The Dimensions of the Extracellular Space in Sartorius Muscle

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ABSTRACT A survey has been made of the amount of muscle water available to inulin, sucrose, and radioiodinated human serum albumin (RISA). The percentage spaces available to the three molecules are of the same order of magnitude, but the sucrose space > inulin space > albumin space. The kinetics of influx and efflux of RISA have been studied, and it appears that a small part of the albumin may be adsorbed in the extracellular phase. Nevertheless the albumin space would appear to give the best index of the extracellular volume.

The scatter in values found for the extracellular space by all methods is very great, ranging from 8 to 40 per cent and renders invalid the use of a mean value for the calculation of intracellular concentrations. The variation within paired muscles is less than between pairs, provided the tissue has undergone no volume change. Increase in total muscle volume when the muscle is placed in a hypotonic solution leads to a decrease in the size of the extracellular space.

All work on the distribution of electrolytes between the external medium and the sartorius muscle depends for its interpretation on an estimate of the extracellular volume of the muscle. This also applies to any attempt to relate the ionic gradients across the muscle membrane with the bioelectric potentials. It is consequently of prime importance to know the dimensions of the extracellular space, and the degree of variation in the dimensions of the space within a pair of muscles. Estimates of the extracellular volume of frog muscle in the literature range from 10 to 12 per cent (1, 2) to 35 per cent of muscle weight (3, 5, 7), depending apparently on the method of estimation, and the species of frog used.

In this study we have attempted to clarify the situation in the following ways. First we have undertaken a comparison of the accuracy of the various methods of estimating the space. Second we have evaluated the scatter in values found in a population of animals, in order to ascertain whether it is legitimate to use a mean value when calculating intracellular concentrations.

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Received for publication, February 24, 1959.
Third we have investigated the effect of change in muscle volume on the dimensions of the extracellular phase.

It would appear that the inulin space is similar in magnitude to the sucrose space, and also to the space available to $^{131}$I-tagged albumin. The scatter in values found is less within paired muscles than between pairs, but over the whole population was found to vary from 8 to 40 per cent. Consequently great care should be taken when evaluating intracellular ionic levels obtained by the use of a mean value for the extracellular space. Results obtained for paired muscles, or when several ions have been estimated on the one muscle, will yield accurate comparisons although the absolute amounts may be in error.

**Methods**

The sartorius muscle of the toad, Bufo marinus, was used throughout this study.

Inulin spaces were determined using a modification of the method of Wilde (11). Muscles were first soaked for 2 hours in Ringer containing 1 per cent inulin, then the inulin was leached out of the muscle into an inulin-free Ringer for a further 2 hours, and the amount of inulin in the Ringer estimated by the anthrone reaction. Sucrose spaces were obtained similarly, save that 3.5 per cent sucrose was made up in Ringer containing 65 m.eq. per liter NaCl (half the usual amount) to avoid any volume change. Sucrose was then leached out into normal Ringer and also assayed by the anthrone technique.

Human serum albumin tagged with $^{131}$I (RISA) was obtained from A.E.R.E. Harwell, and as a gift from Abbott Laboratories, United States of America. It was added to Ringer to give an activity of 25 microcuries per ml. Gamma radiations were measured with an EKCO scintillation counter and rate meter.

RISA spaces were determined by two methods: (1) Muscles were soaked for 3 hours in RISA-labelled Ringer and then effluxed into inactive Ringer; 2 ml. aliquots were counted. Appropriate calibration standards were also counted. (2) After influxing as in (1), each muscle was blotted, weighed, and placed on a small disposable plastic sheet. This was positioned on a shelf some 2 to 3 inches distant from the crystal; variations of geometry due to different sizes and shapes of muscles were found to be negligible at this distance. Standards were made by pipetting as drops known volumes of a 1 in 4 dilution of the RISA-labelled Ringer onto the center of plastic sheets. The volumes (usually 0.1, 0.15, and 0.2 ml.) were chosen to bracket the weights of the muscles used.

At a strength of 25 $\mu$C./ml. of influxing solution, an average muscle gave about 50 c.p.s. (background = 1 c.p.s.). While an activity of 5 $\mu$C./ml. should be adequate for the determination of the extracellular space, we used this higher activity to enable us to plot efflux curves. Refrigeration and the addition of antibiotics permitted the Ringer to be used several times.

The Na$^+$ and K$^+$ content of the muscles was determined with a Beckman flame spectrophotometer.

The statistical procedures used in the evaluation of the results will be described in the text.
In all experiments with a duration of more than 4 hours, a mixture of antibiotics was added to the Ringer solution to ensure sterility (9).

RESULTS

The Identity of the Inulin Space in Muscle

Since the chemical assay of inulin by the anthrone method yields accurate and reliable results, the question as to whether the inulin space represents the total extracellular space depends on the diffusion kinetics of inulin into and out of this space. The equilibration of inulin in frog sartorius muscle has been studied by Boyle et al. (1) who found a half-time for full equilibration of 5 minutes. It would therefore seem that our procedure of allowing 2 hours for complete equilibration should be adequate. The possibility exists, however, that inulin may be adsorbed onto the surface of the cell, or may to some extent penetrate the cell membrane. These effects would lead to erratic results, as neither phenomenon could be expected to take place regularly and so would produce a constant error.

Variation of Inulin Space within Paired Muscles

We have attempted to evaluate the effect of inulin adsorption or penetration in the following ways: first by testing the degree of variation between and within paired muscles, and second by testing the effect of time of soaking on the size of the space. The degree of variation of the inulin space within paired muscles is less than the variation between pairs. Allowing for slight differences in the dissection of paired muscles and asymmetry in the development of connective tissue, one would expect to find similar values for the inulin space of paired muscles. The right leg muscle inulin space was plotted against the left leg inulin space for thirty-seven pairs of muscles, and their correlation was found to be significant at the 1 per cent level.

Variation of Inulin Space between Batches

The mean inulin space (±S.E.) found for estimations carried out over 9 months was 24.8 per cent ± 0.83 (142 observations). The distribution of the inulin space (calculated as a percentage) is shown in Fig. 1, and it will be seen that the values found vary from 8 to 40 per cent. There was a tendency for a batch of toads to show similar inulin spaces, although there was often a marked variation between batches. Thus the mean of 20 observations made on one batch was 16.9 ± 0.82. It will be seen from this that to use the mean of the population would lead to considerable error. The mean of 24.8 per
cent is considerably different from those reported by other authors, and is significantly different from that found previously in this laboratory on a small sample (10). It is consequently our opinion that it is not justifiable to use a group mean in an estimate of the inulin space. Ideally, individual values should be used, or when this is not possible the comparison of results from paired muscles should be used.

**The Entry of Inulin into the Muscle Fiber**

The possibility of inulin penetrating the cell membrane was tested by varying the time of soaking in the inulin-Ringer. One each of five pairs of muscles was soaked in the inulin-Ringer for 2 hours in the usual fashion, and the companion muscle was held in this solution for 18 hours. The time of washout of the inulin was in both instances 2 hours. It may be seen from Table I that

![Table I: The Effect of Time of Soaking on the Size of the Inulin Space](image)

<table>
<thead>
<tr>
<th>Time of soaking, hr.</th>
<th>Inulin space</th>
<th>Na, m. eq. per kg.</th>
<th>K, m. eq. per kg.</th>
<th>Volume change, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12.6</td>
<td>42.4 (29.8)</td>
<td>74.9</td>
<td>+0.2</td>
</tr>
<tr>
<td>18</td>
<td>19.3*</td>
<td>47.8* (28.1)</td>
<td>61.8*</td>
<td>-3.0</td>
</tr>
</tbody>
</table>

Figures in parentheses in Na column refer to intracellular concentrations based on the observed inulin spaces. In this and subsequent tables only figures marked with an asterisk are significantly different from controls.
there was a significant increase in the apparent inulin space of the muscle which was soaked for the longer time. The muscles at 18 hours showed an increase in total Na⁺ content, and a decrease in K⁺ content, compared with the muscles soaked for a shorter time. The intracellular Na⁺ level at the shorter interval calculated on the found inulin space was 29.8 m.eq. per kg. If one accepts the inulin space after 18 hours as a genuine index of the extracellular volume, and calculates the intracellular Na⁺ level on this figure one obtains a Na⁺ level of 28.1 m.eq. per kg. Thus the longer period of soaking has apparently caused no change in the Na⁺ level of the cell.

This experiment suffers from the defect that the 2 hour elution after the prolonged treatment with inulin may not have released all the intracellular inulin. Had longer times of soaking out been resorted to, it would not have been justifiable to refer the ionic analyses to the inulin space, so another experiment was performed to test this point.

Four pairs of muscles were used. One of each pair was soaked in normal Ringer for 18 hours, and then transferred to inulin-Ringer for 2 hours. The companion muscle was held in inulin-Ringer for 2 hours; then both muscles were eluted in normal Ringer serially for 2, 4, and 5 hour periods.

<table>
<thead>
<tr>
<th>Time of elution</th>
<th>Inulin in muscle, gm. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Ringer 18 hrs., then inulin 2 hrs.</td>
<td>Inulin 18 hrs.</td>
</tr>
<tr>
<td>hrs.</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>0.007</td>
</tr>
</tbody>
</table>

The inulin in the wash-out Ringer was estimated, and the results are set out in Table II. It will be seen that there was no significant difference in the amount of inulin eluted from either muscle during the first 2 hour period. If inulin had penetrated the muscle to a greater extent in the long soaked series, it was not released in this time. However, longer times of elution showed larger amounts of inulin coming out in the muscles which were held in inulin for 18 hours than in the controls. The amounts which apparently penetrate the membrane in both series are, however, not great, and it would seem unlikely that they affect the accuracy of the method.

Another experiment was performed to check on the increase in the size of the extracellular space on prolonged soaking. Twelve pairs of muscles were taken, and the inulin space was determined on one of each pair by soaking in inulin-Ringer and eluting in normal Ringer for 2 hours. This muscle was then left in normal Ringer for 20 hours, transferred again to inulin-Ringer,
and another inulin space assayed. The companion muscle was held in inulin-Ringer for 20 hours prior to the estimation of the space. It was found that over the 20 hours in normal Ringer there was an increase in the inulin space of the first group of muscles. That is, the mean inulin space determined initially was 23.0 per cent, and the space found after 20 hours in normal Ringer was 27.3 per cent. This difference is significant at the 1 per cent level. The inulin space determined on the paired muscles held for 20 hours in the inulin-Ringer was 28.8 per cent, which is not significantly different from 27.3 per cent. This is in agreement with the finding in the previous experiment.

It would seem from these experiments that inulin does to some extent penetrate the cell membrane, and that the dimensions of the extracellular space increase on prolonged soaking.

The Variation in Sucrose Space within Paired Muscles

The apparent sucrose space was determined in muscles in the same way as the inulin space. The correlation of right leg sucrose space against left leg sucrose space for sixteen pairs of muscles was found to be significant at the 5 per cent level. Whether this degree of variability of the data which was greater than that found in the inulin figures reflects an inherent inaccuracy in the method will be discussed in the light of subsequent experiments.

The Variation of Sucrose Space between Batches

The mean sucrose space (±S.E.) was found to be 26.5 ± 0.97 per cent (72 observations). The distribution of the percentage sucrose space is shown in Fig. 2, where it will be seen that the values vary from 10 to 40 per cent. The mean sucrose space is close to the mean inulin space (24.8 per cent), and

![Figure 2. The distribution of the percentage sucrose space in muscle.](image-url)
the extreme range of values found is also similar to the range of inulin spaces. The form of the distribution histogram is, however, rather different, as can be seen from a comparison of Figs. 1 and 2. The sucrose distribution was tested for goodness of fit, to a normal curve and was found not to differ significantly from normal.

As was found for the inulin spaces there was a marked variation in sucrose space between different batches of toads. For example the mean of 16 observations on one batch of toads was $15.8 \pm 0.87$ per cent sucrose space, which is significantly different from the mean obtained for the population.

### The Entry of Sucrose into Muscle Fiber

If sucrose can in some instances penetrate the cell membrane, it is obvious that the sucrose space cannot be used as an index of the extracellular volume. This possibility was tested, as was done in the inulin experiments, by soaking one of each pair of muscles in the sucrose solution for 2 hours, and soaking the companion muscle for 12 hours in the sucrose solution prior to determining the space. The results of this experiment are set out in Table III. It will be seen that there was a significant increase in the sucrose space at the longer time of soaking. The muscles showed an increase in Na\(^+\) content, and a decrease in K\(^+\) content compared with the muscles soaked for a short time. If one calculates the intracellular Na\(^+\) content on the basis of the found sucrose spaces, one obtains 25.1 m.eq. per kg., and 33.5 m.eq. per kg. respectively for the muscles soaked for long and short times. It is impossible to state from these data whether the increase in sucrose space is due to the entry of sucrose into the cell, or whether it reflects a genuine increase in the dimensions of the extracellular space. It is probable that both effects are found, as was shown for the inulin space.

### A Comparison of Inulin Space with Sucrose Space

As was mentioned in the preceding section, there was no significant difference between the mean inulin space and the mean sucrose space. Due to the
scatter of the results this agreement could have been fortuitous, and it was
decided to check the point using paired muscles. Several experiments were
performed using from six to eight pairs of muscles in each. One of each pair
was soaked in inulin-Ringer, and the companion was soaked in sucrose. A
"t" test was performed on the grouped results, and the sucrose space was
found to be greater than the inulin space, with the difference significant at
the 0.1 per cent level. When however, the experiments carried out in June
were separated from those performed later it was found that the June group
of eight pairs of muscles showed no difference between inulin and sucrose
spaces.

Thus there would appear to be a tendency for sucrose to penetrate more of
the cell water than inulin, but there is not an extensive, or regular difference
between the two spaces.

The Dimensions of the Albumin Space in Muscle

Evidence has been presented in the previous sections which suggests that
inulin and sucrose may to some extent penetrate the cell membrane. It was
therefore decided to examine the space available to a molecule of the dimen-
sions of serum albumin. The molecular weights of inulin and sucrose are 486
and 342 respectively, which are small molecules compared with albumin
(molecular weight = 69,000). Estimations of the extracellular space using
albumin should therefore be free of the objection that part of the intra-
cellular compartment may be included in the figure obtained. Adsorption
on the surface of the fibers may however, occur, and so give spuriously high
readings. We have attempted to test for this by comparing influx and efflux
curves.

The Influx of RISA into Muscle

The rate of entry of albumin into the muscle was determined by immersing
the muscle in Ringer to which had been added RISA for increasing time in-
tervals, and counting the activity of the muscle between each immersion
period. The muscle was blotted lightly after removal from the active solution,
as it was found that this gave more consistent results than either short washes
in inactive Ringer, or draining the muscle for a standard time against the side
of the container.

The Efflux of RISA from Muscle

Muscles were equilibrated for 3 hours in Ringer containing RISA, then the
efflux of albumin into normal Ringer was followed for 4 hours. At the end of
the run the residual activity of the muscle was assayed, care being taken to use the appropriate standardization to eliminate differences in the geometry of the counting system.

The influx and efflux of RISA have been plotted on a linear scale in Fig. 3, and it will be seen that the curves show a similar time course. When the efflux curve is plotted on a semilogarithmic scale (Fig. 4) it appears to be the sum of a series of at least three exponentials. It is noteworthy that after 4 hours' elution the muscles still contained appreciable amounts of activity.

*The Variation in Albumin Space with Paired Muscles*

The correlation of right leg albumin space against left leg albumin space was carried out as was done for inulin and sucrose, and was found to be significant at the 0.1 per cent level. Fourteen pairs of muscles were used. There was a higher degree of correlation within pairs of RISA-treated muscles, and also within pairs of inulin-treated muscles, than within pairs of sucrose-treated muscles. This may indicate that sucrose gives a less accurate index of the intracellular volume than do albumin and inulin.

*The Variation of Albumin Space between Batches*

The mean albumin space was 21.9 per cent, with a standard error for sixty-four observations of ±0.5. The distribution of the albumin space over the...
population tested is shown in Fig. 5. The distribution was tested for goodness of fit to a normal curve, and was found to be not significantly different. Determinations of inulin spaces were carried out on the same muscles as were used for the albumin space (see below) and the mean inulin space on this population was 26.5 ± 0.6. The distribution of this population of inulin spaces was also found to be normal. The albumin space did not show the same degree of scatter as is shown for that of inulin and sucrose in Figs. 1 and 2, but is similar to the scatter found for the inulin spaces carried out on the same muscles.

A Comparison of Albumin Space with Inulin and Sucrose Space

In this investigation RISA was added as routine to Ringer containing either inulin or sucrose. The muscles were equilibrated in this solution for 3 hours, then blotted, weighed, and the radiation counted. The muscle was then transferred to normal Ringer, and the inulin or sucrose diffusing into it assayed. In this way differences between paired muscles, and variation in blotting technique were obviated in estimating the difference between the two spaces. A "t" test was carried out to check the significance of the difference between albumin and inulin spaces. The difference was significant at the 1 per cent level. The difference between albumin and sucrose spaces was also significant at the 1 per cent level.

An attempt to correlate the three spaces was made in the following experiment. Seven pairs of muscles were taken, and one of each pair was soaked in
albumin plus sucrose-Ringer, and the companion muscle was soaked in albumin plus inulin-Ringer. The results are set out in Table IV.

In conformity with our previous findings the sucrose space > inulin space > albumin space. The difference between sucrose space and inulin space was tested with a "t" test and was significant at the 1 per cent level. The difference between albumin space and sucrose space was also significant at the 1 per cent level. However, the difference between the albumin space and the inulin space was not significant.

**TABLE IV**

<table>
<thead>
<tr>
<th>Inulin space</th>
<th>Sucrose space</th>
<th>Albumin space</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>20.7</td>
<td>22.7</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>22.7</td>
<td>17.4</td>
</tr>
<tr>
<td>28.3</td>
<td>28.2</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>22.0</td>
<td>21.5</td>
</tr>
<tr>
<td>31.1</td>
<td>35.6</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td>29.5</td>
<td>26.5</td>
</tr>
<tr>
<td>25.4</td>
<td>31.4</td>
<td>22.8</td>
</tr>
<tr>
<td></td>
<td>22.8</td>
<td>24.8</td>
</tr>
<tr>
<td>24.1</td>
<td>30.0</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>27.5</td>
<td>21.5</td>
</tr>
<tr>
<td>22.1</td>
<td>28.1</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>23.0</td>
<td>17.5</td>
</tr>
<tr>
<td>29.2</td>
<td>35.8</td>
<td>23.5</td>
</tr>
</tbody>
</table>

**Figure 5.** The distribution of the percentage radioiodinated serum albumin space in muscle.
The Nature of the Variation in Extracellular Volume

The very great degree of variability in the extracellular volume of the sartorius muscle makes the use of a large sample mean in calculating individual intracellular values invalid. It would consequently be of great value if one could relate the dimensions of this space to any other easily measured characteristic of the muscle. To this end we have plotted muscle weight against both inulin space and sucrose space (Figs. 6 and 7). In both instances there was a significant negative correlation between size and extracellular space. That is, large muscles tend to have lower per cent extracellular volumes than small ones. For the inulin space the correlation was significant at the
5 per cent level; for the sucrose space it was significant at the 0.1 per cent level. It will be seen, however, from Figs. 6 and 7 that although most large muscles have small extracellular spaces, there may be a very great degree of variation in the space between small muscles. Consequently one cannot use muscle weight as a criterion for separating muscles into extracellular spatial groups.

A further attempt was made to correlate extracellular space with the total Na⁺ level of the muscle. When inulin space and sucrose space were plotted against the total Na⁺ there was no significant relationship in either case. This was an unexpected finding since one would conceive that a muscle with a large extracellular space would have a higher content of a predominantly extracellular constituent such as Na⁺ than a muscle with a small extracellular space. There was a slight tendency for the plot of inulin space against total Na⁺ to show the expected relationship, but the scatter of the results was so great as to render the correlation non-significant. When extracellular space was plotted against intracellular Na⁺ calculated from the inulin space, there was no suggestion of any correlation. The scatter of intracellular Na⁺ levels ranged from 1 or 2 m.eq. per kg. to 70 m.eq. per kg. The mean of 111 observations was 23.0 m.eq. per kg., and the distribution was normal. This great scatter in intracellular Na⁺ levels is responsible for the lack of correlation between total Na⁺ and extracellular space.

**The Effect of Muscle Volume Change on the Dimensions of the Extracellular Space**

In many experiments dealing with ionic balances and bioelectric potentials, a variation in the composition of the external medium may lead to a change in the muscle volume. Since the volume of the test muscle may change, while the control paired muscle in normal Ringer may not undergo any alteration, the usual comparison of paired muscles is no longer useful. Consequently we have investigated the effect of volume change on the dimensions of the extracellular space in the following experiments.

Muscles were placed in a low Na-Ringer (65 m.eq. per liter) containing inulin, and the inulin space was determined on the muscle after the swelling induced by the hypoosmotic solution had reached a steady state. The companion muscle was held in a normal inulin-Ringer for a similar time. The Na⁺ content of the muscles, and the degree of swelling were also noted. The effect of low Na-Ringer was also compared with the effect of a low Na-Ringer to which choline chloride or sucrose had been added to maintain the osmotic pressure. In the experiment in which sucrose was added, the sucrose was estimated, and used as an index of the extracellular space. The results of these experiments are set out in Table V.
It will be seen that in each instance the swelling of the muscle has resulted in a decrease in extracellular space. It must be presumed from this that the swelling of the muscle fibers has produced a decrease in the packing fraction, as well as an over-all increase in the volume of the tissue.

**Table V**

<table>
<thead>
<tr>
<th>Ringer solution</th>
<th>Volume change per cent</th>
<th>Inulin space m.l.</th>
<th>Sucrose space m.l.</th>
<th>Total Na m.eq/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half Na</td>
<td>26.7</td>
<td>8.8</td>
<td>11.9</td>
<td>36.3</td>
</tr>
<tr>
<td>Normal Ringer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half Na</td>
<td>37.4</td>
<td>8.9</td>
<td>17.1</td>
<td>30.6</td>
</tr>
<tr>
<td>Half Na, plus choline chloride</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half Na</td>
<td>34.9</td>
<td>14.4</td>
<td></td>
<td>26.1</td>
</tr>
<tr>
<td>Half Na, plus sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table VI**

<table>
<thead>
<tr>
<th>Ringer solution</th>
<th>Volume change per cent</th>
<th>Inulin space m.l.</th>
<th>Albumin space m.l.</th>
<th>Total Na m.eq/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half Na</td>
<td>21.0</td>
<td>11.7</td>
<td>10.0</td>
<td>12.1</td>
</tr>
<tr>
<td>NaX3</td>
<td>-7.2</td>
<td>20.4</td>
<td>13.7</td>
<td>172</td>
</tr>
<tr>
<td>Half Na</td>
<td>20.9</td>
<td>15.7</td>
<td>13.5</td>
<td>19.3</td>
</tr>
<tr>
<td>Normal Ringer</td>
<td></td>
<td>25.4</td>
<td>21.9</td>
<td>60.4</td>
</tr>
</tbody>
</table>

In Table VI are collected the results of experiments in which alterations in the osmotic pressure of the Ringer are compared with both inulin and albumin spaces. These spaces were determined on the same muscle, in the usual manner. It will be seen that in these experiments also the volume increase has caused a reduction in the extracellular space. The inulin space is consistently greater than the albumin space as noted in the earlier section. In the high Na-Ringer in particular the disparity is considerable.

**Discussion**

The impression of uncertainty found in the literature regarding the size and variation in the extracellular space would seem to derive from doubts about the accuracy of the methods of assay, and a study of insufficiently large
samples. It would seem from this study that inulin, RISA, and sucrose all give a reasonable index of the extracellular space, although the use of RISA would seem the method of choice. This method is only open to the objection that a slight adsorption on to the surface of the fibers appears to occur, but this is not sufficient to cause appreciable error. A similar finding has been reported by Jackson and Pace (6), who used albumin to estimate the interstitial water of mitochondrial preparations.

Our results suggest that inulin may to some extent penetrate the cell water, thus tending to give too high a reading. This was found to occur to a considerable extent in nephrectomized sheep (8), in which a change in cell permeability is found. The degree of penetration found under our experimental conditions is, however, not sufficient to invalidate the method. Sucrose also appears to penetrate the cell, since the sucrose space is largest. It is of interest to compare our albumin efflux curves with those obtained for sucrose by Johnson (7). Elliott (4) has shown that the inulin space in brain tissue is less than the sucrose or thiocyanate space, which he ascribes to different extracellular compartments.

The great variability of the extracellular space, as measured by all three methods, has been commented on in the Results section. It suffices to say here that this variation renders invalid the use of a mean value in the estimation of extracellular space. The use of paired muscles, which would normally lead to accurate comparisons, though perhaps incorrect absolute amounts, cannot be resorted to if there is any difference in volume change between the paired muscles. The brief series of experiments reported here shows clearly that increase in total muscle volume leads to a decrease in the dimensions of the extracellular space.

**BIBLIOGRAPHY**