^-}^2\,F^2} \tag{5}$$

where R is the gas constant, T is the absolute temperature, z_{A^-} is the ionic valence, and F is the Faraday (Hodgkin, 1951). This equation assumes independent ion movement and thus provides an estimate of the rate of simple ionic diffusion through the membrane. This calculated (conductive) flux is substracted from the observed (tracer) flux in order to estimate the electrically silent component of the observed flux. For example, the membrane in Fig. 1 had a value of G_m of 105 ± 60 nS cm^{-2} after thinning to a optically black state. Since t_{A^-} is unknown, Eq. (11) gives an upper limit on J_{A^-} of 2.5×10^{-14} mol cm^{-2} sec^{-1}, two orders of magnitude lower than the observed J_A. Thus, in this experiment at least 99% of the auxin flux is nonionic.

The membrane conductances in all of our experiments ranged from 1.2 to 1100 nS cm^{-2}, with an average value of 12 ± 8 nS cm^{-2} (SE, $n=25$). The greatest source of variability was in the egg lecithin-decane membranes. However, there was no correlation between the membrane conductance and the auxin concentration and no correlation between the membrane conductance and the auxin flux, which was >95% nonionic under all conditions.

In order to obtain a better estimate of the membrane conductance to ionized auxin, we did one series of experiments in which the only major ions were A^- and BIS-Tris$^+$, 3.0 mM, pH 6.7. Under these conditions, the conductance of egg lecithin-decane bilayers, measured 30–50 min after thinning, was 6 ± 2 nS cm^{-2} (SE, $n=5$). Even at this high auxin concentration, the conductance was similar to that of the "best" control membranes in auxin-free solutions containing various salts and buffers. Even if all the conductance were due to A^-, then J_{A^-} calculated from Eq. (5) would be about 2×10^{-15} mol cm^{-2} sec^{-1}, and $P_{A^-}^M$ (i.e., $J_{A^-}/[A^-]$) would be less than 10^{-9} cm sec^{-1}.

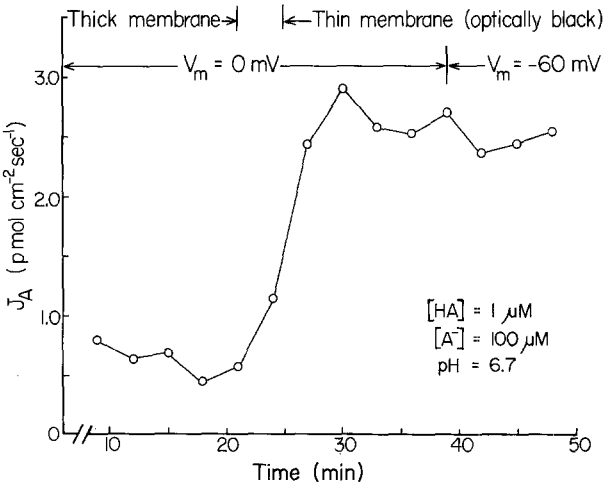

Fig. 1. One-way flux of auxin through a lipid bilayer membrane made of phosphatidyl serine in decane (30 mg/ml). Aqueous solutions contain 90 mM NaCl plus 5 mM MES buffer, pH 6.7. The transition from thick (colored) to thin (optically black) state occurred 21–25 min after membrane formation. At 39 min the membrane potential was clamped at -60 mV (rear side negative). The direction of the one-way flux is from rear to front.

Membrane and Unstirred Layer Permeability to Auxin

Figure 2 shows the one-way flux of auxin (J_A) as a function of $[A^-]$ at constant [HA]. The membranes were made of egg lecithin in decane (25–35 mg/ml). The solid line is calculated from Eq. (1), using values of $P_{HA}^{UL}=P_{A^-}^{UL}=6.5\times10^{-4}$ cm sec^{-1} and $P_{HA}^M=3.4\times10^{-3}$ cm sec^{-1}. These values of P^{UL} and P_{HA}^M were determined by eye to give the "best fit". As will be shown below, similar values of P^{UL} and P_{HA}^M are obtained by linear regression, using Eqs. (2) and (3).

At pH 4 the rate of auxin transport is about equal to that expected for diffusion through the unstirred layer alone. The unstirred layer thickness is defined as D/P^{UL}, where D is the aqueous diffusion coefficient. Since $D\simeq7\times10^{-6}$ cm^2 sec^{-1}, the data suggest an unstirred layer thickness of about 110 μm, similar to values previously obtained by us and others with

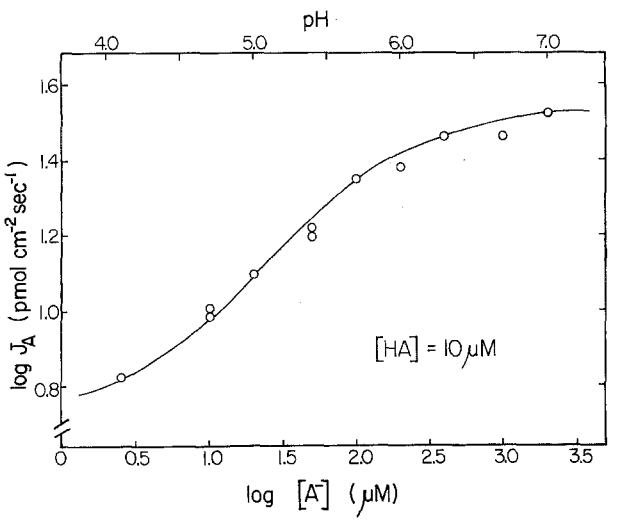

Fig. 2. One-way flux of auxin (J_A) as a function of ionized auxin (A^-) concentration at constant nonionized auxin (HA) concentration. The membrane is egg lecithin in decane (30–35 mg/ml). The aqueous solutions contain 90 mM NaCl plus 5–10 mM buffer. Each point represents a single membrane. The solid line is calculated from Eq. (1), assuming $P^{UL}_{HA} = P^{UL}_{A^-} = 6.5 \times 10^{-4}$ cm sec^{-1} and $P^{M}_{HA} = 3.4 \times 10^{-3}$ cm sec^{-1}.

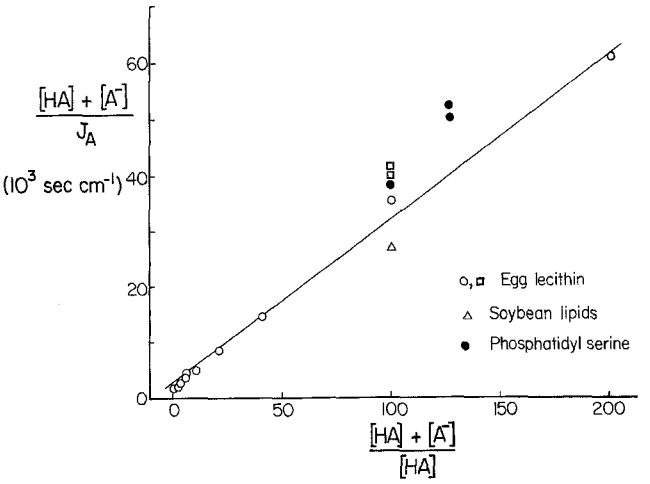

Fig. 3. Auxin flux as a function of auxin concentration, plotted according to Eq. (2). Auxin concentrations ranged from 1–10 μM HA and 2–3000 μM A^-. The open circles (egg lecithin-decane) are the same data as shown in Fig. 2. Linear regression analysis of these data yields a P^{M}_{HA} (1/slope) of $(3.3 \pm 0.2) \times 10^{-3}$ cm sec^{-1} (mean ± SD). Shown also are data for phosphatidyl serine and soybean lipids. The aqueous solutions contain 90 mM NaCl plus 5–10 mM buffer in all experiments except those indicated by open squares, which were either A^- plus BIS-Tris$^+$ (3.0 mM) or A^- plus BIS-Tris$^+$ (3.0 mM) plus KCl (5.0 mM).

this type of system (e.g., Andreoli & Troutman, 1971; Gutknecht & Tosteson, 1973; Finkelstein, 1976).

Over the pH range 4.7–5.7 the flux is roughly proportional to $[A^-]$, even though electrical measurements indicate that A^- does not cross the membrane at a significant rate. The increase in flux is due to the rapid equilibration of tracer between A^- and HA, which allows A^- to "facilitate" tracer diffusion through the unstirred layer. A number of previous studies have described this type of facilitated diffusion, which may occur whenever a tracer exists in two or more chemical forms, one of which cannot cross the membrane, e.g., CO_2 and HCO_3^- (Gutknecht, Bisson & Tosteson, 1977), and halogen and halide (Gutknecht, Bruner & Tosteson, 1972).

At pH > 6 the auxin flux becomes limited by the rate at which HA crosses the membrane, because $P^{UL}_{A^-}[A^-]$ becomes larger than $P^{M}_{HA}[HA]$. Thus the flux saturates at high pH and further increases in $[A^-]$ cannot increase the flux. By fitting Eq. (1) to the data, the value of P^{M}_{HA} of 3.4×10^{-3} cm sec^{-1} is obtained. This is more than six orders of magnitude higher than the upper limit of $P^{M}_{A^-}$, estimated from the membrane conductance.

A more accurate way of estimating P^{M}_{HA} is shown in Fig. 3, in which the data are plotted according to Eq. (2). One advantage of this approach is that P^{M}_{HA} (1/slope) can be estimated by linear regression. A second advantage is that Eq. (2) normalizes the flux with respect to concentration. Thus, we can pool the data for all auxin concentrations, provided that permeability is independent of concentration. This is useful because the membranes containing negatively charged lipids (e.g., phosphatidyl serine) were unstable at auxin concentrations > 100 μM.

Linear regression analysis of the egg lecithin data (shown also in Fig. 3) (open circles) yields $P^{M}_{HA} = (3.3 \pm 0.2) \times 10^{-3}$ cm sec^{-1}. Linear regression analysis of the same data plotted according to Eq. (3) (not shown) yields $P^{UL} = (6.9 \pm 2.4) \times 10^{-4}$ cm sec^{-1} (mean ± SD).

Figure 3 also shows flux data for two other types of membranes, i.e., phosphatidyl serine, and soybean lipids. The soybean lipids contain about 22% lecithin, as well as phosphatidyl serine, phosphatidyl inositol, and phosphatidyl ethanolamine (Gambale, Gliozzi & Robello, 1973). Figure 3 shows that the membrane permeabilities to auxin are similar within a factor of 1.5 for both the zwitterionic and negatively charged lipids. Figure 3 also shows that ionic strength has no effect on the auxin permeability of egg lecithin-decane membranes.

Hydrocarbon/Water Partition Coefficient

The partition coefficient (K_p) for nonionized auxin between decane and water was measured at pH 2.7, ($[A^-]/[HA] = 0.01$). The aqueous solution was buffered with HCl (ca. 2 mM) and contained NaCl

(100 mM) and auxin (0.1 mM). The value of K_p was $(9.7 \pm 1.8) \times 10^{-4}$ (mean ± SE of three measurements).

Carrier-Mediated Auxin Transport

In one experiment we tested the effect of a long-chain secondary amine (Amberlite LA-2) on auxin transport. Amberlite LA-2 (Rohm and Haas, Philadelphia, Pa.) is a liquid anion exchanger which can be built into lipid bilayer membranes by simply adding it to the membrane forming solution. In lipid bilayers Amberlite behaves as "titratable carrier" which facilitates the coupled (nonconductive) transport of monovalent anions and protons (Gutknecht & Walter, 1979). Since Amberlite has been shown to have a high affinity for certain organic anions (Shean & Sollner, 1966), we expected that it would facilitate the diffusion of ionized auxin through bilayers. In this experiment the membranes were made from a mixture of egg lecithin and Amberlite, 50 mg each per ml decane, which gives a lecithin/Amberlite mole ratio of 1:2. The auxin flux was measured at pH 7.0 under conditions identical to those in Fig. 2, i.e., 2.0 mM auxin, 90 mM NaCl, and 5 mM BIS-Tris buffer.

At pH 7.0 the auxin flux through lecithin-Amberlite bilayers was 283 ± 32 pmol cm^{-2} sec^{-1}, almost an order of magnitude higher than the maximum flux shown in Fig. 2. The flux was >99% nonconductive, as indicated by the membrane conductance, i.e., 370 ± 130 nS cm^{-2}, and Eq. (5). The total auxin permeability, i.e., $J_A/[A_T]$, was about 1.4×10^{-4} cm sec^{-1} and would be about 30% higher if corrected for the unstirred layer permeability.

In the absence of additional data, it is difficult to distinguish between a carrier-mediated cotransport of A^- and H^+ and a simple diffusion of HA alone. However, we have shown previously that Amberlite facilitates the cotransport of anions and protons, as expected from its ability to extract protons and anions from an aqueous phase into an organic phase (Shean & Sollner, 1966). At pH 7.0 the auxin permeability of lecithin-Amberlite bilayers is about an order of magnitude higher than the Br^- permeability (Gutknecht, Graves & Tosteson, 1978). Anions having lower field strengths, e.g., I^- and SCN^-, show permeabilities higher than Br^- (Gutknecht & Walter, 1979). Thus, our results can be readily explained as a carrier-mediated A^- transport involving the protonated form of Amberlite LA-2. On the other hand, Amberlite does not increase the membrane permeability to urea, water, sodium or sulfate (Gutknecht et al., 1978; A. Walter, *unpublished data*). Thus, the eight- to ninefold increase in auxin flux at pH 7.0 cannot be due to a nonspecific increase in membrane permeability to nonionized auxin.

Discussion

Auxin Permeability of Lipid Bilayers and Plant Cell Membranes

The diffusion of many nonelectrolytes through egg lecithin-decane bilayers obeys Overton's rule, i.e., $P_d \propto DK_p$ (Finkelstein, 1976). When a pure hydrocarbon such as hexadecane is used as the model solvent for K_p measurements, the slope of P_d *vs.* DK_p is 1.0 (Orbach & Finkelstein, 1980), which indicates that pure hydrocarbon is a suitable model solvent for the rate-limiting barrier in a lecithin-decane bilayer. The relationship holds true for K_p's ranging from 2×10^{-6} (glycerol) to at least 0.12 (salicylic acid) (Hogben et al., 1959; Gutknecht & Tosteson, 1973; Orbach & Finkelstein, 1980). The relation also holds for molecules ranging in size from acetamide (mol wt = 59) to codeine (mol wt = 299). Our value of P_{HA}^M for auxin (3×10^{-3} cm sec^{-1}) is exactly that predicted by the K_p (1×10^{-3}) and D (7×10^{-6} cm^2 sec^{-1}). In other words, nonionized auxin fits the pattern established for other nonelectrolytes in this particular lipid bilayer system.

Our value of P_{HA}^M is similar to the nonionized auxin permeability of sphingomyelin-tocopherol bilayers, i.e., 3.7×10^{-3} cm sec^{-1} (Bean, Shepherd & Chan, 1968)[1]. The auxin permeability of planar bi-

[1] Bean et al. used a spectrophotometric method to measure bilayer permeabilities to auxin and other fluorescent compounds, many of which are quite liophilic. The highest permeabilities they reported were $(2-3) \times 10^{-4}$ cm sec^{-1} for indole, indole-3-ethanol, and 5-hydroxyindole. These values are surprisingly low for such lipophilic molecules. Since their net fluxes were rather insentive to stirring rate, as well as to the ratio of membrane area to aperature depth, they concluded that "... the membrane is the only restricting factor on the diffusion of substances showing permeability coefficients similar to or lower than those of indole-ethanol in sphingomyelin-tocopherol membranes."

We find this argument unconvincing and believe that many of their permeability values are dominated by unstirred layer effects for the following four reasons: First, their apparent permeability to indole-3-ethanol was $(2.7-3.6) \times 10^{-4}$ cm sec^{-1}. If we assume the unstirred layer is rate limiting and then calculate a permeability based on the depth of the aperatures in their partitions, we get a range of permeabilities of $(1.8-3.0) \times 10^{-4}$ cm sec^{-1}, similar to their observed values. Second, they observed similar permeabilities to indole, indole-3-ethanol, and 5-hydroxyindole. These are unlikely to be membrane-limited permeabilities because the addition of a hydroxyl group to indole should decrease the membrane permeability at least 200-fold (Finkelstein, 1976; Wright & Bindslev, 1976; Orbach & Finkelstein, 1980). Third, their observed permeability to indole-3-ethanol was not affected by lipid compo-

layers made from sphingomyelin-cholesterol-tocopherol or brain lipids-tocopherol is at least an order of magnitude lower (Bean et al., 1968). Similarly, Finkelstein (1976) found that the nonelectrolyte permeability of sphingomyelin-cholesterol-decane bilayers was more than an order of magnitude less than egg lecithin-decane bilayers. The auxin permeability of the plant cell, *Hydrodictyon*, is about 10^{-3} cm sec^{-1} (Raven, 1975), which falls within the range observed for these "loose" and "tight" lipid bilayer membranes.

From electrical measurements we estimate the permeability of lecithin-decane bilayers to the auxin anion (A^-) to be less than 10^{-9} cm sec^{-1}, at least six orders of magnitude less than the permeability to nonionized auxin. This enormous difference between $P^M_{A^-}$ and P^M_{HA} is predicted from electrostatic considerations (Finkelstein & Cass, 1968) and has been observed previously with other weak acids, e.g., salicylic acid and various other uncouplers (Gutknecht & Tosteson, 1973; Dilger & McLaughlin, 1979). The permeability of the plant cell, *Hydrodictyon*, to ionized auxin is about 10^{-6} cm sec^{-1} (Raven, 1975), much higher than the permeability of an unmodified lipid bilayer. Thus, ionized auxin transport through plant cell membranes may occur via specialized carriers or channels (Rubery & Sheldrake, 1974).

In plant cells a carrier-mediated cotransport of H^+ and A^- was recently suggested by Rubery (1978). The lecithin-Amberlite bilayer provides a simple model for this type of coupled transport (Gutknecht & Walter, 1979). If plant cell membranes contain a titratable anion carrier with properties similar to Amberlite, then a net flux of A^- could be driven by either a pH gradient or an A^- gradient, but not by an electrical potential gradient. In addition, nonionic diffusion of HA will occur in parallel to any carrier-mediated transport of A^-. As we have shown, the rate of nonionic diffusion of HA also depends on A^-, due to chemical reactions in the unstirred layers. Thus, a quantitative description of the relative roles of the two types of transport *in vivo* is a difficult task (Rubery, 1978).

Effect of Surface Charge on Auxin Transport

Rubery and Sheldrake (1973) have examined the relation between auxin uptake and pH in several plant cells and tissues. They found that the uptake of auxin, as well as many other weak acids (Simon & Beevers, 1952), resembles a titration curve with the maximum rate of uptake at low pH. However, the curve is displaced so that the half-maximal flux is about 1 pH unit above the pK. They suggest that this displacement is due to a negative charge on the membrane or in the cell wall, which causes a decrease in the pH near the membrane surface and a consequent increase in the apparent pK of the weak acid. Similar arguments have been made by Bean et al. (1968) and Jackson and Cohn (1977). These arguments are incorrect, however, because the negative surface charge causes equal and opposite effects on the aqueous concentrations of H^+ and A^-. Thus, surface charge should have no effect on the concentration of HA which is the permeant form (*see* Raven, 1975; Goldsmith, 1977, p. 445; McLaughlin, 1977, p. 125). Our results show, as expected, that the auxin permeability of negatively charged bilayers made of phosphatidyl serine (PS) or soybean lipids is similar to the permeability of egg lecithin (PC) bilayers.

Figure 4 shows the observed displacement of the auxin uptake *vs.* pH curve in the alga *Hydrodictyon*. The data points are the observed net influxes of auxin (Raven, 1975), the dashed line is the "titration" curve expected if only HA crosses the membrane, and the solid line is the theoretical result expected for HA permeation plus A^- diffusion and reaction to form HA at the membrane surface. The solid line is calculated from Eq. (1) and choosing by eye the "best fit" values of $P^M_{HA} = 1.8 \times 10^{-3}$ cm sec^{-1} and $P^{UL} = 1.3 \times 10^{-3}$ cm sec^{-1}. This assumed value of P^{UL} in *Hydrodictyon* is about twice the P^{UL} observed in our lipid bilayer system. This is reasonable because the cytoplasm is near neutral pH and well buffered. Con-

sition. At the same time, their permeability to nonionized auxin differed by a factor of 36 between the "loosest" membrane (sphingomyelin-tocopherol) and the "tightest" membrane (brain lipid-tocopherol). Fourth, their value for the salicylic acid permeability at pH 7 is almost five orders of magnitude lower than our value for egg lecithin bilayers (Gutknecht & Tosteson, 1973). Unfortunately, neither the test solute concentrations nor the buffer identity were given by Bean et al. However, if we assume their salicylate concentration was low and their solutions were adequately buffered, then we can estimate from their data a very reasonable salicylic acid (HA) permeability of 0.1 cm sec^{-1} for brain lipid-tocopherol membranes, compared to 0.7 cm sec^{-1} for our lecithin-decane bilayers. Our calculation is based on the assumption that at pH 7 the membrane rather than the unstirred layer limits the net flux of this permeant weak acid (Gutknecht & Tosteson, 1973). The auxin permeability values we have quoted from Bean et al. are derived by using a similar set of assumptions, i.e., only HA crosses the membrane and at $pH \gg pK$ the net flux is rate limited by the membrane rather than the unstirred layer.

Bean et al. noted that, when they changed the pH, the net fluxes of weak acids and weak bases changed in the direction, but not the magnitude, expected for permeation of only the nonionized species. This led them to conclude that the concentration of the nonionized species was "... not the only factor involved in controlling the diffusion gradient and permeability." According to our interpretation of their results, the "other factor" is the diffusion of the ionized form and the chemical reactions involving A^-, HA, and H^+ (or buffered H^+) in the unstirred layers.

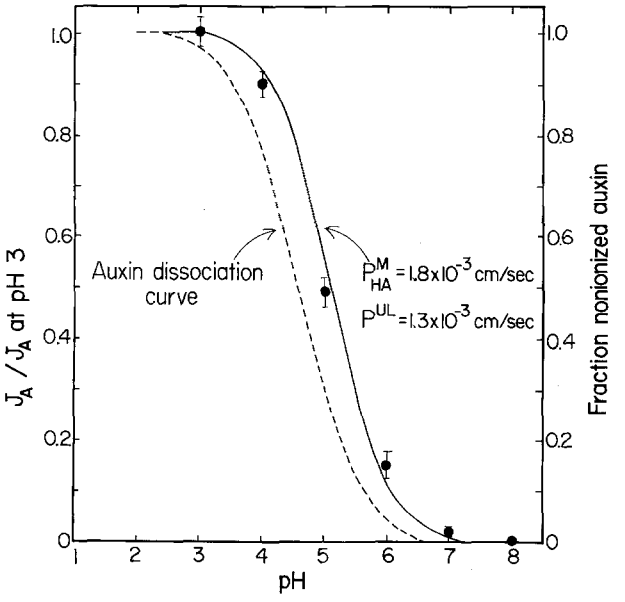

Fig. 4. Comparison of auxin uptake by the alga *Hydrodictyon* with that predicted by two different models. The flux data (±SE) are from Raven (1975), expressed as a fraction of the auxin influx at pH 3.0. In the *Hydrodictyon* experiment, J_A at pH 3.0 was 7.3 $\times 10^{-12}$ mol cm^{-2} sec^{-1} and [HA]+[A$^-$]=10 μM at all pH values (MES or HEPES buffer, 5 mM). The dashed line shows the influx predicted for permeation of HA only with no chemical reactions in the unstirred layer. The solid line is the influx predicted for the permeation of HA only with conversion of A$^-$ to HA in the unstirred layer, assuming $P^M_{HA} = 1.8 \times 10^{-3}$ cm sec^{-1} and $P^{UL} = 1.3 \times 10^{-3}$ cm sec^{-1}.

sequently, as HA crosses the membrane it is immediately converted to A$^-$, and the cytoplasmic unstirred layer resistance is, in effect, reduced (Gutknecht & Tosteson, 1973).

We believe that our model for diffusion plus chemical reactions in the unstirred layer can explain the displacement of the weak acid uptake *vs.* pH curve in *Hydrodictyon*, as well as in other plant cells. Higher ratios of P^M_{HA}/P^{UL}_A will produce larger displacements between the observed curves and the HA dissociation curves. In a few types of cells, a displacement of the auxin uptake *vs.* pH curve is not observed (Rubery & Sheldrake, 1973). There are at least two possible explanations. First, if experiments are conducted in unbuffered solutions, e.g., yeast cells in distilled water, then the formation of HA from A$^-$ may be limited by the diffusion of H$^+$ through the unstirred layer, and the facilitation of the net auxin influx by A$^-$ will be reduced (Gutknecht & Tosteson, 1973). Alternatively, if $P^M_{HA} < P^{UL}_A$, then the membrane permeability will be rate limiting under all conditions and chemical reactions in the unstirred layer will not play an important role in the transport process.

Conclusions

The diffusion of auxin through lipid bilayer membranes is "facilitated" by the diffusion of A$^-$ through the unstirred layers and interconversion of A$^-$ and HA at the membrane surface. Consequently, the auxin flux is larger than that expected for simple diffusion of HA alone. The transport process shows saturation kinetics because the membrane transport of HA is rate limiting only at high pH, i.e., when $P^{UL}_A[A^-] > P^M_{HA}[HA]$. The model which describes our results can explain the observed displacement of the auxin uptake *vs.* pH curve in various plant cells.

A variety of other plant hormones, competitive inhibitors, and herbicides are moderately lipophilic weak acids, e.g., abscisic acid, giberellic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and 2,3,5-triiodobenzoic acid (Rubery & Sheldrake, 1973, 1974). Thus, chemical reactions in the unstirred layers probably play an important role in the transport of these compounds, as well as in the transport of auxin.

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