STUDIES ON THE PHYSICAL STATE OF WATER IN LIVING CELLS AND MODEL SYSTEMS. XI. THE EQUILIBRIUM DISTRIBUTION COEFFICIENTS OF PENTOSES IN MUSCLE CELL WATER: THEIR DEPENDENCE PRIMARILY ON THE MOLECULAR WEIGHTS OF THE PENTOSES AND LESSER DEPENDENCE ON THEIR STEREOSPECIFICITY

G. N. LING and M. M. OCHSENFELD
Department of Molecular Biology, Pennsylvania Hospital, Eighth and Spruce Streets, Philadelphia, PA. 19107.

● *Studies of the metabolism of four pentoses (D-, and L-arabinose; D-, and L-xylose) in frog muscle at 0° C revealed that all are metabolized at extremely slow rates. As a result, the metabolic degradation of these pentoses does not significantly affect their equilibrium distribution in muscle cells at this temperature at least. Of the four stereoisomers, three (L-xylose, D-arabinose, L-arabinose) were found exclusively or almost exclusively in the cell water, demonstrating a rectilinear distribution pattern; the equilibrium distribution coefficients (q-values) obtained from the slopes of these rectilinear distribution curves are 0.256, 0.274. and 0.271 respectively. The fourth pentose, D-xylose, is apparently partially adsorbed. With the aid of the equation for solute distribution according to the association-induction (AI) hypothesis, the data for this sugar can also be fitted with a theoretical curve calculated on the basis of a q-value close to those for the other three pentoses. The close similarity of the q-values of pentoses which are sterically different, but have identical molecular weights, provides further confirmation of the "size rule': a prediction of the polarized multilayer (PM) theory of cell water, according to which, the q-values are as a rule primarily determined by the molecular weights of the solute and to a lesser extent dependent on its stereospecificity.*

Many solutes are found in living cells at concentrations lower than in their surrounding medium. Na^+ and non-metabolized sugars are well known examples. A large number of them demonstrate a straight line equilibrium distribution pattern, i.e., if the equilibrium concentration of the solute in the cell water is represented as $[S]_{in}$ and that of the external solution, $[S]_{ex}$, a plot of $[S]_{in}$ (as ordinate) against $[S]_{ex}$ (as abscissa) produces a straight line with a slope of less than one (Troshin, 1966; Ling, 1984). In a preceding paper (Ling, 1988), it has been shown how this rectilinear distribution of solutes with below-unity slopes cannot be explained by the membrane pump theory but is in full accord with the Sorption Theory of Troshin (1966) and the association-induction hypothesis of Ling (1984).

Troshin, in his "Sorption Theory", attributed the phenomenon of partial solute exclusion from living cells to the existence of living cells as colloidal "coacervates" with reduced solubility for these solutes. In support Troshin demonstrated similar solute exclusion in non-living "coacervate" models (Troshin, 1966). The Sorption Theory did not explain (i) how, in physical terms, is water in coacervates, living or nonliving, different from normal liquid water, nor (ii) why water in living cells should have reduced solubility for some solutes (e.g., sucrose) (Troshin, 1966, p. 115) but normal solubility for others (e.g., urea) (Hill, 1930).

The polarized multilayer (PM) theory of cell water was published in 1965 as a subsidiary theory of the broader theory of the living

cell, the association-induction hypothesis (Ling, 1962, 1965). While in agreement with Troshin's recognition of the unusual nature of cell water and its great significance in cell physiology, the PM theory has been concerned from its very inception with the physical mechanisms that underlie the unusual behaviors of cell water. Thus according to the PM theory, the bulk of water in living cells and in cogent model systems differs from normal liquid water because this water exists in the state of polarized multilayers, in consequence of direct and indirect interaction with the exposed NH and CO groups of the fully-extended polypeptide chains pervasively present in all cells and anchored directly or indirectly to a cytoskeletal framework. Water in the state of polarized multilayers has reduced solubility only for large and complex molecules (e.g., sucrose) that do not fit into the dynamic structure of polarized multilayers, but has normal or even above-normal solubility for solutes like urea and ethylene glycol that are small and/or fit sterically into the dynamic water structure. This dependency of the degree of exclusion on the molecular size of the solute is called the "size rule".

In the present communication, we describe results of experimental study of the equilibrium distribution in frog muscle of four pentoses with different steric structures but exactly the same molecular weight. Our purpose was to test if the four all distribute themselves according to the size rule and rectilinearly with approximately the same slope, which equals the equilibrium distribution coefficient or q-value. However, before this can be achieved, we must demonstrate that none of these four pentoses is degraded by isolated frog muscles at a significantly high rate. Were it otherwise, the slopes of the distribution curves will not yield the correct q-values but values lower.

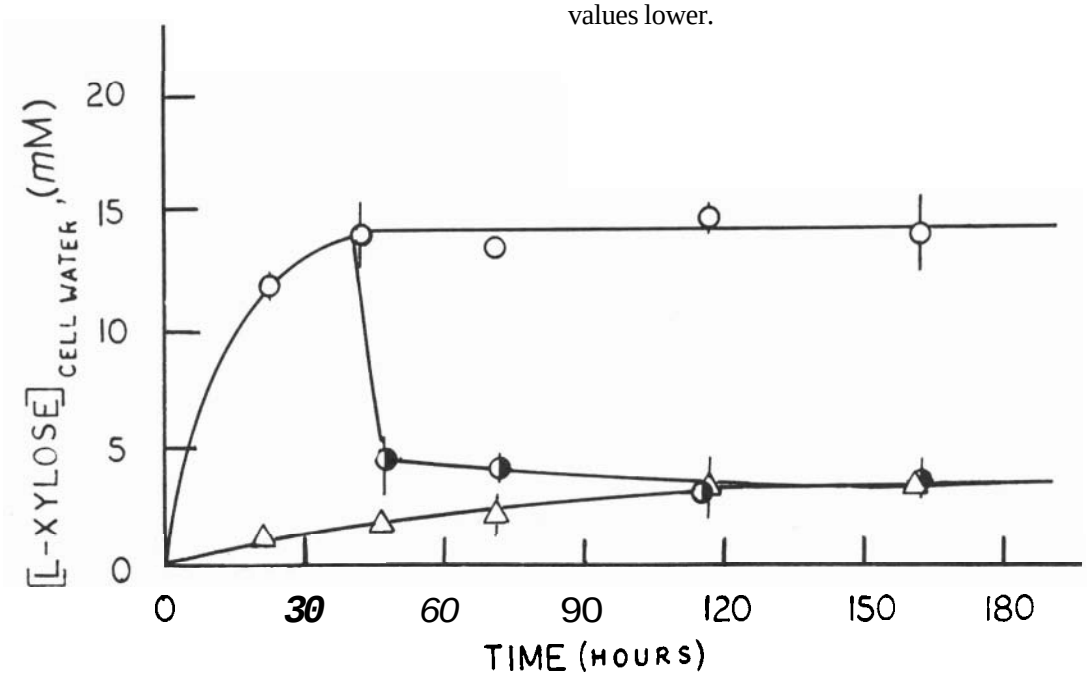

FIGURE 1. Time Course of L-xylose uptake by frog sartorius muscles (O°C). Sartorius muscles were incubated in Ringer solutions containing either 60 mM (O) or 10 mM (A) Lxylose. After 41 hours, one half of the muscles remaining in the 60 mM Lxylose solution were transferred to a fresh Ringer's solution containing 10 mM L-xylose (O). Four muscles were removed at times indicated on the abscissa. The ordinate represents the L-xylose concentration in the cell water after correction for extracellular space fluid. The standard error of the means is represented by the distance between the horizontal bars when its magnitude exceeds the size of the symbol.

MATERIALS AND METHODS

Sartorius muscles were isolated from North American leopard frogs (Rana pipiens) and incubated in a Ringer's solution containing varying concentrations of four pentoses. The pentose studied were all obtained from Sigma Chemical Co., St. Louis, Mo.: D-arabinose, Lot 116F-0609; L-arabinose, Lot 96F-0466; D-xylose, Lot 76F-0193; L-xylose, Lot 124F-0337.

The basic incubation medium contained 115 mM Na^+, 2.5 mM K^+, 1.2 mM Mg*, 0.71 mM Ca^{++}, 107 mM Cl, 2.0 mM $H_2PO_4^-$, 1.2 mM HPO_4^-, 1.2 mM SO_4^-, 6.64 mM HCO_3^-. When the concentration of pentose in the environment was varied, an osmotically equivalent amount of NaCl was removed.

Incubation was at O°C ± 0.5° C with shaking. Following incubation for a period of six days, extracellular space fluid was removed from the muscles using the centrifugation procedure described by Ling and Walton (1975). The muscle was then frozen in liquid nitrogen and broken into two parts.

Water content was determined on one part of each tissue sample by drying 24 hours at 101° C; the pentose concentration of the other part of the tissue sample was measured colorimetrically by standard procedures (modification of method of Roe and Rice, 1948) after extraction of the muscle tissue in 5% trichloroacetic (TCA). Incubation medium was also diluted with TCA. Background blanks, obtained by incubation of muscles in pentose-free solutions, were also determined.

To determine the rate of metabolism of the pentoses, paired small frog muscles (sartorius, semitendinosus, tibialis anticus longus, ileofibularis) were isolated and sorted into two groups, each group containing one each of the diffrerent types of muscles. Both groups of muscles were chilled in 15 cm long test tubes. To each tube was then added 0.5 ml of cold Ringer solution (of composition described above), containing 30 mM of one of the 4 pentoses. To one group, called the initial group, was then added rapidly 10 ml of distilled water and promptly heated in a boiling water bath for 20 minutes. A teflon marble was placed on the open end of each test tube to prevent evaporation and loss of water from the tube.

After cooling, to the tube content was then added 2 ml of 0.3 N $Ba(OH)_2$ and also 2 ml of a solution of $ZnSO_4$. The $ZnSO_4$ solution was of such a predetermined concentration that it will cause neither more nor less than the total precipitation of all the Ba^{++} in the solution. After thorough mixing, the solution was filtered and the collected clear supernatent solution assayed for its pentose content according to the method of Roe and Rice (1948). The second group of muscles in the 0.5 ml pentose Ringer solution (the final group) was incubated in a 0° C bath for 6 or 7 days with gentle shaking. At the end of this incubation, the tube contents were treated in exactly the same way as the initial groups and their pentose contents assayed.

RESULTS

At O°C, all 4 pentoses under study were metabolized by frog muscles at extremely slow rates as shown in Table I. These findings are in agreement with the knowledge that these pentoses are not metabolized (by monogastric mammals, possessing one-compartment stomach as in humans, in contrast to ruminants) (Pigman, 1957, p. 795 and 796) and with our own prior finding that at this low temperature even D-glucose (the main food of many living cell types including the frog tissues) is not metabolized by frog muscles (Ling et al., 1969).

Figure 1 shows that L-xylose reaches (reversible) equilibrium in frog muscle after about 40 hours of incubation at O°C at an initial L-xylose concentration of 60 mM. At an initial L-xylose concentration of 10 mM, it took much longer. This low permeability of

frog muscle to L-xylose stands in contrast to the much higher permeability to D-xylose, which reaches diffusion equilibrium in less than 18 hours at the same temperature (Ling unpublished, see also Ling et al., 1973, p. 23). To insure attainment of equilibrium distribution for all four pentoses, they were routinely incubated for six days.

Figure 1 also demonstrates the much more rapid efflux of Lxylose from the muscles after they were first incubated in a Ringer solution containing 60 mM L-xylose for 41 hours and 50 minutes and then transferred to another Ringer solution containing 10 mM L-xylose. The L-xylose concentration in the muscles rapidly fell from 14 mM to 4.5 mM in only 5 hours.

Figure 2 and Table II present the equilibrium distribution data of the four pentoses, D- and L-arabinose, and D- and L-xylose in frog muscle at O°C after six or seven days of incubation. All four sets of data can be fitted by straight lines with linear correlation coefficients above +0.9. The slopes of the D-

TABLE I. The Rate of Consumption of Pentoses by Isolated Frog Muscles at O°C. Paired small frog muscles were isolated and separated into two groups, each group, weighing about 0.5 g., contains one muscle of each type of a total of 4 types (sartorius, tibialis anticus longus, semitendinosus, ileofibularis). Initial and Final group refers to each of the two groups. Total volume (of water) represents the sum of 80% of the muscle fresh weights and 0.5 ml of pentose-containing Ringer solution, in which the muscles were incubated. The concentrations given are represented as millimoles per liter of the total volume of water. Consumption rates, however, were calculated on the basis of one gram of fresh muscle weight. Four sets of means ± standard deviations were given under Consumption Rates.

Sugar	Duration (hours)	Muscle weight (g.)	Initial total vol. (ml)	Initial concentration (mM)	Final total vol. (ml)	Final concentration (mM)	Consumption Rate (μmoles/g./hr.)
D-arabinose	144	0.535	0.922	16.93	0.878	17.62	-0.00182
	144	0.516	0.909	16.87	0.906	17.48	-0.00686
	159	0.528	0.918	16.50	0.914	16.0	0.00630
	144	0.555	0.872	17.20	0.880	17.0	0.00050
							0.00387 ± 0.00275
L-arabinose	144	0.493	0.890	17.33	0.884	16.65	0.00985
	144	0.520	0.912	16.55	0.906	16.60	-0.00067
	159	0.529	0.919	16.60	0.919	16.60	0.00000
	144	0.522	0.904	16.85	0.914	15.91	0.00918
							0.00757 ± 0.00418
D-xylose	144	0.481	0.880	16.96	0.878	16.48	0.00649
	144	0.471	0.873	17.17	0.904	15.66	0.01220
	159	0.497	0.894	17.50	0.899	16.10	0.01490
	144	0.504	0.882	17.00	0.889	16.10	0.00950
							0.01080 ± 0.00310
L-xylose	144	0.481	0.881	16.35	0.886	15.35	0.01150
	144	0.476	0.877	16.62	0.882	15.94	0.00758
	159	0.470	0.872	17.81	0.872	17.20	0.00769
	144	0.465	0.873	18.08	0.887	17.00	0.01050
							0.00917 ± 0.00187

arabinose, L-arabinose and L-xylose curves are close to each other and respectively **0.274, 0.271** and **0.256.** The slope of the D-xylose curve, on the other hand, is distinctly higher than the others and is roughly **0.4.**

DISCUSSION

At a steady state, the rate of influx of a solute S into a fixed population of muscle cells must equal the sum of the rate of efflux of S and that of its metabolic degradation. Under this condition, one can write

$$k_1 [S]_{ex} = k_2 [S]_{in} + k_3 [S]_{in} = (k_1 + k_3) [S]_{in}, \quad (1)$$

where k_1 and k_2 are the inward and outward permeation rate constants for the solute S and are given in units of μmoles per hour per one millimolar concentration gradient. k_3 is the rate constant for the rate of metabolic degradation in the same unit.

In case where k_3 is equal to zero (i.e. S is not metabolized at all),

$$k_1 [S]_{ex} = k_2 [S]_{in} . \quad (2)$$

Under this condition, the system has entered into an equilibrium state and accordingly,

$$[S]_{in} / [S]_{ex} = k_1 / k_2 = q . \quad (3)$$

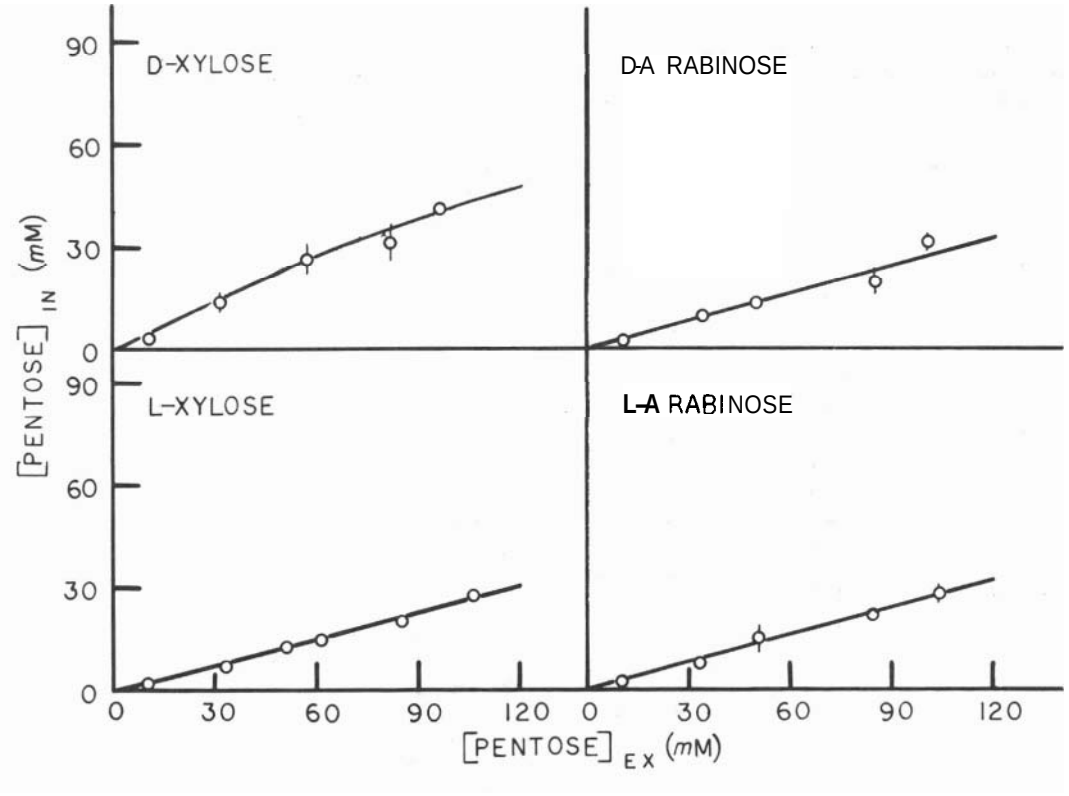

FIGURE 2. The Equilibrium Distribution of four pentoses in the water of frog sartorius muscle as described in the text. The ordinate represents the pentose concentration in the cell water after corrections for the extracellular space fluid. The standard error of the means is represented by the distance between the horizontal bars when the value exceeds the diameter of the circle. Points are experimentally determined. For D-, L-arabinose and L-xylose the straight lines are drawn from the data points by the method of least squares; for D-xylose, the curved line is theoretically calculated on the basis of Equation 6.

In the case where the solute S is partially excluded from the cell water (i.e. $q < 1$), clearly $k_1 < k_2$. However, if the solute is being metabolized at a significant rate, then at this steady state,

$$[S]_{in} / [S]_{ex} = k_1 / (k_2 + k_3) = q'. \quad (4)$$

q', the nonequilibrium q value (not to be confused with the apparent equilibrium coefficient, or p-value) is lower than the true q-value. Therefore, if we are trying to find out about the physical state of cell water through the determination of the q-value of a suitable probe molecule, clearly one must be certain that the probe is not being metabolized at an appreciable rate. In other words, k_2 must be very much larger than k_3.

In the present study, we have made no elaborate efforts to determine k_2 with a high degree of precision, since that would not be necessary. Here a rough estimate of k_2 would be quite sufficient. Such a rough estimate of k_2 is already on hand.

Of the four pentoses, time course studies like those shown in Figure 1 have provided an approximate estimate of k_2 for the least permeant pentose among the four studied, L-xylose. From the data given in Figure 1, we know that it took about 5 hours for the intracellular L-xylose concentration to fall from the high level of 14 mM to 4.5 mM, eventually levelling off in the new medium at about 3.4 mM. The half time of efflux of L-xylose ($t_{1/2}$) is estimated at 2 hours. From this value, one can readily determine k_2:

TABLE II. The fifth column represents the q-values determined by the method of least squares except D-xylose in which case the volume 0.423 is truly a p-value (see test and legend of Figure 3). r of the sixth column is the linear correlation coefficient between $[S]_{ex}$ and $[S]_{in}$. Number in parentheses represents the total number of sets of values in the calculation of r.

	$[S]_{ex}$ (mM)	$[S]_{cell}$ (μM/gm)	$[S]_{in}$ (mM)	q	r
D-Arabinose	10.8	1.79 ± 0.45	2.32 ± 0.58		
	34.1	7.32 ± 0.91	9.39 ± 1.17		
	49.5	10.10 ± 1.14	13.42 ± 1.52	0.274	+0.928(23)
	85.1	14.82 ± 3.16	19.45 ± 4.16		
	101.2	24.01 ± 1.86	31.60 ± 2.45		
L-Arabinose	10.3	1.62 ± 0.41	2.09 ± 0.54		
	33.1	6.10 ± 0.94	7.80 ± 1.22		
	50.8	11.24 ± 2.98	14.88 ± 3.92	0.271	+0.942(23)
	84.4	16.60 ± 1.11	21.80 ± 1.49		
	104.1	21.51 ± 1.82	28.16 ± 2.38		
D-Xylose	11.2	1.15 ± 0.55	3.09 ± 0.71		
	31.7	10.37 ± 2.12	13.47 ± 2.68		
	56.9	20.45 ± 3.50	26.59 ± 4.53	(0.423)	+0.932(24)
	81.5	23.81 ± 4.02	30.98 ± 5.20		
	96.6	32.20 ± 1.68	41.68 ± 2.17		
L-Xylose	11.3	1.38 ± 0.066	1.81 ± 0.087		
	33.7	5.08 ± 0.63	6.57 ± 0.82		
	56.0	9.35 ± 0.88	12.25 ± 1.15	0.256	+0.970(40)
	61.0	10.83 ± 0.41	14.52 ± 0.519		
	84.7	15.22 ± 1.10	19.79 ± 1.43		
	106.5	21.04 ± 0.92	27.65 ± 1.21		

Equilibrium Distribution of Pentoses in Frog Muscle

$$k_2 = \ln 2/\ 2 = 0.694\ /\ 2 = 0.347\ hr^{-1}$$

DISCUSSION

or more explicitly, k_2 = 0.347 pmoles / g / hr. / millimolar gradient. With this information on hand, we only need to estimate $[S]_{in}$ and obtain k_3 from the data given in the last column of Table I to make a comparison of the values of k_2 and k_3.*

As explained in the legend of Table I, the initial and final concentrations are in units of the total water within the system, i.e. the sum of the water in the muscle cells, which equals 80% of the muscle weights, and the volume of the Ringer solution added (0.5 ml). Since the average final L-xylose concentration was 16.37 mM, the average q value measured, 0.267 and the muscle weights were about 0.5 g., the intracellular pentose concentration, $[S]_{in}$ is then

$$\frac{16.37\times(0.5+0.5\times0.8)\times0.267}{0.5+0.5\times0.8\times0.267} = 6.48\ mM,$$

and $k_2\,[S]_{in}$ is equal to 0.347 X 6.48 = 2.25 pmoles / g / hr. Therefore,

$$k_3\,[S]_{in}\ /\ k_2\,[S]_{in} = 0.00917/2.25 = 0.4\%.$$

*The time course studies described in Figure 1 were carried out exclusively on thin and flat sartorius muscles, whereas the metabolic studies described on Table I were on sartorius muscles in addition to other cylindrical and thicker muscles (see p. 4). Since the time for an external pentose to travel through the interstices of the thicker muscles takes a longer time than through the thinner sartorius muscles, one might wonder if a judicious comparison had been made between k_2 and k_3 determined. This concern is, however, of no significance due to the *incomparably slower* rate of metabolism of pentoses by the cells with t equal to many days when compared to the time needed by pentoses to penetrate the interstices of the thicker muscles with t equal to only 2 or 3 hours (see Ling and Kromash, 1967).

Since L-xylose not only has the lowest permeability rate among the four pentoses, it has also, relatively speaking, a fairly high rate of metabolism, the fact that k_3 is only 0.4% of that of k_2 leaves no doubt that metabolism of all four pentoses is so slow that k_3 can be considered to be entirely trivial. The ratio of $[S]_{in}$ over $[S]_{\infty}$ in fact represent the true q-value of the probe pentose under study.

According to the AI hypothesis, a battery of stereoisomers like the four pentoses studied offers a useful set of tools to probe the physical state of water in living cells. According to theory, these stereoisomers with identical molecular weights should all distribute in the water of the same type of cells with similar q-values. If adsorption on a specific type of site should occur, one would expect, from the stringent requirements of stereospecificity in adsorption, it would be limited to a specific isomer; certainly not all the stereoisomers can be adsorbed on the same sites and to the same degree. The data presented in Figure 2 and Table II are in full agreement with this anticipation. Apparently D-xylose alone is adsorbed to a significant degree. The three other pentoses, L-xylose, D-arabinose, and L-arabinose appear to be found exclusively in the cell water exhibiting closely similar q-values. We shall next review additional work that supports the conclusion that D-xylose in the cells is partially adsorbed.

As mentioned above, at O°C, frog muscle does not metabolize even D-glucose which is the main food source for frog muscle (Ling et al., 1969). After extensive washing at 25°C in an insulin-free preincubation Ringer's solution, labeled D-glucose distributes itself rectilinearly in a subsequent incubation at O°C. However, if the preincubation solution contains insulin, then to the rectilinear fraction of labeled D-glucose is added an adsorbed fraction. At equilibrium, the total labeled D-glucose concentration in the cell then obeys the following equation:

$$[S^*]_{cell} = \alpha q_s [S^*]_{ex} + \frac{[f_s]\,\tilde{K}\,[S^*]_{ex}}{1 + \tilde{K}\,[S^*]_{ex} + K\,[S]_{ex}}, \quad (6)$$

where α is the percentage of water in the cells. q, is the equilibrium distribution coefficient of labelled D-glucose in the cell water. $[S]_{ex}$ is the concentration of non-labelled D-glucose in the external medium. $[S]_{cell}$ and $[S^*]_{ex}$ are the equilibrium concentrations of the labeled sugar in the cell (in μmoles/gram of fresh cells) and in the external medium (in molarity) respectively. $\tilde{K}$ and K are the adsorption constants for labelled and non-labelled D-glucose. $\tilde{K}$ and K are essentially equal (Ling and Will, 1969; Ling, 1984, p. 368). However, the effectiveness of insulin in promoting the D-glucose adsorption also requires the presence in the ***preincubation*** solution of either D-glucose or some other structurally similar sugars or derivatives called **"primers"**. Indeed, of the 40 compounds tested, only 7 were found to be effective primers: D-glucose, 2-deoxy-D-glucose, 3-0-methyl-D-glucose, D-galactose, D-mannose, D-fructose, and D-xylose. Other sugars including L-xylose, D-arabinose, and L-arabinose are essentially inactive, although a marginal activity could be detected in L-arabinose (Ling and Will, 1976).

The phenomenon of priming was explained as follows: insulin acts only at the higher temperature as a "cardinal adsorbent" initiating a propagated unmasking of D-glucose adsorption sites. This propagated unmasking occurs only when D-glucose or another primer is present with insulin at the higher temperature (26° C) in the preincubation solution. The primer can then occupy the sugar binding site next to the insulin binding cardinal site and in so doing allow the sequential opening up to more primer molecules of a chain of sites made potentially available by insulin binding (see Ling, 1984, Figure 11.14). Sites so unmasked and held "open" by the primer molecules can accommodate in a subsequent incubation at 0° C, labeled D-glucose. Indeed the labelled D-glucose merely ***exchanges*** for the adsorbed primer molecules, a process requiring kinetic energy not beyond that available at O°C. Careul comparison of the structural requirements for an effective primer indicates that virtually all the OH, H, and CH_2OH of D-glucose play some role of different significance. The most important ones, however, are the availability of a free OH group on C_1 and an upward oriented OH on C_3 (Ling and Will, 1976). Both requirements are of course met by D-xylose but not the three other pentoses studied, L-xylose, D-arabinose, and L-arabinose.

It was also found that effective primers are themselves accumulated in an adsorbed form in insulinized muscle and in the cell water according to Equation 6. Indeed in 1965, Ling had published the result of some early study on the accumulation of labeled D-xylose in freshly isolated, unwashed frog muscle. Reproduced here as Figure 3, the experimental points are accurately described by Equation 6 with [f], equal to 25.6

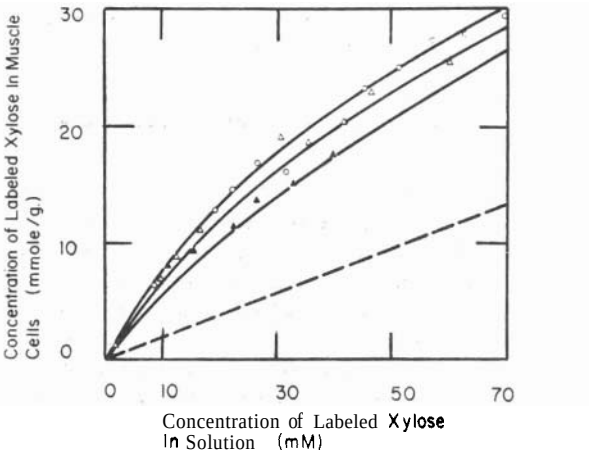

FIGURE 3. The equilibrium distribution of labeled D-xylose in frog muscle at O°C in the presence of no (-O-O-), 10 mM (-A-A-) and 30 mM nonlabeled D-xylose. Solid lines are theoretical calculated on the basis of Equation 6 and [f] = 25.6 μmoles/g. of cells, (-▲-▲-) = 80%, K_s = 29.6 $(M)^{-1}$ (reproduced from Ling, 1966, by permission of Fed. Proc.)

μmoles/g. of fresh cells, $\tilde{K}_{D\text{-xylose}}$ equal to 29.6 $(M)^{-1}$ and a q-value for D-xylose in the cell water equal to 0.24 (Ling, 1966, p. 968), which is not too far from the q-values of the other three pentoses studied here: D- and L-arabinose, and L-xylose. The solid line going through or near most of the data points of D-xylose in Figure 2 is also theoretically calculated on the basis of Equation 6, with $[f]_s = 33$ μmoles/g., $\tilde{K}_{D\text{-xylose}} = 8.2$ $(M)^{-1}$, and $q_{D\text{-xylose}} = 0.270$.

One then asks, "Why is the D-xylose curve in Figure 3 quantitatively different from the D-xylose distribution curve in Figure 2?" There is a simple and plausible answer: the frog muscles used in Figure 3 were incubated at 0° C for a total duration of only 17 hours, while the muscles in Figure 2 had been incubated for six days. Thus, the insulin originally present in the muscle before isolation was apparently still present at a higher level than after six-day incubation in an insulin-free Ringer's solution. In harmony with this view, Ling et al. (1969) have demonstrated that the level of labeled D-glucose accumulated was strongly dependent on the level of insulin present. That insulin promotes D-xylose accumulation in mammalian muscle was also observed by Kipnis and Cori (1957).

In summary, the study of the equilibrium distribution of four pentose stereoisomers has confirmed the "size rule" according to which the q-value is primarily dependent on the molecular weight of the probe molecule. This study also has established the usage of a battery of stereoisomers, as a superior means of determining the q-value and hence the intensity of the degree of polarization of the bulk phase water in living cells.

The foregoing work was supported by the National Cancer Institute Grant, 2-R01-CA16301.

REFERENCES

Hill, A. V. (1930) *Proc. R. Soc. London Ser. B. 106:*477.

Kipnis, D. M., and Cori, C. F. (1957) *J. Biol. Chem.* 224: 681.

Ling, G. N. (1962) *A Physical Theory of the Living State: The Association-Induction Hypothesis,* Blaisdell, Waltham, Mass.

Ling, G. N. (1965) *Ann. N. Y. Acad. Sci. 125:*401.

Ling, G. N. (1966) *Fed. Proc. 25:*958.

Ling, G. N. (1984 *In Search of the Physical Basis of Life,* Plenum Publishing Corp., NY.

Ling, G. N. (1988) *Physiol. Chem. Phys. and Med NMR 20:*281.

Ling, G. N., and Kromash, M. H. (1967) *J. Gen. Physiology 50:*677.

Ling, G. N., and Walton, C. L. (1975) *Physiol. Chem. Phys. 7:*215.

Ling, G. N., and Will, S. (1969) *Physiol. Chem. Phys. 1:*263.

Ling, G. N., and Will, S. (1976) *Physiol. Chem. Phys. 8:*115.

Ling, G. N., Miller, C. and Ochsenfeld (1973) *Ann. N. Y. Acad. Sci. 204:*6.

Ling, G. N., Neville, M. C., Shannon, P., and Will, S. (1969) *Physiol. Chem. Phys. 1:*85.

Pigman, W. (1957) *The Carbohydrates, Chemistry, Biochemistry and Physiology,* Academic Press, *N.Y.*

Roe, J. H., and Rice, E. W. (1948) *J. Biol. Chem. 173:*507.

Troshin, A. S. (1966) *Problems of Cell Permeability* (M. G. Hall, transl.) (W. F. Widdas, ed.), Pergamon Press, London.

Recemved March 16,1989; *review received May* 8, 1989; *revised May* 10, 1989.