Estimation of extracellular spaces of smooth muscle using different-sized molecules

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Barr, Lloyd, and Richard L. Malvin. Estimation of extracellular spaces of smooth muscle using different-sized molecules. Am. J. Physiol. 208(5): 1042-1045. 1965. — The apparent fraction of tissue water available as solvent was determined for a number of test substances: urea, arabinose, mannitol, sucrose, raffinose, inulin, and radioiodinated serum albumin. The apparent extracellular spaces decrease with increasing molecular size. Urea seems to come to concentration equilibrium in all tissue water. Arabinose and mannitol values are close together at about 70% tissue water. Similarly sucrose, raffinose, and inulin values cluster at about 48% tissue water, while RISA can reach only about 20%. The amounts of sodium, potassium, and chloride in canine intestinal smooth muscle are similar to values given for other smooth muscle. Intracellular ion concentrations similar to those of other tissues can be calculated if the true extracellular space is between that measured by RISA and sucrose.

The question of how to determine the distribution of water between extracellular and intracellular compartments is important for the study of the distribution of ions within a tissue, osmotic phenomena, and water metabolism. The basic question of what measurements to make remains unanswered.

The usual method of determining what fraction of tissue water is extracellular is to measure the volume of distribution of some "impermeant" solute molecule. The primary difficulty is that there is no independent method to validate the assumption that a molecule is in fact impermeant. Internal criteria can be met: for example, measurements should be made after enough time for equilibrium to occur, and several impermeant solutes should all have the same apparent volume of distribution. However, recent studies on smooth muscle (2, 5), cardiac muscle (8), and gastric mucosa (3) support the hypothesis that the apparent volume of distribution increases in small steps with decreasing molecular size, i.e., that there are more than the two classic tissue water compartments.

The purpose of this paper is to provide data describing, for a series of molecules, the time courses of diffusion into a relatively homogeneous tissue, the circular muscle layer of canine jejunum. It appears from the data reported below that there are at least four water compartments in this smooth muscle.

METHODS

Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg iv). A 20-cm length of intestine beginning approximately 20-40 cm from the stomach was isolated and removed. The circular smooth muscle layer was dissected free from the mucosa and longitudinal layers, while the intestinal tube was mounted on a glass rod. The dissection was done in Krebs-Henseleit (K-H) solution (6) and took approximately 20 min. Thirty to forty rings 1-2 mm wide were then cut from the remaining circular smooth muscle tube and placed in a bath of K-H solution at 38 C.

A mixture of 95% O₂ and 5% CO₂ saturated with water vapor was bubbled through the K-H solution. After being preincubated for 1 hr the tissue was rapidly transferred to a K-H solution containing one of the following in trace amounts: radioiodinated serum albumin (RISA), urca-C¹⁴, D-arabinose-1-C¹⁴, D-mannitol-1,6-C¹⁴, uniformly labeled sucrose-C¹⁴, and inulin carboxylic-C¹⁴ acid, or 21 mM/liter raffinose. In a few experiments 21 mM/liter sucrose or 48 g/liter inulin was added to the bath along with the tracer. The labeled medium was also maintained at 38 C and aerated with 95% O₂ and 5% CO₂. At timed intervals rings of tissue were removed from the bath, quickly blotted dry on clean filter paper, placed in tared 10-ml volumetric flasks, and weighed. The wet weights of the tissue were approximately 100 mg. Some flasks were then placed in an oven at 105 C overnight so that wet and dry weights could be determined. The remaining flasks were filled with distilled H₂O and kept at 4 C in order to leach out the test substance from the tissue. It was found that 18 hr was sufficient time for
leaching. The concentration of the test substance in the supernatant fluid was determined by either chemical or isotopic analysis. Aliquots of the incubation solution were withdrawn at intervals and also analyzed for the test substance. No change in the bath concentration of any test substance was found during the incubation period. RISA activity was measured using a deep-well scintillation counter. The activity of C14-labeled compounds was determined with a liquid-scintillation counter. The chemical concentrations of inulin, raffinose, and sucrose were determined according to the method of Shreiner (9), Na and K were measured as previously described (7), and chloride by electrometric titration. In all experiments in which raffinose was added to the medium there was also present one other substance labeled with C14. Thus, in at least one experiment, raffinose determinations were made on the same tissue as were arabinose, mannitol, inulin, or sucrose. In addition, some tissues were analyzed chemically and isotopically for inulin or sucrose. No differences in results were observed between the isotopic and chemical analyses. All results are expressed as a fraction of the apparent volume of distribution at any given time divided by the total water volume of the tissue.

RESULTS

The apparent volumes of distribution were calculated for different times as the test substances diffused into the tissues. Table 1 presents the data from all experiments. Since it was possible to follow only two compounds in any single experiment, the variability between dogs contributed to the statistical significance of the differences between the means of the different substances. Figure 1 shows graphically the changes in the apparent volume of distribution of all compounds studied as a function of incubation time.

None of the compounds reached its “plateau” value in less than 80 min of incubation. That this was not complicated by water movements is evident from the fact that there was no change in the water content of the tissue during the entire period of incubation. At the end of 5 min the tissue water was 83.9 ± 0.9 % of the wet weight, and at the end of 240 min it was 83.6 ± 0.3 %.

Nonelectrolyte spaces. Urea penetrates the tissue water compartments rapidly. In fact, it appears to come to a higher concentration in the tissue than in the bath, i.e., its final volume of distribution is greater than the volume of tissue water. Bozler has reported similar findings in other tissue (9). However, it seems probable that the urea is at the bath concentration in all the tissue water and an additional amount is bound.

Arabinose and mannitol, as may be seen from Fig. 1, diffuse into the tissue with quite similar time courses and both have access to such a large fraction of the tissue water that some penetration into cells must occur. However, although the data are not definitive on this point, the curves seem to plateau below the level of equidistribution in all cell water.

The curves for sucrose, raffinose, and inulin are clustered together. Again, as with urea, the existence of virtual equilibrium in these respective spaces seems amply demonstrated. The size of these spaces is not far from values reported for inulin spaces in other smooth muscles (2, 5).

However, it is important to note that the order of the curves downward, including the one for RISA, is determined exactly by molecular size. In regard to the RISA, there is nothing about the entry curve to reject the assumption that it may be used to estimate accurately extracellular space. Its curve, in fact, approaches a plateau as early as the other much smaller molecules.

None of the molecules diffused into the muscles with the kinetics of a one or two well mixed compartment model for the tissue. Neither did they follow curves for diffusion into sheets of homogenous material. Moreover, there was no ordering of the curves nor clear differences between the time course resulting when the fractions of values in Fig. 1 were divided by the 240-min values and plotted. Such curves represent the approach of each compound toward equilibrium in its own space.

Ion content. The electrolyte concentrations expressed in milliequivalents per kilogram wet weight in the smooth

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**TABLE 1. Summary of all measurements of volumes of distribution**

<table>
<thead>
<tr>
<th>Compound</th>
<th>5 Min</th>
<th>10 Min</th>
<th>20 Min</th>
<th>40 Min</th>
<th>60 Min</th>
<th>80 Min</th>
<th>160 Min</th>
<th>240 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>0.556±0.30</td>
<td>0.716±0.08</td>
<td>0.926±0.14</td>
<td>1.063±0.17</td>
<td>1.068±0.17</td>
<td>1.067±0.13</td>
<td>1.132±0.023</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>0.400±0.15</td>
<td>0.433±0.11</td>
<td>0.473±0.11</td>
<td>0.516±0.14</td>
<td>0.609±0.14</td>
<td>0.725±0.14</td>
<td>0.709±0.021</td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.374±0.12</td>
<td>0.418±0.11</td>
<td>0.454±0.11</td>
<td>0.495±0.11</td>
<td>0.539±0.12</td>
<td>0.592±0.14</td>
<td>0.692±0.016</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.439±0.17</td>
<td>0.449±0.14</td>
<td>0.457±0.13</td>
<td>0.483±0.13</td>
<td>0.491±0.13</td>
<td>0.506±0.14</td>
<td>0.478±0.013</td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td>0.397±0.14</td>
<td>0.360±0.11</td>
<td>0.395±0.11</td>
<td>0.417±0.08</td>
<td>0.439±0.08</td>
<td>0.471±0.09</td>
<td>0.491±0.018</td>
<td></td>
</tr>
<tr>
<td>Inulin</td>
<td>0.309±0.18</td>
<td>0.366±0.18</td>
<td>0.432±0.09</td>
<td>0.427±0.11</td>
<td>0.466±0.11</td>
<td>0.455±0.011</td>
<td>0.469±0.012</td>
<td></td>
</tr>
<tr>
<td>RISA</td>
<td>0.078±0.09</td>
<td>0.068±0.11</td>
<td>0.124±0.09</td>
<td>0.155±0.10</td>
<td>0.177±0.11</td>
<td>0.183±0.010</td>
<td>0.193±0.010</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents volume of distribution divided by total tissue water ± standard error of the mean. Numbers in parentheses denote number of experimental observations.
FIG. 1. Time course for change in volume of distribution for different-sized molecules.

FIG. 2. Estimation of intracellular concentrations for Na, K, and Cl as a function of the fraction of tissue water assumed to be extracellular.

Muscle were found to be Na⁺, 72.6 ± 2.3 SEM; Cl⁻, 57.6 ± 6.3 SEM; and K⁺, 75.4 ± 3.0 SEM. In Fig. 2 are plotted the calculated intracellular concentrations of these ions as a function of the fraction of water assumed to be extracellular. Values used for composition of extracellular fluid are plasma values in milliequivalents per kilogram H₂O taken from Spector (10), i.e., Na⁺, 161; K⁺, 4.8; and Cl⁻, 114; H₂O is 930 ml/kg. Since the extracellular concentrations of Na⁺ and Cl⁻ are higher than those for the mean tissue water concentrations, the calculated intracellular concentrations decrease with increasing extracellular space. The sum of the concentrations for the three ions also decreases. However, for K⁺ the converse is true. As assumed extracellular space is increased the calculated intracellular concentration for K also increases.

Obviously no space larger than the Na⁺ space can be the true extracellular space. Thus arabinose and mannitol penetrate to greater volumes than could be extracellular.

DISCUSSION

There seems to be no doubt that arabinose and mannitol diffuse into intracellular water. The question then arises as to whether these compounds are approaching extracellular concentrations in all cell water compartments. The amount of arabinose in the tissues certainly appears to have reached a plateau of less than that required by equidistribution. Although the amount of mannitol in tissues may still be rising after 4 hr, the rate is quite slow. In fact, it is reasonable to assume that arabinose and mannitol are diffusing into a part but not all of the intracellular compartments. This part might be the cytoplasm but not the nucleus or vice versa. Similar suggestions might be made about the sarcoplasmic reticulum or mitochondria but of course these spaces are comparatively small. A second possibility is that arabinose and mannitol curves represent penetration into all cell water and an active transport outward from the intracellular compartment. Finally, it is also possible that these substances leak into cells traumatized by the dissection. However, this latter seems quite unlikely because the spaces are independent of the size of the preparations.

The fact that sucrose, raffinose, and inulin give essentially the same extracellular space (400–450 ml/kg tissue H₂O) lends support to the assumption that for this tissue they provide a good means of estimating extracellular space. The agreement between the results for them is
particularly significant because they vary considerably in molecular size. Nevertheless, one cannot exclude the possibility that the true extracellular space is smaller and that some of the considerations discussed above for arabinose and mannitol apply for sucrose, raffinose, and inulin as well.

In this light, it is possible to consider the RISA space the true extracellular space. The RISA curve in Fig. 1 seems to have plateaued, and it is certainly difficult to imagine that the true extracellular space is smaller than that available to RISA. On the other hand it again is possible for some regions of the extracellular compartments to be closed to the diffusion of RISA. One can only speculate what such a space might be. Electron micrographs of canine intestinal smooth muscle show packing of the collagen fibers and the extracellular protein near the cells (9). RISA might be able to diffuse into this region only very slowly. However, it is not now possible to decide whether such packing of extracellular protein occurs in living muscle or is an artifact of fixation. In any case it does seem safe to assume that the extracellular space of this muscle is not less than the RISA space and not more than the inulin-raffinose-sucrose space.

That the order of molecular sizes of the test substances correlate with the order of the 240-min space values of Table 1 is suggestive, as Bozler has pointed out, of a system of blind diffusion channels whose diameters vary considerably (2). This notion is certainly more attractive than an indefinitely large number of membrane-bounded compartments.

Intracellular ion concentrations. There are no a priori reasons to consider the \([Na]^+\) of 15 mEq/kg H2O and \([K]^+\) of 170 mEq/kg H2O calculated from the inulin space to be more valid than those calculated from the RISA space: \([Na]^+\) 68 mEq/kg H2O and \([K]^+\) 110 mEq/kg H2O. All that can be said is that the RISA values are closer to those calculated for other mammalian smooth muscles. The inulin space yields values closer to those calculated for skeletal muscle.

The amount of chloride in smooth muscle has long been known to be higher than in other tissues. It is too high to allow the assumption of a Donnan equilibrium for Cl and K. Even assuming the sodium space to be the extracellular space, the chloride equilibrium potential is much lower than the potassium one. It is also lower than resting potentials of circular smooth muscle of the feline intestine (10). If the true extracellular space is smaller the apparent chloride excess increases. The chloride data can be explained of course if considerable amounts of chloride are bound. Another explanation which should not be ignored is that chloride ions are actively transported into the cells. This latter explanation has another assumption inherent in it, i.e., the number of other intracellular anions, presumably organic, is considerably less than in other cell types.

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REFERENCES