ON THE ABSORPTION OF LEAD INTO THE CELLS OF NITELLA

BY

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Introduction.

**Collander** has shown in several papers on *Chara ceratophylla* (Wallr.) and other characeae that the concentration of ions in the cell sap is greatly in excess of that found in the water of the habitat.

According to **Collander** (1930) the leaf cells of *Ch. ceratophylla* contain up to 40 µl sap, which is easily got out in a pure state, and they are therefore well suited for permeability determinations in which the substances permeating can be determined analytically. In the paper quoted the normal composition of the sap was determined and compared with the water of the natural habitat (4—5 % saline).

All the ions determined were found in the sap in higher concentration than in the surrounding water. The total salinity of the sap amounted to about 15 %o. Since the sap contains very little protein (< 1 %o) or other organic compounds (< 3 %o) the ions cannot be organically combined, as is borne out also by the high electric conductivity. By means of a compensation dialysis it was shown further that the mud, from which the plants are assumed to absorb part of their nutritional substances, exhibited much lower concentrations of salt than the sap. It is evident therefore that the salts are transported from a dilute external solution towards a more concentrated internal. In later papers (36, 39) **Collander** turns more and more to the view that the ions are actively absorbed by the cells by a special "adenoid" mechanism. The process requires the expenditure of energy on the part of the plants.

In an attempt to elucidate the mechanism of the active absorption **Brooks** (1938) observed on *Nitella* that radio-potassium (42K) was for some hours during the transport concentrated within the protoplasm.

**Mullins** (42) continuing the work of **Brooks** examined whether the very numerous granules in the protoplasm of *Nitella* could have anything to do with absorption of ions. He placed cells in 10 millimolar KCl (containing 42K) and centrifuged them after a suitable period during a suitably varied time and at varying rates. As the granules have a higher specific gravity he got them massed together in the lower parts of the cells. After centrifuging he counted the activity of the upper and lower halves of the cells.
separately and found that the active potassium was to a certain extent concentrated within the granules.

My experiments serve as a control and extension of the observations of Mullins. I worked with radio-lead (Thorium-B, atomic weight 212, half life 10.6 hours). This isotope was studied by Hevesy and Paneth (quoted from *A Manual of Radioactivity* 1938) who showed that a chemical separation between the isotope and ordinary lead is impossible. They are chemically identical. Cells have a very considerable affinity to lead and the activity which corresponds to less than $10^{-10}$ gram-atom, can be measured with sufficient accuracy in a Geiger-Müller counter.

The active lead is obtained from a thorium preparation, placed in a small container below a negatively charged platinum foil. Gaseous thorium emanation is continually formed and attracted by the platinum on which it is transformed to $^{212}$Pb. A practically maximal quantity is reached in a little over 3 days when the rate of decay will become equal to the rate of formation. The lead is dissolved in $\frac{1}{10}$ HCl and neutralized afterwards with an equal amount of NaOH. The first experiments were made with the active lead alone, the later ones with a mixture of a certain amount of ordinary lead with this. In this way the relation between the measured activity and the corresponding quantity of lead could be defined.

The *Nitella* cells were found to be very suitable for these experiments. Single intermodal cells were isolated and could live on indefinitely. Cells of 70—100 mm length and about $\frac{1}{2}$ mm diameter were selected. They were placed in narrow glass tubes sealed at one end ($120 \times 4$ mm), and the outside fluid was kept mixed by air bubbles. During the experimental period samples were taken from the fluid by a Carlsberg pipette (vol. 11.56 µl), dried and counted. The Geiger-Müller counter employed in the laboratory is described by Hilde Levi (1941) and by Holm-Jensen (1943). The countings per minute were corrected for counting error, zero effects and the natural loss as characterized by the half life period. Usually about 1000 impulses were counted giving a sufficient accuracy of about 3%.

### Centrifugation Technique.

Mullins centrifuged his cells for a long time (up to 8 hours) at a low speed. I found in a series of preliminary experiments that centrifuging for 7 to 10 min. at 3000/min. will give the most complete displacement of granules without any visible damage to the cells, which were centrifuged in the same tubes in which they were exposed to the experimental solutions. After centrifuging in this way the cells retained their turgor completely, and microscopic
observation showed an undisturbed distribution of protoplasm and chloroplasts and no change in the protoplasmic flow. A small precipitate, presumably granules, was visible for a short time at the lower end of the cell.

Centrifuging longer at the same rate brought a number of chloroplasts down and experiments with centrifuging at lower rates (2000/min.) caused the cells to die slowly without giving any effective precipitate of granules.

After centrifuging the cell is cut across, the cut surface touched with a narrow capillary tube and sap collected and weighed. The protoplasm is brought out by drawing the cell between two fingers exerting considerable pressure and is likewise collected in a capillary tube.

**Description of an Experiment.**

The active lead is dissolved from the platinum electrode in 0.2 ml n/10 HCl and neutralized with 0.2 ml n/10 NaOH. The solution is transferred to an experiment tube and filled up with distilled water to 1.8 ml. After mixing by air an initial sample is taken by a Carlsberg pipette of 11.56 µl. The sample is blown out into a counting dish and dried. Three living Nitella cells, 70—100 mm long, are put into the tube and samples taken at suitable intervals by means of the same pipette. In this case at 13°0, 14°0, 17°0 and finally at 19°0. After the final sample the cells are centrifuged, cut and samples of sap, upper and lower protoplasm collected in capillary tubes. These are weighed, the contents blown out into counting dishes and the tubes again weighed to 0.1 mg.

In this case we have two samples of sap 3.9 mg and 6.0 mg, upper protoplasm 4.3 and 8.7 mg, lower 4.5 and 8.5 mg. The countings corrected as above gave the following results in impulses /min./ 11.56 µl.

<table>
<thead>
<tr>
<th></th>
<th>Initial sample</th>
<th>13°0 cells put in</th>
<th>14°0</th>
<th>17°0</th>
<th>19°0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuged</td>
<td>1680</td>
<td>414</td>
<td>36</td>
<td>45</td>
<td>51</td>
</tr>
<tr>
<td>7 min.</td>
<td>26</td>
<td>365</td>
<td>1000</td>
<td>795</td>
<td></td>
</tr>
<tr>
<td>15 min.</td>
<td>19</td>
<td>400</td>
<td></td>
<td></td>
<td></td>
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</table>

The experiment shows that lead disappears rapidly from the bathing fluid and is to some extent concentrated in the protoplasm while only a little
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penetrates into the sap. Apparently the lead is preferentially absorbed by the granules, since the protoplasm from the lower end of the cell contains after centrifuging 67 to 73% of the whole.

The values for the sap may become too high and for the protoplasm too low on account of the difficulties inherent in a complete separation of the two.

Three similar experiments gave for the protoplasm from the lower half of the cells, 66%, 73% and 55%.

Three control experiments in which no centrifugation was made showed the following accidental distributions 51%, 61% and 57% for the halves containing the highest activity.

In order to determine absolute quantities of lead experiments were made with mixtures of known amounts of lead acetate and radiolead, and for these it was necessary to determine the lead concentrations which would not damage the cells.

A number of isolated Nitella cells were distributed into 5 test tubes containing increasing concentrations of lead acetate namely: distilled water, 0.001, 0.01, 0.1 and 1 millimole respectively. In the lowest concentrations including 0.01 mM the cells could live for more than 10 days. In 0.1 mM they died in 5 days and in 1 mM in two days.

An experiment was made with 1.8 ml 0.1 mM Pb(\(\text{CH}_3\text{COO}\))\(\text{2H}_2\text{O}\) to which was added 0.4 ml active lead solution.

<table>
<thead>
<tr>
<th>Initial sample</th>
<th>2370 imp./min./11.56 (\mu)l</th>
</tr>
</thead>
<tbody>
<tr>
<td>12(^{20}) cells put in</td>
<td>62</td>
</tr>
<tr>
<td>13(^{45})</td>
<td>46</td>
</tr>
<tr>
<td>14(^{30})</td>
<td>47</td>
</tr>
<tr>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>21 final sample</td>
<td>17</td>
</tr>
<tr>
<td>Sap</td>
<td>27</td>
</tr>
<tr>
<td>Protoplasm from upper half</td>
<td>265</td>
</tr>
<tr>
<td>» » lower »</td>
<td>546</td>
</tr>
</tbody>
</table>

The quantities of lead are calculated as follows. The number of impulses corresponding to the total quantity of lead added is \(\frac{2370}{11.56} = 2200 = 451000/\text{min.}\) representing 0.18 micromole (\(\mu\)M). 1000 impulses correspond therefore to

\[
\frac{1800}{451} \times 10^{-4} = 4.0 \times 10^{-4} \mu\text{M lead.}
\]

Allowing for the samples taken out the final quantity of lead in the bathing fluid works out as
\[
\frac{17.3 \times 2180}{11.56} = 3270 \text{ impulses or } 13 \times 10^{-4} \mu M \text{ lead.}
\]

For the total quantity of sap in the 6 cells experimented on we have
\[
\frac{27 \times 48}{11.56} = 112 \text{ impulses or } 0.45 \times 10^{-4} \mu M \text{ lead.}
\]

The protoplasm from the upper halves of the cells gives
\[
\frac{265 \times 12}{11.56} = 276 \text{ impulses or } 1.1 \times 10^{-4} \mu M \text{ lead and the protoplasm from the lower halves}
\]
\[
\frac{546 \times 12}{11.56} = 568 \text{ impulses or } 2.25 \times 10^{-4} \mu M \text{ lead.}
\]

The total quantities of protoplasm and sap were made out by weighing 10 cells and separating plasma and sap in capillary tubes. The results were:
- 10 cells with an average length of 70 mm weigh ................. 170 mg
- The protoplasm from them ........................................... 40 mg
- The sap ................................................................. 80 mg

Of the lead originally present 99% have disappeared and special experiments showed that almost the whole of this was adsorbed to the cells.

The percentage distribution of the lead between upper and lower protoplasm was in this case 33—67%; in two further experiments the results were 40—60% and 30—70%.

**Summary.**

The experiments support the assumption that the granules may represent a link in the mechanism for ion absorption from the external fluid and hold the ions temporarily combined.

**Literature.**


