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Effect of Deuterium Oxide on Junctional Membrane Channel Permeability

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Summary. The effect of deuterium oxide on junctional membrane permeability to dichlorofluorescein was examined to determine the mode of transfer of the dye from one cell interior to another in the septate giant axon of earthworm. Dichlorofluorescein was shown to diffuse through the nexus passively and in a hydrated form. Additionally, evidence suggested an alteration of the cell-to-cell channel structure by deuterium/hydrogen exchange. Dichlorofluorescein was rendered impermeant at 6 °C in D_2O and 4 °C in H_2O. Action potentials, however, were capable of propagation from cell to cell at 4 °C in D_2O and H_2O. The results are consistent with a hydrophilic channel where solute molecules diffuse through the junction (nexus) in a hydrated form. The temperature blocks are presumably brought about by increasing hydration shells around solute and channel proteins with cooling until the solute is rendered too large to diffuse.

Key Words junctions · permeability · deuterium oxide · diffusion · hydrated channels · earthworms

Introduction

Much evidence exists for an aqueous channel in the nexus or gap junction (Loewenstein & Kanno, 1964; Casper et al., 1977; Makowski et al., 1977; Unwin & Zampighi, 1980; Loewenstein, 1981). These channels are believed to be formed by subunit connexon proteins (Goodenough, 1975). Physiological data from mammalian heart (Weidmann, 1966) has revealed the junctional membrane conductance to be many orders of magnitude higher than the plasma membrane. Intercellular coupling in the form of electrotonic spread has been demonstrated in nervous tissue (Bennett, 1977) and other tissues such as epithelium (Loewenstein, 1975). The simplest interpretation of these results has been a high conductance pathway between cells. Quantifying junctional conductance and permeability relative to the plasma membrane has been performed on cardiac muscle and invertebrate septate axons (Weidmann, 1966; Weingart, 1974; Brink & Barr, 1977; Brink & Dewey, 1978) again yielding results consistent with an aqueous pathway. Further, the junctions appear to discriminate on the basis of molecular size and charge (Loewenstein, 1975; Flagg-Newton, Simpson & Loewenstein, 1979; Brink & Dewey, 1980).

If the junction has a water-filled channel which appears to discriminate on a basis of molecular size and charge (Brink & Dewey, 1978, 1980; Flagg-Newton et al., 1979), then solvent exchange (D_2O) should have predictable effects on junctional permeability as a function of temperature for a water soluble probe.

The present conceptual models for the structure of water indicate that water molecules form clusters of various sizes with lifetimes of 0.1 nsec, and the size of these clusters increases with decreasing temperature (Arnett & McKelvey, 1969). With cooling, the clustering of D_2O molecules around a solute is enhanced as compared to H_2O. Increased cluster sizes of D_2O and increased radii around a solute cause the conductivity of a D_2O solution to be reduced. For example, in solution D_2O decreased K^+ conductance by 20% at 20 °C. In a deuterated channel within a membrane bathed in D_2O one might predict a reduction in permeability for a solute molecule much like the reduction in solution if solvent clustering and solute hydration (solvent isotope effects) were greater in D_2O. A solute molecule might be expected to become impermeant at a warmer temperature in D_2O *vs.* H_2O because the hydration shell for the solute and the channel is significantly larger in D_2O than H_2O. Deuterium exchange for hydrogen (primary isotope effect) on channel proteins might also be expected to have an effect on channel function. Exchange of deuterium for hydrogen has the potential to alter tertiary or quaternary structures of protein (Schauf & Bullock, 1979). This effect might cause the functional diameter of a channel to be altered. Since the exchange will have little effect

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on clustering and hydration around a protein, changes in temperature dependence should be minimal when compared to H_2O. Thus permeability of a cell-to-cell channel may be increased or decreased by deuterium exchange, but the temperature dependence would be unaltered.

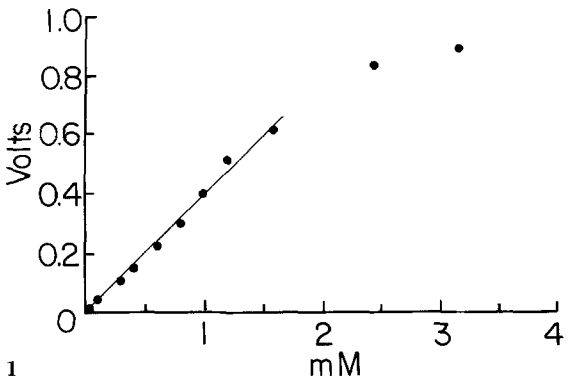
To test the effects of deuterium oxide on solute mobility through cell-to-cell channels, the fluorescent dye dichlorofluorescein was injected into earthworm median giant axons and the permeability of the septa to the dye was monitored at various temperatures in H_2O and D_2O saline. Action potential propagation across the septa was also monitored in D_2O and H_2O from 25 to 4 °C to assess electrical coupling across the junction.

The earthworm giant septate axon is an excellent system to study because it contains septa (Gunter, 1975) which lie perpendicular to the long axis of the constituent cells and the septa contain nexuses (Brink & Dewey, 1978; Kensler, Brink & Dewey, 1979). The nexal junctions are of the A-type (Kensler et al., 1979). This junction has been shown to have a high conductance with a linear *I–V* curve over a 20 mV range (Brink & Barr, 1977) and high permeability relative to the plasma membrane (Brink & Dewey, 1978).

Materials and Methods

Nerve cords were dissected (Brink & Barr, 1977) and bathed in H_2O saline or D_2O saline (Bio-Rad 99.8% pure). The pH of H_2O saline was 7.4, and the deuterium ion concentration (*pD*) of D_2O equaled the pH plus 0.4 (Wang & Copeland, 1973). The cords were incubated for at least 3 hr and as long as 24 hr in both salines before use. The saline was gently stirred during the incubation period. The earthworm giant axon has been described as a partially myelinated axon containing nodes 500 μm apart (Gunther, 1975). Thus the most limiting case for D_2O exchange would be exchange at the nodes. In myelinated fibers with 2-mm node spacing and an axon diameter of 20 μm, exchange of cytoplasmic H_2O with D_2O was calculated to occur within 15–30 min (Spyropoulos & Ezzy, 1959; Garby & Nordquist, 1955). For the earthworm fiber, exchange via nodes occurs with even greater speed because the nodes are closer together. Using a model with 500 μm spacing between nodes exchange is almost complete with 10 min of exposure. With more extensive exchange surface area, the time needed for equilibration is decreased.

Junctional permeability was followed by using a photomultiplier to monitor dye fluorescence across the septa and through the axoplasm as a function of time. A Farrand monochromator in conjunction with a photomultiplier was mounted on a Zeiss light microscope. The *x*-axis drive of the stage was driven by a 5 rpm motor (Synchron) and appropriate gearing (Mathis) to move the stage at a constant velocity of 100 μm/sec. The photomultiplier system monitored light through a slit with a width of 20 μm and length of 10 μm at 400 ×. Changes in fluorescence distribution were monitored along the long axis of the axon with this system. If flux of a molecule across the septa is to be calculated, then the relationship between the fluorescence of the molecule and the concentration must be established. Figure 1 illustrates the photomultiplier output for various concentrations of dichlorofluorescein. Glass tubes 100 μm wide were filled with the dye so that a direct comparison was made between axon and standards. Figure 1 first shows that over a wide range the photomultiplier output was linearly related to concentration. The fluorescence is quenched markedly at high concentration (2 mM).



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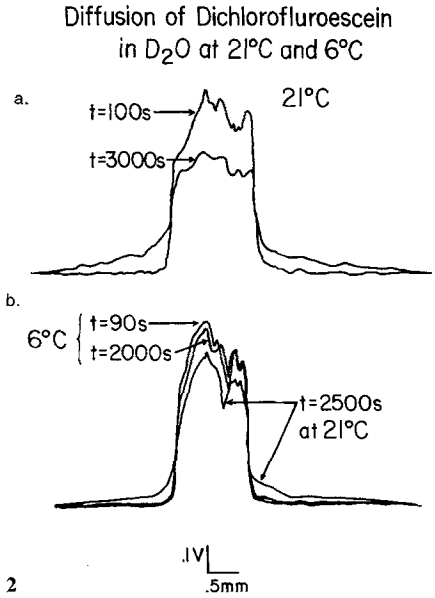

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Fig. 1. Photomultiplier output *vs.* dye concentration in 100 μm diameter pipettes is linear throughout a great deal of the concentration range. At concentrations greater than 1.8 mM, the relationship between photomultiplier output and concentration changes. The linearly between 0 and 1.8 mM allows direct conversion of photomultiplier output to concentration

Fig. 2. Fluorometric scans of axons injected with dichlorofluorescein in D_2O saline. The dye was iontophoresed into the axons and diffusion in the axoplasm and across the septa monitored. Diffusion at 21 °C in D_2O over a 2,900-sec interval is shown. (*b*) The lack of diffusion at 6 °C in D_2O saline for a 1,910-sec interval is illustrated. The preparation was warmed to 21 °C for an additional 2,500-sec and diffusion proceeded

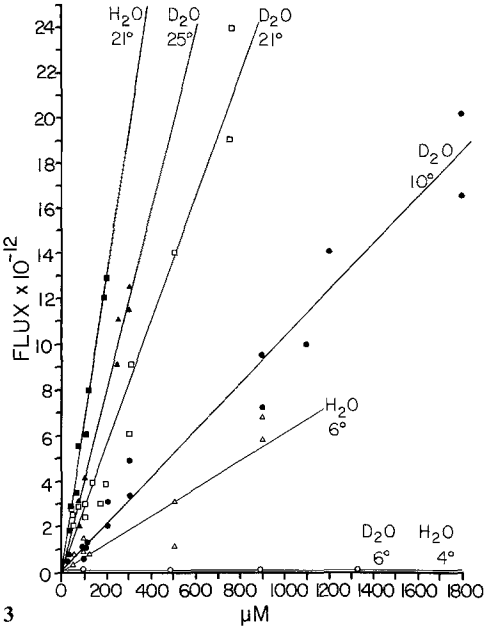
Dye injection was accomplished by iontophoresis of the dye into the axon (Brink & Barr, 1977). All injections were done at room temperature. Within 5 sec of termination of dye injection the nerve cord was immersed in a large saline water bath of appropriate temperature. The preparation was transferred to a temperature-controlled room containing the fluorometric apparatus. The preparation was scanned in the temperature room within 90 sec of dye injection termination. Temperature in the saline water bath and room was maintained to within 0.5 °C. Analysis of fluorometric scans was carried out in the same way as described by Brink and Dewey (1978, 1981). The area under the curves (Fig. 2) was calculated, and shifts in the area from one side of the septum to another represented the flux of that dye. The concentration difference across the septum was used to calculate flux and, subsequently, permeability. It was assumed that transnexal membrane potential was zero and that 4.5% of the septum was nexus (Brink & Dewey, 1978).

For propagation studies an axon was injected with carboxyfluorescein or dichlorofluorescein to visualize a septum. Microelectrodes were placed on either side of the septum where the distance between electrodes was 500 μm to 1.2 mm. Temperature was controlled by a Cambion bipolar temperature controller. A YSI Model 31 conductivity bridge was used to measure ionic conductivity of 100 mM KCl in H_2O and D_2O at pH = 7.0. The temperature of the conductivity cell was controlled by the Cambion temperature controller.

Results

Typical fluorometric scans of dichlorofluorescein diffusion at 21 °C in D_2O are shown in Fig. 2*a*. In Fig. 2*b* diffusion in D_2O was first monitored at 6 °C, and no diffusion occurred over a 2,000-sec period. Subsequent temperature rise to 21 °C for 2,500-sec showed the junction to be permanent. Thus at 6 °C the diffusion rate of dichlorofluorescein is below the resolution limit of the apparatus ($<1\times10^{-9}$ cm/sec). In H_2O saline temperature block was not achieved until 4 °C. The shift in area from one side of the septa to another with time is proportional to flux across the septa for the dye (Fig. 2), thus allowing the computation of flux and permeability for the septa (Brink & Dewey, 1978) by quantifying the area shifts. The calculated permeability was $P_n=3.8\times10^{-5}$ cm/sec and 3×10^{-5} cm/sec for the left and right septa, respectively, in Fig. 2*a*. In Fig. 2*b* at 6 °C, no value was calculable except after exposure to 21 °C $P_n=2.6\times10^{-5}$ cm/sec and 2.4×10^{-5} cm/sec for the two septa.

The relationship between flux and concentration gradient across the septa was proportional as Fig. 3 illustrates. The data points are given in con-



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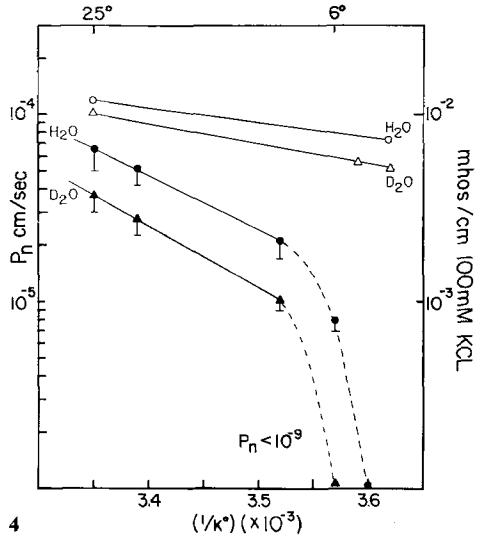

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Fig. 3. Flux of dichlorofluorescein *vs.* the concentration gradient across the septa. The flux of the dye at 21, 6 and 4 °C in H_2O saline is plotted. The flux concentration relationship is plotted at 25, 21, 10 and 6 °C in D_2O saline. The data was best fit by a straight line in all cases. Nonlinear functions were not capable of fitting the data as well as straight lines. The correlation coefficient ranged between 0.99 and 0.93

Fig. 4. An Arrhenius plot of junctional permeability in D_2O and H_2O. The standard deviations are shown with each mean. Each mean was determined from an η of at least 8 and as great as 12. Note the break point in permeability below 10 °C. This break represents the point at which the solute is too big and channel too small to permit diffusion. The ionic conductivity of 100 mM KCl (pH = 7.0) was measured in H_2O and D_2O at various temperatures as the graph illustrates. Temperature was controlled with a Cambion temperature control unit. The electrodes were platinum with a 1-cm spacing. The units of conductivity are mhos/cm

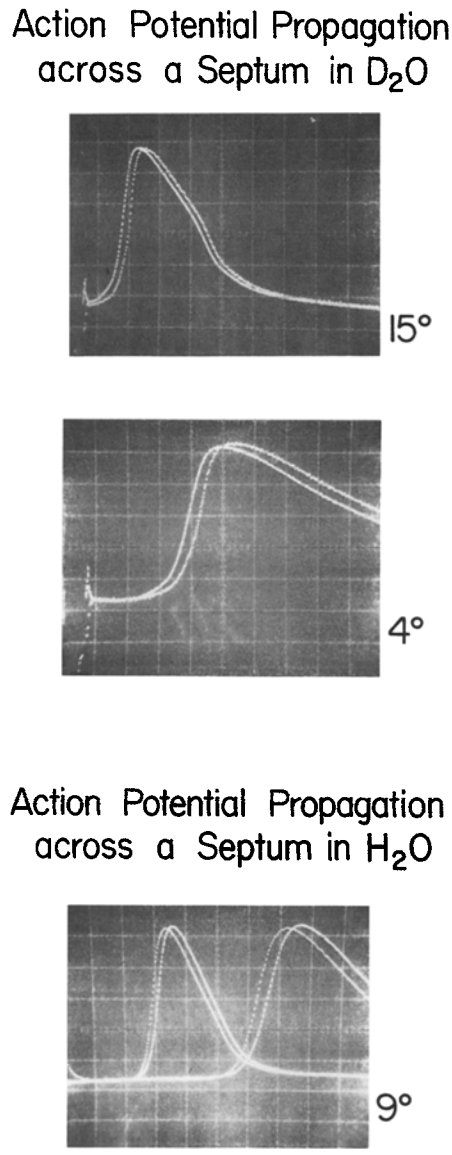

Fig. 5. Action potential propagation across a septum at 15 °C and 4 °C in D_2O saline is illustrated. The delay time was 200 μsec at 15 °C and 450 μsec at 4 °C. The distance between electrodes was 1.0 mm. Septum position was determined by dye injection. The velocity at 4 °C was 2.2 m/sec and 5 m/sec at 15 °C. The vertical calibration in both cases was 20 mV/cm and the sweep speed was 1.0 msec/cm. In the last panel action potential propagation was observed in H_2O with a 1.0-mm distance between electrodes. The temperature was 9 °C. The vertical calibration was 20 mV/cm and the sweep speed was 1.0 msec/cm and 0.5 msec/cm, respectively. The H_2O preparation was also able to propagation action potentials at 4 °C. Stimulation was accomplished by application of extracellular current pulses at 1.0 cm distance from the recording site

junction with the best fit line. A straight line gave the best fit with a correlation coefficient varying from 0.99 to 0.93. Curvilinear functions were not at any point able to fit the data as well as linear functions. At 25, 21 and 10 °C the flux concentration relationship was linear for both D_2O and H_2O, indicating a thermally dependent passive diffusion through the junction. The concentration gradient range varied over two orders of magnitude and no saturation phenomenon was observed. Additionally, the flux of dichlorofluorescein was blocked regardless of the concentration gradient at 6 °C in D_2O saline (Fig. 2*b*) while in H_2O saline a block was not forthcoming until 4 °C. The flux data for 21 and 10 °C in H_2O is not illustrated because much of the data overlaps with D_2O flux data at 25 and 21 °C. The flux *vs.* concentration relationship was also linear for 21 and 10 °C in H_2O. Each point in Fig. 3 represents a single measurement of flux across a septum. In all cases shown the flux was measured as the shift in area from one side of a septum to the other from the initial scan ($\leqq$90 sec) to another scan usually 600–1,200 sec later. In all cases the flux was followed further in time, and no change in permeability was observed in time for any one preparation (Brink & Dewey, 1978).

The log of junctional permeability for dichlorofluorescein was plotted against the inverse of temperature (1/K°) for both D_2O and H_2O data in Fig. 4. The permeability decreased with cooling in both solvents. In D_2O saline the permeability was suppressed and showed greater temperature dependence. At 25 °C the reduction was 42% while at 10 °C it was reduced 54%. The amplitude of the reduction of permeability is greater than that predicted for solvent isotope effects alone. One likely possibility for the extra reduction was deuterium exchange which had as its result an effective reduction in the nexal membrane channel diameter. The Q_{10} for the nexus permeability in D_2O and H_2O was 2.6 and 2.3, respectively, between 20 and 10 °C, thus indicating a greater temperature dependence in D_2O saline than H_2O. The Q_{10} given by Ramon and Zampighi (1980) for crayfish lateral axon septa was 3.0 in H_2O saline with slow cooling. These values are high relative to that predicted for a large aqueous channel and may indicate that the junctions are partially uncoupled as a result of increases in axoplasmic Ca^{++} with cooling (Loewenstein, 1981). Ionic conductivity of 100 mM KCl at pH 7.0 in H_2O and D_2O is also plotted in Fig. 4. The effects of D_2O on the conductivity of 100 mM KCl are explainable on the basis of solvent isotope effects (Hardy & Cottington, 1949; Heppolette & Robertson, 1960; Schauf & Bullock, 1979). At 25 °C the conductivity was reduced by 17% and at 5 °C the reduction was 30%. The units are given on the right vertical axis as mhos/cm. The Q_{10} values for ionic conductivity were 1.40

in D_2O and 1.27 in H_2O between 20 and 10 °C. The ratio of the H_2O and D_2O Q_{10}s was 0.90 and 0.88 for nexus permeability of dichlorofluorescein. The similarity of the ratio changes indicate that the dye is experiencing the same influences within a channel with cooling that strong electrolytes do in solution. If hydration and clustering were the same within a channel for both solvents, then the ratio would be 1.0. The hiatus in the declining slope of permeability *vs.* temperature below 10 °C indicates that some rate limiting process had effectively decreased nexal permeability to a nondiffusible state.

Action potential propagation was not eliminated by D_2O saline even at 4 °C as Fig. 5 illustrates. Deuterium oxide did cause a slowing of propagation and altered the shape of the action potential. A direct comparison can be made between the two top panels of Fig. 5 and the bottom one, a similar experiment except in H_2O at a temperature of 9 °C. The electrode separation is the same for both H_2O and D_2O. The delay time for propagation across the septum at 15 °C was 200 μsec, equalling a velocity of 5 m/sec, and at 4 °C the delay was 450 μsec and the calculated velocity was 2.2 m/sec in the D_2O while in H_2O at 9 °C the velocity was 8 m/sec. The Q_{10} equaled 2.2 for propagation across the septa in D_2O.

Discussion

Many of the differences in the physical properties between the two solvents such as hydrogen bonding length, dipole movement, and molecular dimensions are small (Arnett & McKelvey, 1969). Others are significantly different. They are heat capacity, viscosity, melting and boiling points (Nemethy & Scheraga, 1964; Schauf and Bullock, 1979). As has already been pointed out because there are similar properties, hydrogen atoms on a protein can easily exchange with deuterium atoms in D_2O, possibly altering its structure (primary isotope effect, Schauf & Bullock, 1979; Katz & Crespi, 1970).

Some of the more disparate features of D_2O are responsible for the solvent isotope effect (Schauf & Bullock, 1979). The viscosity of D_2O is significantly higher than that of H_2O (0.9 cP for H_2O *vs.* 1.1 cP for D_2O at 20 °C, Arnett & McKelvey, 1969). Changes in hydration radii and increased clustering and cluster size are thought to be plausible explanations for the increase in viscosity (Heppolette & Robertson, 1960). The difference in viscosity is an important reflection of the solvent isotope effect. In this case the temperature dependence of a process like nexus permeability would be greater in D_2O than H_2O if hydration played an important role in the process. Figure 4 illustrates an example of the solvent isotope effect.

Dichlorofluorescein is not a strong electrolyte like K^+. The lone carboxyl group has a pK of 3.0 (Brink & Dewey, 1980). Therefore the primary hydration shell might well be less developed than an ion like K^+ or absent along many regions of the molecule. But any region which is polar or charged will form hydration shells (Arnett & McKelvey, 1969). The reduced ionic conductivity of 100 mM KCl in D_2O is explained on the basis of viscosity and hence the behavior of the solvent. Weak acids also shift their pK values in D_2O. This shift is usually equal to or less than 0.8 pK units (Laughton & Robertson, 1969). A shift of this magnitude still leaves the majority of dye molecules in an ionized form at or about neutral pH or *pD*. Charge groups within the channel might also be expected to be affected. With a pK of 7.3, as Spray, Harris and Bennett (1982) suggest, the junction would be almost completely turned off at neutral *pD*. The junctional conductance went from maximum to minimum over 0.5 pH units in killifish and axolotl.

Gramicidin channels have been shown to decrease their conductivity 20% at 20 °C for K^+ ion in D_2O which has prompted Tregold and Jones (1979) to argue that the ion moves through a solvent-filled channel in a hydrated state and that the reduction was solely due to solvent viscosity. Changes in channel conductance at various temperatures were not reported. Also, Finkelstein and Rosenberg (1979) have estimated the gramacidin channel to contain six water molecules. However, the percent reduction of junctional membrane permeability to dichlorofluorescein in D_2O at any one temperature is greater than predicted if the solvent effect were alone in its influence. At 25 °C permeability was reduced 42%, whereas a reduction of 20% might be expected if solvency were solely responsible for a permeability reduction as seems to be the case for gramicidin channels (Tregold & Jones, 1979). The simplest explanation for the excessive reduction is structural change in the junctional membrane channels due presumably to deuterium-hydrogen exchange. Other possibilities are effects on gating mechanisms unseen in this preparation, but appropriate techniques have shown them to exist elsewhere (Spray, Harris and Bennett 1981). The magnitude of the temperature dependence in both solvents is also much greater than that predicted from simple solvent effects (Ramon & Zampighi, 1980). This might reflect a junctional

barrier whose mechanisms are as yet not understood (Ramon & Zampighi, 1980).

Another approach may also explain the results. Lauger (1979) has elucidated a channel model as a series of binding sites, each of which represents a potential energy minima for a solute molecule. The temperature dependence of the binding sites would greatly influence the temperature coefficients for channel permeability. A comparison of temperature dependence of various enzyme substrate reactions shows greatly varied values of Q_{10}. Two examples are lactate dehydrogenase with a Q_{10} of 1.8 at high concentrations of substrate and pyruvate kinase which shows a Q_{10} of 2.7. With lower substrate concentrations lower Q_{10} values are attained, well below 2.0 (Hochachka & Somero, 1973). The temperature range was 5–15 °C. If it is assumed that deuterium has little effect on the substrate binding site affinity, then Q_{10} difference in D_2O and H_2O should be minimal or effected only by solvent isotope effects in the media surrounding the site. Deuterium substitution for hydrogen that alters the binding site-substrate affinity may reduce or enhance that process. An example of affinity enhancement is the STX disassociation constant for the STX binding site on Na^+ channels. In H_2O it is 1.2 nM while in D_2O it is 0.67 nM (Hahin & Strichartz, 1981), a 50% reduction. Comparing the rate constants for STX binding Hahin and Strichartz (1981) found that K_1 ($M^{-1}sec^{-1}$) was unchanged in H_2O and D_2O. But K_2 (sec^{-1}) was halved by exposure to D_2O. Thus the ratio of $K_{2H_2O}/K_{2D_2O}=2.0$. The rate constants are defined as $K_1=1/\tau_{on}-1/\tau_{off})/[STX]$ and $K_2=1/\tau_{off}$. The time constants (τ_{on} and τ_{off}) are measures of the reduction and subsequent recovery of I_{Na} in the presence of STX in both H_2O and D_2O saline. Another example of deuterium oxide effects on rate constants is given by Klinman (1976) for yeast alcohol dehydrogenase. The ratio of K_{H_2O}/K_{2D_2O} (K_{cat}, sec^{-1}) for aldehyde substrate is 0.5 while alcohol gives a value of 1.2. In fact, D_2O has varied effects on rate constants for many chemical reactions (Klinman, 1976; Schowen, 1976) making it difficult to predict the D_2O effect on the kinetics of a channel model.

An anion in a channel which interacts with binding sites and in between is subject to solvent isotope properties (solvation) could display the effects seen here, that is a larger reduction in junctional membrane permeability at a given temperature than predicted by solvent effects but with a temperature dependence superimposed on the excessive reduction. The increased reduction may represent a more stable binding conformation for substrate (dye) and site brought about by the higher bond energy of the deuterium atom (0.24 kcal/mole at 25°) which in effect slows the rate of transit through the junction. If it is assumed that the permeability is a measure or proportional to the hypothesized binding site affinity, then activation energy can be calculated (Hochachka & Somero, 1973). The activation energy difference between D_2O and H_2O is $\simeq 1$ kcal/mole, indicating about four deuterium bonds. Whether this represents four binding sites or one with four deuterium bonds is unknown. It is impossible to distinguish between multiple sites or a single one.

The 42% reduction in permeability in D_2O at 25 °C could be caused by a structural alteration in the channel, making its diameter smaller, or a deuterium effect on a binding site. A third possibility consistent with the structural alteration hypothesis is increased hydration shells effecting diffusion through the channel due to interaction of the solute and channel walls which begins to approximate the substrate-binding site mechanism where the solvation shells (or partial shells) of the substrate and channel interact in a fashion similar to solvent-solvent interaction but within the confines of the channel (hydrogen or deuterium bonds).

The temperature block of dichlorofluorescein is hard to reconcile in terms of binding site-substrate interaction. There are examples of kinetic binding affinity decreases by almost an order of magnitude with acute temperature change (Prosser, 1973; Hochachka & Somero, 1973), but the decreased affinity is at some temperature other than the acclimation temperature of an animal. Here the animals are acclimated to 5 °C.

The effect of temperature on channels which allow single file motion of ions might be expected to show Q_{10} values similar to bulk solution if one assumes that the groups making up the wall (oxygen) act much like the oxygen of water. Thus for a channel where this kind of interaction occurs, little or no change in temperature dependence in D_2O would result because no D_2O molecules could act as a hydration shell within the channel. Lithium conductance in gramacidin channels in H_2O *vs.* D_2O (Tregold & Jones, 1979) might well be explained with the previous scenario. The lack of hydration shells within channels and around solutes would result in a loss of solvent isotope effects.

The release of Ca^{++} into the axoplasm with cooling is also an explanation (Loewenstein, 1981). However, the total axoplasmic Ca^{++} (Brinley, 1980) within an axon may be below the Ca^{++} threshold for uncoupling which is $10^{-7}-10^{-6}$ M

(Loewenstein, 1981). The high Q_{10} values may also mean that the dye is being restricted from more and more channels with cooling due to size. The smaller channels become more impermeant to the probe at warmer temperatures than the large channels due to hydration. Increasing acidity also causes uncoupling (Turin & Warner, 1977). The value of intracellular pH necessary to affect junctional conductance is 6.0 (Turin & Warner, 1977). The worms used in this study were cold adapted (5 °C). It seems unlikely that their acclimation state is one where junctional membranes are partially or totally uncoupled.

The magnitude of the temperature dependence in both solvents is large, but the ratio of Q_{10}s has a value other than unity, indicating that solvent effects are acting in conjunction with other phenomenon. However, from this data it seems that dichlorofluorescein diffuses through a deuterated junctional membrane in a hydrated (deuterated) form (Heppolette & Robertson, 1960; Laughton & Robertson, 1969; Schauf & Bullock, 1979).

Figure 6 summarizes heavy water effects on nexal membrane permeability. The percent reduction of nexal permeability in D_2O *vs.* temperature as compared to the percent reduction of ionic conductivity in D_2O for KCl is plotted. The reduction of gramicidin channel conductance (Tregold & Jones, 1979) at 20 °C in D_2O is also plotted as is G_{Na} for *Myxicola* (Schauf & Bullock, 1979). The dashed line represents the predicted function if hydrogen atom-deuterium atom exchange were the only phenomenon present. The exchange would not alter the temperature dependence of the process (Schauf & Bullock, 1979).

The percent reduction of nexal membrane permeability is much too great to be explained by solvent effects alone. Figure 6 illustrates that both primary isotope effects and solvent effects are present. The rate of reduction of permeability is greater for the nexus than the reduction of ionic conductivity for KCl in D_2O. This may be a reflection of the influence of hydration along the nexal membrane channel wall. The data indicates that the molecule dichlorofluorescein moves through the nexus in a hydrated form. The differential temperature block at 6 °C in D_2O and 4 °C in H_2O is also consistent with the molecules diffusing through the junction in a hydrated form. The hydration shell within the nexal channel and around the molecule finally yield a case where the effective molecular size is greater than the hydrated channel size.

It is worth noting again that in Fig. 3 in all cases the flux was linearly proportional to the concentration gradient. The concentration only covers two orders of magnitude, but over that range it does not illustrate any saturation of flux, as might be expected if a carrier were responsible for transport across the nexus. Deuterium might also be expected to completely shut down a carrier because of the potential for exchange and hence structural alteration, but D_2O simply slowed diffusion.

The dimensions of dichlorofluorescein are such that it is almost planar and between 1.0–1.1 nm in diameter in the unhydrated state. Assuming hydration is of critical consequence as this data suggests, the functional size of the channel could be quite large as the data of Schwartzmann et al. (1981) shows (1.6–2.0 nm). In the aforementioned

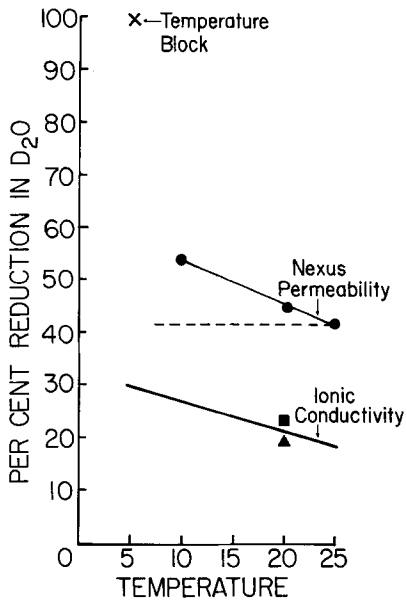

Fig. 6. The percent reduction of a process, in this case ionic conductivity of 100 mM KCl and nexus permeability to dichlorofluorescein in D_2O, is plotted *vs.* temperature (solid line). ▲ represents the reduction of gamicidin channels in D_2O at 20 °C (Tredgold & Jones, 1979) and ■ represents $\bar{G}_{Na}$ in *Myxicola* axon membranes in D_2O (Schauf & Bullock, 1979). Over a 20 °C range, ionic conductivity is reduced from 15 to 30% in D_2O, indicating an increase in viscosity and thus presumably an increase in hydration radii around solute ions over that experienced in H_2O. Nexus permeability is affected similarly, showing increased reduction in permeation from 42% at 25 °C to 54% at 10 °C. The percent reduction at any one temperature is greater than predictable from solvent effects alone. The increase of 42% at 20 °C is 22% greater than predicted by solvent effects. This extra increase is most likely due to hydrogen/deuterium exchange. Deuterium oxide affects the nexus decreasing its permeability through hydrogen/deuterium exchange but also allows anions to pass in a hydrated form. The dashed line represents the result of a primary isotope effect acting alone. It would add a constant onto the temperature dependence of a process. *X* marks the point of permeation block for dichlorofluorescein in the nexus

study neutral probes were used, many of which would not be expected to be heavily hydrated.

In summary, the data indicates that there is a hydrogen-deuterium exchange which lowers junctional permeability. Whether this effect creates a smaller channel or influences binding sites within the channels is unknown. However, the dye molecules transverse the junction in a hydrated state. This is shown by the greater temperature dependence of the junctional membrane channel permeability in D_2O saline. The block of dye diffusion across the septa while still allowing action potential propagation indicates the junction is functioning at 4 °C, and it is size of the solute which has made it impermeant at 6 °C. The Q_{10} for the propagation process was less than the values for dye permeation in H_2O and D_2O. The data is consistent with the model of an aqueous-filled channel.

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