THE ACCUMULATION OF ELECTROLYTES

VII. ORGANIC ELECTROLYTES

PART 2*

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The first part of this paper demonstrates a marked preponderance of inorganic cation equivalents in saps from the leaves of Rheum, Rumex, and Oxalis. In this connection it seemed desirable to examine the data in the literature. There are very few analyses of plant saps sufficiently complete for our requirements and these deal chiefly with cells free from chlorophyll, which are not very useful for our present purpose. But there are numerous cases in which leaves or entire plants were ashed and analyzed (these will be referred to as analyses of total ash).

A selection of the published leaf ash analyses has been treated in such a way as to show the proportion of cation to anion equivalents, (a) in the total ash, and (b) in the cell sap. In arriving at the latter values (Column 15, Table I, p. 284) the probable composition of the sap has been calculated by means of certain corrections applied to the total ash analyses, according to the scheme outlined below.

It was recognized that to calculate the composition of the sap deductions should be made from the data for total ash to account for the following:

(a) Polar substances precipitated in the cells (e.g. calcium oxalate, carbonate, pectate, and silica).

(b) Substances which yield on ashing inorganic substances, origi-


1 See, for example, the analyses of orange juice, lemon juice, etc., in the literature.

2 Fe₂O₃ and SiO₂ are usually reported in ash analyses, but since little is known of their mode of entrance into the plant except that they are probably colloidal dispersely dispersed, they have been omitted from both total ash and sap analyses.

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<table>
<thead>
<tr>
<th>Plant†</th>
<th>Ash as % of dry weight</th>
<th>Equivalents per 100 gm. of ash</th>
<th>Total cation equivalents in total ash</th>
<th>Total cation equivalents in sap</th>
<th>Total anion equivalents</th>
<th>Ratio Cation : Anion equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
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<tr>
<td>1. Rumex acetosella</td>
<td>8.14</td>
<td>0.676</td>
<td>0.230</td>
<td>0.7357</td>
<td>0.03168</td>
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<tr>
<td>2. Rheum rhabdocarpum</td>
<td>7.93</td>
<td>0.3070</td>
<td>0.0250</td>
<td>0.1411</td>
<td>0.2707</td>
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<td>3. Trifolium pratense</td>
<td>8.11</td>
<td>0.6964</td>
<td>0.1832</td>
<td>0.2330</td>
<td>0.3792</td>
<td>0.3515</td>
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<td>4. Quercus robur</td>
<td>3.05</td>
<td>0.7051</td>
<td>Trace</td>
<td>0.9318</td>
<td>0.6698</td>
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<tr>
<td>5. Arum maculatum</td>
<td>8.35</td>
<td>0.4321</td>
<td>0.0381</td>
<td>0.2480</td>
<td>0.5119</td>
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<tr>
<td>6. Morus alba</td>
<td>7.48</td>
<td>0.6653</td>
<td>0.1000</td>
<td>0.1840</td>
<td>0.6178</td>
<td>0.5878</td>
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<td>7. Spinacia oleracea</td>
<td>16.27</td>
<td>0.4985</td>
<td>0.0130</td>
<td>0.1950</td>
<td>0.3698</td>
<td>0.3560</td>
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<td>8. Anemone salicina</td>
<td>9.7100</td>
<td>0.3920</td>
<td>0.0020</td>
<td>0.1420</td>
<td>0.1410</td>
<td>0.1210</td>
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<td>9. Olea europaea</td>
<td>4.56</td>
<td>0.5590</td>
<td>0.0000</td>
<td>0.2560</td>
<td>0.2069</td>
<td>0.0550</td>
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<tr>
<td>10. Nicotiana</td>
<td>—</td>
<td>1.1010</td>
<td>0.2987</td>
<td>0.1760</td>
<td>0.8975</td>
<td>0.0060</td>
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<td>11. Syringa vulgaris</td>
<td>3.47</td>
<td>0.6751</td>
<td>0.5729</td>
<td>0.7935</td>
<td>0.4767</td>
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<td>12. Stellaria media</td>
<td>13.18</td>
<td>0.7586</td>
<td>0.2260</td>
<td>0.5000</td>
<td>0.0790</td>
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<td>13. Beta vulgaris</td>
<td>15.09</td>
<td>0.8500</td>
<td>0.3700</td>
<td>0.4820</td>
<td>0.4494</td>
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<td>14. Ficus carica</td>
<td>8.26</td>
<td>0.3166</td>
<td>0.1781</td>
<td>0.3560</td>
<td>0.6540</td>
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<td>15. Medicago sativa</td>
<td>8.71</td>
<td>0.8917</td>
<td>0.0394</td>
<td>0.8385</td>
<td>0.3104</td>
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<td>16. Daucus carota</td>
<td>13.61</td>
<td>0.2602</td>
<td>0.8203</td>
<td>0.3500</td>
<td>0.1931</td>
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<td>17. Chichorium intybus</td>
<td>12.46</td>
<td>0.2750</td>
<td>0.0248</td>
<td>0.3100</td>
<td>0.1990</td>
<td>0.1487</td>
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<td>18. Citrus aurantium</td>
<td>10.53</td>
<td>0.3515</td>
<td>0.1681</td>
<td>0.0140</td>
<td>0.2832</td>
<td>0.2681</td>
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<td>19. Atropa bellaodora</td>
<td>10.81</td>
<td>0.6726</td>
<td>0.5629</td>
<td>0.5842</td>
<td>0.3203</td>
<td>0.2994</td>
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<td>20. Primula farinosa</td>
<td>11.73</td>
<td>0.4269</td>
<td>0.2813</td>
<td>0.9208</td>
<td>0.6104</td>
<td>0.5913</td>
</tr>
</tbody>
</table>

* Except No. 1 for which see p. 293.
† For English names see footnote 43, p. 292.
nally present in non-polar linkages (e.g. chlorophyll yielding MgO and proteins yielding SO₄).

(c) Substances insoluble in water but dissolved in the non-aqueous part of the protoplasm which yield on ashing inorganic oxides (e.g. lecithin yielding P₂O₅, calcium diglyceril phosphate yielding both P₂O₅ and CaO).

(d) Inorganic anions and cations in combination with proteins in the protoplasm. But any proteinates in the protoplasm must have been formed by the interaction of MOH with the acid part of the protein and this is practically equivalent to the entrance of MOH so no deduction will be made (M is any inorganic cation).

(e) Inorganic anions and cations in solution in the intercellular liquid.

(f) Inorganic ionogenic substances in the protoplasm.

There is unfortunately no way of estimating these last two corrections. But it may be assumed that the amounts involved are small compared with the amounts of inorganic substances in the cell sap.

We shall now consider each of the ash constituents in turn in order to determine approximately what corrections should be applied.

Sodium and Potassium.—The idea that the alkali metals can be held inside cells in non-polar linkages seems to be fairly prevalent. But the recent investigations of Weevers,⁴ Penston,⁵ Lloyd,⁶ Maquenne and Demoussy,⁷ Canals,⁸...
Canaye, and Cabanes,9 Kostytischew and Eliasberg,9 and Hill and Kupalov10 indicate that this is not the case.11 This conclusion seems probable on chemical grounds, for it is clear that according to the modern theory of valence it would be very difficult to form compounds of sodium and potassium (second and third members of the a subgroup of Group 1) in which the metal is covalently instead of electrovalently bound.12 The organic compounds of these elements which have been investigated are salt-like in character (with one or two possible exceptions). Those in which the metal is not directly linked to a carbon atom, such as salts of organic acids, are in general soluble and are hydrolyzed more or less according to the strength of the acid. Those in which the metal is apparently joined to a carbon atom are such as the alkali alkyls (e.g. NaCH₃), the alkali aryls (e.g. NaC₆H₅), the alkali alkyl aryls (e.g. C₆H₅–CN₃), are extremely unstable, some of them uniting explosively with oxygen when exposed to the air and all of them being decomposed violently by a trace of moisture. They are also salt-like in organic solvents. It was formerly supposed by Schlenk and Holtz13 that the colorless compounds of this group were non-polar but it has since been established by Hein and his coworkers14 that like colored members they are ionized in suitable solvents. For example, sodium ethyl and other colorless compounds of this class conduct electrolytically in zinc ethyl which is itself a non-conductor. Accordingly, as Rodebush15 points out, if we could liquefy a compound such as sodium ethyl without decomposition we have every reason to suppose that it would show the ionization and conductance of a fused polar salt. Other groups of metal to carbon linkage compounds are formed by the addition of the metal to unsaturated groups, such as –C=–C– and –C=–N– and –C=–O–, and by the addition of alkali alkyls and aryls to –C=–C–. Although the physical chemistry of these compounds has not been investigated

12 Fajans, K., Naturwissenschaften, 1923, 11, 165.
13 Schlenk, W., and Holtz, J., Ber. chem. Ges., 1917, 60, 262.
carefully it may be assumed that they also are polar. In any case they are
decomposed by water very readily.  
This suggests that the possibility of the occurrence of non-polar compounds of
sodium and potassium in plant tissues is remote, as indeed Kostytschew and
Eliasberg concluded on experimental grounds.

It is possible also that a part of the alkalies appearing in the ash may be derived
(a) from insoluble salts. This is unlikely because of the general solubility of
alkali salts in water. (b) From salts dissolved in the oily part of the protoplasm.
In this connection it has been suggested in a former paper that HX, the sub-
stance in the protoplasm, which furnishes anions to transfer cations between
the outside and the vacuole, may be a diglycerol phosphoric acid. Hundeshagen has
shown that the alkali salts of such compounds are more soluble in some non-
polar solvents than in water, and Chibnall and his coworkers have found similar
substances in the protoplasm of a variety of cabbage (Brassica oleracea, L.) and
crow’s foot grass (Dactylis glomerata, L.) in the form of the calcium salts. It
is, of course, quite improbable that any of the purely inorganic salts of sodium
or potassium will be as soluble in the oily part of the protoplasm.

Probably also in some leaves a certain amount of alkali proteinates is dispersed
in the protoplasm. In others the protein is cationic.

Some recent work by Sidgwick and Brewer (Sidgwick, N. V., and Brewer,
F. M., J. Chem. Soc., 1925, 127, 2379) suggests that organic compounds in which
sodium or potassium are covalently linked in chelate rings are possible. These
compounds are the result of the reaction between the metal hydroxide and beta
diketones and similar compounds. Usually the compound is salt-like in char-
acter, but in a few cases derivatives have been obtained which are non-polar
since they dissolve in toluene and other non-hydroxyl organic solvents, and have
definite melting points. The authors assign the following structure to the benzoyl
acetone derivative,

\[ \text{H} \quad \text{CH}_3 \quad \text{C} \quad \text{O} \quad \text{O} \quad \text{C} \quad \text{CH}_3 \\
\text{HC} \quad \text{Na} \quad \text{NCH} \quad \text{GH} \quad \text{C} \quad \text{O} \quad \text{C} \quad \text{CH}_3 \]

in which the metal is bound covalently between two chelate rings. These sub-
stances are very unstable in the presence of water.


Since alkali and alkali proteinate are formed by the interaction of alkali hydroxide with an acid group, no correction will be applied to the ash analyses to account for them. In any case the amount of alkali combined with X is certainly small and the calculation below indicates that the correction for alkali proteinate does not exceed 5 per cent. According to Chibnall and Grover the proteins extracted from leaves are similar to glutelins but as far as we know the predominant plant proteins are globulins. According to Kodama one of these, edestin, has a combining weight of 5402 at pH 9.34. The pH outside the protoplasm may possibly reach this value due to photosynthesis. Svedberg has found that the molecular weight of edestin is 208,000. Green, on the basis of the Debye-Hückel theory, has calculated that its “valence type” around pH 7.0 is about 25, which makes it either quinquaque or 25 valent. The latter figure gives a combining weight 8320. The true combining weight no doubt lies between this figure and 5402. But taking 5402, then \( \frac{75}{5402} \) = 0.014 equivalent of alkali cations might conceivably be present in the protoplasm in combination with protein. But Chibnall considers that the amounts of protein he was able to extract from leaves are less than the actual amounts present, due to losses of various kinds, so that it may be assumed that 150 gm. of protein are present in 1000 gm. of dry leaves. This amount could hold in combination 0.028 equivalent of alkali cations or roughly 0.5 per cent of the total alkali present. This calculation ignores the fact that at least some Ca and Mg proteinates must also be present.

Magnesium.—Magnesium appears to be deposited in plant tissue as phosphate, but this is so rare as to be negligible. Some magnesium may also be bound by protein, but here again, magnesium proteinate is formed by the interaction of Mg(OH)\(_2\) and the acid portion of the protein molecule and no correction has been applied. The only other important non-sap occurrence of magnesium is in the chlorophyll. In the case of green leaves a correction has been computed for each case on the basis of the average amount of chlorophyll in green leaves as given in the literature. Thus Willstätter and Stoll found that for the leaves of five common trees, the average chlorophyll content was 0.8 per cent of the fresh leaves or 2.5 per cent of the dry leaves. Similar values have been found by Lubimenko and by Sjöberg.

31 Kodama, K., J. Biol. Chem., Japan, 1922, 1, 419.
31 Green, A., J. Biol. Chem., 1932, 96, 47.
35 Lubimenko, V., Compt. rend. Acad., 1924, 179, 1073.
36 Sjöberg, K., Biochem. Z., Berlin, 1931, 240, 156.
In general the ash is about 10 per cent of the dry weight of the leaves so that
100 gm. of ash which corresponds to 1000 gm. of dry leaves would contain the
magnesium derived from 10 gm. of chlorophyll. But chlorophyll a or b contains
about 2.7 per cent Mg which corresponds to 0.27 gm. of Mg or 0.0225 equivalent
of Mg in 100 gm. of ash.

Apparently therefore a small but not negligible correction ought to be applied
to the magnesium ash results for green leaves, and this has been done by assuming
in each case that the chlorophyll content is 1 per cent of the dry leaf weight of
the leaves and calculating from the published data the weight of leaves necessary
to produce 100 gm. of ash. For example, the pure ash of Beta vulgaris is given
as 15.09 per cent in Wolff, and hence 669 gm. of dry leaf correspond in this case
to 100 gm. ash, and the Mg correction would be 0.0149 equivalent.

Calcium.—Calcium occurs in polar, but insoluble form, as the oxalate, sulfate,
and phosphate in plant tissues and according to Chibnall and Channon27 as Ca
salts, of diglyceryl phosphoric acid, dissolved in the cytoplasm. The results of
Chibnall and Channon indicate in the case of cabbage leaf that about 2/3 of
the Ca present is water-soluble. Kostychev and Berg28 find for a series of plants
only about 1/3 of the Ca to be extracted by water, the remainder being present
in insoluble ionogenic form. Calcium may also be present in combination with
proteins, as a proteinate and possibly also combined in a non-polar manner.

In our calculation to sap composition, in order to avoid the inclusion of calcium
not originally present as ions, we have corrected the ash data by excluding Ca
entirely.

Iron.—There is very little evidence for the assumption that iron is present as
a cation in leaf sap, although the ash yields appreciable amounts of Fe2O3. How-
ever, Jones29 has suggested recently on the basis of microprecipitation reactions
that ionogenic iron is widespread in vegetable tissues. This is contrary to the
work of Chibnall and Channon who found no “soluble” iron in cabbage leaf, and
of Maquenne and Cerighelli30 who found that the expressed and centrifuged
juices of romaine, lettuce, and other vegetables, high in iron content, contained
very little iron. On the basis of these latter results we have corrected the ash
data for iron by omitting it from the cations.

Chloride.—As far as we can determine no compounds have been isolated in
plants in which chlorine was either present in an insoluble polar compound or
coordinate bound in an organic molecule. Jung31 who has examined numerous

28 Kostychev, S., and Berg, V., Planta, 1929, 8, 56.
30 Maquenne, L., and Cerighelli, R., Bull. Soc. chim., France, 1921, 29, series
4, 899.
1920, 128, 297.
plant tissues concludes that the chlorine is always present in plants as chloride. He found most of the chloride in the leaves.

Wood found that in the xerophytic plants *Atriplex* and *Kochia*, which take up a great deal of NaCl from the soil, the amount taken up by the leaves was roughly a function of the amount in the soil, and that most of it was in the veins, not in the cells. It is well known that seashore plants also take up sodium chloride in large quantities, and Wood’s work at once suggests that where the chloride content is abnormally high, particularly when the sodium content is also high (which seems to be nearly always the case), a great part of it is present as sodium chloride in the veins, and should not be counted as cell sap. It is not possible to correct for this condition quantitatively. But it is a point to be borne in mind wherever the chloride and sodium contents are unusually high. Obviously the presence of much NaCl in the leaf veins will lower the ratio cations ÷ anions.

*Sulfate.*-Brunswik found that crystals of calcium sulfate were abundant in the leaves and young stems of the Tamaricaceae, but generally, sulfate is not present in crystals precipitated in the cells. A certain amount of sulfur is present in some cases in the form of essential oils, but as these are volatile not much sulfate ion will find its way into the ash from this source. Probably in leaves the chief source of sulfate ion which was not originally present as such in the cell sap is the sulfur coordinately bound up in the protein of the protoplasm and the sap. Assuming, as before, that there are 150 gm. of protein present for each 100 gm. of ash, if the sulfur content is taken as 1 per cent, the sulfate which might be derived from this source would be 0.1 equivalent. However, as Bertrand and Silberstein have shown, up to 50 per cent of the sulfur present in the plant is lost when the plant is ashed instead of being oxidized by wet methods, so that the deduction on this account might be 0.05 equivalent. However, in order to avoid overcorrection of an anion no correction has been applied.

*Phosphate.*—In some plants calcium or magnesium phosphate crystals occur, but this is rare.

The chief source of phosphoric acid in leaves is probably the phospholipins (phosphatides) or related substances of the protoplasm, such as the Ca diglycerol phosphatidate found by Chibnall and Channon in the leaf protoplasm of *Brassica oleracea*. Chibnall and Channon found that about 75 per cent of the total phosphate of the leaf is water-soluble, but their ether-soluble phosphoric acid derivatives accounted for only about 10 per cent of the remainder. André has treated dried and powdered lilac and chestnut leaves with alcohol and ether, to extract what he calls the organic phosphorus. It appears from his results that about 10 per cent of the total phosphorus, according to this criterion, is organic.

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In addition to the well-known phosphatides and derivatives, such as Chibnall found in leaves, and which are known to be present in larger quantities in seeds, Hansteen-Cranner, Grafe, and Magistris have claimed to have extracted by water alone phosphatides from living plant tissues such as carrots, beets, etc., without injuring the tissue. But this is disputed by Steward.

On the whole it appears that about 80 to 90 per cent of the phosphate in the ash was originally present in the cell sap as such, and in order to avoid any suspicion of over-correction we shall make no deduction from the published phosphate figures. However, we shall regard the phosphoric acid as a dibasic and not a tribasic acid because the pH of plant saps is almost always below 7.0 where the \( PO_{4}^{-2} \) ion can exist only in infinitesimal amounts.

**Silica.**—Silica is almost always present as deposited SiO\(_{2}\), but it is clear that in the intracellular liquid it must be either dissolved or collooidally dispersed, since it makes its way from the roots and deposits in part in the leaves. As long ago as 1878 Lange concluded that it is present as colloidal salt dispersed free silicic acid, and this conclusion is accepted by Nanji and Shaw in their work on silica in straw. These writers, however, believe that about 10 per cent of the silica might be present in the form of carbohydrate esters, but no such compounds were isolated.

In any case it may be assumed with confidence that there are no silicate ions in the cell sap, since, as Hägg has recently shown, the dissociation constants of \( H_{2}SiO_{4} \), which he considers to be the only true silicic acid, are of the order of \( 10^{-9} \) and \( 10^{-19} \).

Accordingly we shall assume that the silica of the ash analyses contributed no anions to the cell sap.

**Nitrate.**—It is conceivable that there is present in the sap nitrate ion. This does not appear in the ash analyses. It is difficult to estimate what correction should be made but it may be concluded that it is small. Thus, according to Campbell's results for twenty-five weeds the nitrate nitrogen did not exceed 0.80 per cent of the dry weight of the plant tissue. In most cases it was much less and at maturity it was almost always absent.

There are present also basic nitrogen compounds, such as ammonia and organic bases which are cationic at the Ca encountered in leaf saps. These also disappear in ashing so that the error due to the failure to account for anionic nitrogen is offset to some extent.

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Ionogenic Inorganic Substances in General.—The processes of drying and ashing employed in reducing plant tissue to an analyzable form are drastic. Thus various sulfur compounds are probably oxidized to \( \text{SO}_3 \) or \( \text{H}_2\text{SO}_4 \). Phosphoric acid is freed from the organic combinations in which it is usually found in plant materials, and probably certain amounts of pyrophosphoric and metaphosphoric acids are produced. All these acids because of their lower volatility are capable of decomposing chlorides, so that if any appreciable amounts of free acids are formed chloride ion may be replaced. This is an additional reason for not making a correction for sulfate or phosphate.

Further, in the course of drying the plant material there is the possibility that magnesium chloride and possibly calcium chloride may be hydrolyzed in part to basic chlorides with a corresponding loss of chloride ion. However, since nearly all the water is driven off in the neighborhood of 100°C, this loss is probably not serious, for as a number of investigators have shown the hydrolytic decomposition of hydrated magnesium chloride is not marked below 200°C in air.\(^4\) Hydrated calcium chloride is even more stable.

Summarizing, we conclude that to transform ash analyses of total ash into analyses of sap the following corrections should be made. Ferric or ferrous iron and silicate should be omitted since the amounts dissolved in the cell sap are negligible. Calcium, although some may be dissolved in the sap, should be omitted since the amount is doubtful. A deduction should be made in the case of magnesium to allow for the amount contained in chlorophyll. No deduction should be made from the sulfate or phosphate figures; but phosphate should be taken as bivalent. No correction should be applied to sodium, potassium, or chloride.

Table I\(^4\) (p. 284) gives figures derived from the data in the literature. In the original these results were expressed as percentages of

\(^4\) For references see Mellor, J. W., A comprehensive treatise of inorganic and theoretical chemistry, London, Longmans, Green and Co., 1923, 4, 300.

\(^4\) The data from which the figures in Table I are derived were taken from Wolff, E., Aschen-Analysen von landwirtschaftlichen Producten, Berlin, Wiegandt und Hempel, 1871. All relate to leaves except the figures for \textit{Rumex acetosella} which apply to the whole plant in which the volume of root and stem is small as compared to that of the leaf (as explained on p. 293 the whole plant may be included without error). The English names are as follows: (1) field sorrel; (2) rhubarb; (3) red clover; (4) oak; (5) wake robbin; (6) mulberry; (7) spinach; (8) oat; (9) olive; (10) tobacco; (11) lilac; (12) chickweed; (13) beet; (14) fig; (15) alfalfa; (16) carrot; (17) chicory; (18) orange; (19) deadly nightshade; (20) primrose.
oxides in the pure ash. Here they are expressed as equivalents of the corresponding basic or acidic radical per 100 gm. of pure ash. They are therefore not conventional concentration terms. These cannot be estimated.

Let us first consider the total ash. Columns 1 to 6, and 8 to 10 are self-explanatory. Column 11 is the sum of Columns 3, 4, 5, and 6. Column 13 is the sum of Columns 8, 9, and 10. Column 14 is obtained by dividing the values in Column 11 by those in Column 13.

Let us now consider the composition of the sap as calculated from the total ash by the method summarized on p. 292. Column 7 gives the values for magnesium after deducting what was probably present in the chlorophyll. Column 12 is the sum of Columns 3, 4, and 7: Column 15 is obtained by dividing the values in Column 12 by those in Column 13. To the extent that the corrections are right, Column 15 gives the actual preponderance of inorganic cations over anions in the sap. These results are comparable therefore with those given in Part 1, for the leaf saps of Rheum, Rumex, and Oxalis. The selection of data yields ratios of cations/anions both higher and lower than the average 3.8 given in the table on p. 239 of Part 1. For the most part plants showing a fairly high ratio of cations to anions in the sap were selected for Table I as being of more interest from our present point of view, but it should be pointed out that in only a very few of the hundred or more cases examined in the literature were the anion equivalents greater than cation equivalents in the corrected figures for sap, and in no case was this true of the total ash.

The above analyses are of leaves only (except Rumex acetosa)44). When organic substances manufactured in a green cell migrate to a colorless cell of the same plant and are analyzed along with the green cells they may be regarded as on practically the same basis as if they still remained in the green cell. Hence the colorless cells of the leaf, or of the root and stem of the same plant, may be included in the analysis along with the green cells.

It is, however, misleading to take analyses of colorless cells by themselves (e.g. fruits, roots, fungi, etc.) since these cells may have absorbed inorganic cations paired with organic anions. Usually a high ratio of cations to anions is found in such cells (in the banana fruit,

Musa sapientum, the ratio of cation to anion equivalents in the total ash is 8.64).

In this connection we may consider the situation in animal cells. Hill and Kupalov, and likewise Fenn and Cobb, state that there is an excess of inorganic cation equivalents in frog muscle. Page found this to be true of eggs of the sea urchin (Arbacia) and of the starfish (Asterias) which are devoid of yolk. It also applies to the white of hen's egg but it is evident from Needham's summary that in the yolk there is so much sulfur and phosphorus in organic combination (yielding inorganic anions on ashing) that the anion equivalents preponderate. As the yolk is used up in development the situation changes and the cation equivalents predominate more and more. It is of interest to note that such a predominance is also found in the human embryo.

**DISCUSSION**

As already stated (Part 1, p. 235) our experiments on Valonia were thought to indicate that the cations enter chiefly as MOH and react in the sap with an organic acid, HA, to form KA. This would mean that the penetration of cations is necessarily accompanied by the formation of organic salts which should be capable of detection when they exist in sufficient quantity.

In the experiments described in Part 1 of this paper such salts were found and their presence in other cases (Part 2) is shown by the fact that the inorganic cation equivalents exceed the anion equivalents. As already mentioned this excess has been expressed in two ways in Table I: as the ratio of cations to anions in the total ash, and as the ratio of cations to anions in the cell sap. The sap data are chiefly interesting from the standpoint of accumulation where the relation between the soluble electrolytes of the sap and the external solution

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is important. At present, however, we are concerned chiefly with the question whether an excess of inorganic cations over inorganic anions has entered the leaf cells. From this point of view all the inorganic constituents (except iron and silica which for reasons mentioned before are excluded) should be counted, because, regardless of the state in which they happen to be in the leaf cells at the time of analysis, obviously at some stage in the plant's growth they have entered as part of a dissolved electrovalent compound. (For example, many green cells contain calcium oxalate crystals, and this calcium, not in solution in the cell sap, must be counted, since there is no reason to suspect that its mode of entrance is different from that of calcium which may be present in another cell as dissolved calcium chloride.)

The same is true of the anions which form inorganic elements such as sulfur and phosphorus, and hence all sulfate and phosphate reported in the ash should be counted. However, it must be admitted that the anion total is probably too low because, as Bertrand and Silberstein have shown, in the dry ashing up to 50 per cent of the sulfur is lost. Further, as pointed out below, some of the SO$_4^{--}$ and HPO$_4^{--}$ present may represent chloride which has been displaced during ashing.

Hence in determining whether cations have entered as hydroxides we should use the figures for total ash.

Before concluding that excess of cation equivalents means the entrance of cations combined with hydroxyl we must examine other alternatives. We may now consider the various possibilities in turn.

(a) Ionic Exchange.—When the external solution loses a molecule of MOH it is equivalent to losing M$^+$ and gaining H$^+$ and when MOH reacts in the sap with HA to form MA it is equivalent to losing H$^+$ from the sap and gaining M$^+$. Hence the net result is the same as if H$^+$ from the sap were exchanged for M$^+$ from the external solution, the ions passing as such through the protoplasmic surface.

For reasons elsewhere set forth in detail it seems improbable that a

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49 "Entrance" in this connection means simply passage from the aqueous external solution through the outer non-aqueous protoplasmic surface. It does not imply penetration as far as the vacuole although this usually occurs. However, a certain amount of magnesium passes into the protoplasm and is combined in the chlorophyll molecule. This is counted as having entered the cell.

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passage of ions as such through the protoplasmic surface plays an important part in the penetration of electrolytes and hence in the subsequent discussion it will be regarded as a negligible factor.

(b) Entrance of Organic Electrolytes.—In the case of cells devoid of chlorophyll, whether plant or animal, the excess of cation equivalents might be thought to indicate the entrance of cations in combination with organic anions. But this cannot be the case with cells containing chlorophyll which manufacture organic from inorganic materials and can at best absorb only minute amounts of organic substances from the soil.

(c) Entrance of Nitrates.—If a cation should pass into the protoplasm in combination with nitrate the latter might be reduced and built up into the protein molecule, leaving the cation free to pair with an organic anion elaborated in the vacuole. The ash would then show an excess of inorganic cations over anions. To what extent this process can occur is as yet unsettled. Certainly nitrate ion enters the plant and is used in protein synthesis. (This entrance could occur by exchange, in the same way as we believe that Cl⁻ is exchanged for HCO₃⁻ in Valonia.) The amount of nitrate ion present in leaves is seldom more than a trace, and in many cases it is entirely absent. Thus in the sap extracted from Rheum, Rumex, and Oxalis we found no definite reaction for nitrate ion. (This, of course, does not mean that no nitrates were taken up by the roots for it is well known that such nitrates may not appear in the leaves presumably because they are reduced in the root or stem to ammonia which enters the leaf cells as such, or as NH₄OH, or as some other compound.) However, if this ion goes into the protein molecule, we should scarcely expect to find more than a trace.

If it may be assumed that all the protein of leaves has been derived from $M\text{NO}_3$, it is possible to estimate the maximum amount of $M$
(cation) which may have entered the leaf cells in this manner. Thus, as pointed out previously, according to the results of Chibnall and Grover\textsuperscript{50} there appears to be on the average 150 gm. of protein per 100 gm. of pure ash in leaves. If 16 per cent of this is nitrogen, this corresponds to \[ \frac{150 \times 16}{14 \times 100} = 1.7 \text{ equivalents of nitrate nitrogen.} \]

Table I shows this is usually greater than the cation total in the sap. Further the weight of evidence\textsuperscript{52} suggests that the primary products required for the synthesis of proteins (the amino acids) are produced in the leaf and are transferred to other parts of the plant for the completion of the synthesis. On this basis it is clear that far more than enough nitrate could enter the leaf to account for all the cations which have entered.

But the experiments of Prianischnikow\textsuperscript{51} and others indicate that ammonia is taken up in preference to nitrates so that when \( \text{NH}_4\text{NO}_3 \) is present the external solution becomes acid.

It seems possible that in the leaf cells studied by us some of the nitrogen was taken up in the form of \( \text{NH}_4 \) and \( \text{NH}_3 \), as well as in the form of \( \text{NO}_3 \). In this case the ratio of cations to anions might not be greatly affected.\textsuperscript{53}

But there is no reason to suppose that if potassium entered as nitrate the rate would be affected by changes of pH as was found in experiments on \textit{Valonia}. Here it seems very improbable that the entrance of nitrates plays an important rôle. Moreover unless there is a considerable loss of nitrogen (which is highly improbable) the cell as a whole would contain at least as many equivalents of protein nitrogen as of potassium if the latter entered solely as nitrate: this is certainly not the case in \textit{Valonia}.

\textit{(d) Entrance of Bicarbonates.}—If the cations entered in combination with bicarbonate ion, and if \( \text{CO}_2 \) derived from the latter were subsequently used in the photosynthesis of carbohydrates, the cations could pair with organic anions in the cell. This situation, of course, would

\textsuperscript{50} See Onslow, M. W., Principles of plant biochemistry, Cambridge University Press, 1931, chapter V.

\textsuperscript{52} Presumably cations would be used up first since to make protein \( \text{NO}_3 \) must be converted to \( \text{NH}_3 \); this would lower the ratio of cation equivalents to anion equivalents.
give an excess of inorganic cations on ashing. The proportions of bicarbonate ion to CO$_2$ on the one hand, and to carbonate ion on the other, depend, of course, on pH, but it happens that in sea water when the pH is changed from 8 to 9 the bicarbonate fraction of the system changes very little$^{54}$ so that we should expect that if the cations enter as bicarbonate little effect would be observed in the case of *Valonia* on changing the sea water pH from 8 to 9. Actually, however, there was a more than three fold increase in the rate of potassium entrance when the pH was raised from 8.2 to 8.5 to 8.8.

Other experiments on *Valonia* show that CO$_2$ enters very rapidly with little or no penetration of bicarbonate. M. M. Brooks$^{57}$ states that the sap of cells exposed to bicarbonates became more alkaline (the pH being measured after the removal of CO$_2$ from the sap) and ascribes this to the entrance of bicarbonate but it is evident that the same result would be produced by the penetration of KOH.

We therefore suppose that if the ionic activity product (H) (HCO$_3^-$) should at any time become greater outside equality would be promptly restored by the penetration of CO$_2$ rather than by that of bicarbonate. Since the cell is constantly producing CO$_2$ its movement is outward except when photosynthesis is taking place.

It seems probable that such considerations also apply to the green cells of the leaf.

(e) Entrance of Sulfate and Phosphate.—Since both sulfur and phosphorus appear in organic combination in the protoplasm it is possible that cations may have entered in combination with sulfates or phosphates, which have subsequently been utilized in the protoplasm, leaving the cation free to pair with an organic anion. In the case of *Valonia*, however, this seems impossible, for the total amounts of organosulfur or organophosphorus compounds in the cell are certainly far too small to account for all the cation which has entered the cell.

Summarizing then we believe that the above considerations show

$^{54}$ Cf. Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925-26, 9, 255. There is little change in the concentration of HCO$_3^-$ or CO$_3^{2-}$ when the pH changes from 8 to 9.


that the suggestion that cations enter mainly in combination with hydroxyl ion is consistent with all the known facts of penetration in *Valonia* and also in green plant cells.

Adopting this view our present picture of the entrance of cations is as follows. MOH unites with a constituent of the protoplasm HX, as suggested by certain models, forming MX which reacts in the sap with a weak organic acid HA to form MA and that A is then exchanged for Cl or other inorganic anions coming from the external solution.

This process may be practically complete as in *Valonia*, or may be much less extensive as in the case of the leaves studied by us, and in those cited from the literature, which give a high ratio of cation to anion equivalents. As intermediate cases we may mention *Chara ceratophylla*, Wallr., as described by Collander, and *Nitella clavata*, as described by Hoagland and his associates, where the ratio is about 1.1.

**SUMMARY**

Analyses have been made of the inorganic constituents of the juices expressed from the leaves of *Rheum, Rumex*, and *Oxalis*.

It has been shown that in all cases there is a large excess of inorganic cations over anions in the sap, the average ratio of cations to anions being 3.8 (Part 1, p. 239).

The ash analyses of plant tissues (chiefly leaves) reported in the literature have been examined critically, and it has been shown that the preponderance of inorganic cations over inorganic anions in the ash and in the sap is general.

It has been concluded that the excess of inorganic cations is consistent with the view that cations pass into the protoplasm chiefly in the form of hydroxides, and are accumulated either in the form of organic salts (such as the oxalates) or in non-polar linkage.

It has been concluded that practically all the potassium and sodium found in plant ash must have been present originally in the form of soluble ionogenic compounds, but that a considerable part of the cal-

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60 Zscheile, F. P., Jr., *Protoplasma*, 1930, 11, 481.
Calcium and magnesium may have been present originally in the form of insoluble salts or as components of non-polar compounds.

The methods whereby the cations, particularly potassium, may have been accumulated have been discussed, and it has been concluded that as it does not seem very probable that they enter chiefly as nitrates or bicarbonates we may suppose that they go in to a large extent as hydrates: this is highly probable in the case which has been most carefully investigated (*Valonia*).