

THE ACCUMULATION OF ELECTROLYTES

VII. ORGANIC ELECTROLYTES

PART 1

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It has been suggested that potassium may enter *Valonia* chiefly as KOH which reacts with an organic acid HA in the sap to form KA, after which A is exchanged for chloride so that potassium accumulates as KCl. This is not contradicted by any known facts and it is supported by a variety of evidence; inasmuch as this has recently been discussed¹ it need not be cited here. Other cations may act similarly in this and in other cells.

This means that the entrance of cations is accompanied by the formation of organic salts whose presence could be demonstrated if they existed in sufficient quantity. For this reason it seemed desirable to examine the sap of certain plants in which considerable amounts of organic acid could be expected.

The plants studied were *Rheum rhabonticum*, L. (the rhubarb used as a vegetable), *Rumex acetosella*, L. (field or sheep sorrel), and *Oxalis corniculata*, L. (wood sorrel) mixed with a small amount of *Oxalis filipes*, Small.

A complete analysis of the plant sap for all ions, including the organic anions, is unfortunately not possible at the present time since to deal quantitatively with complex mixtures of organic anions a very large amount² of material must be available. But the analyses here given throw some light on the question at issue.

¹ Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 967. Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1933-34, **17**, 727.

² For reasons which will be discussed in Part 2 we confined our investigation to leaves: these were available only in limited amounts.

Experimental Procedure

After collection the plants were kept in a moist cool place. Only the thinner parts of the leaves were used for the extraction of sap: the leaf petioles were rejected, and in the case of *Rheum* the thick midvein of the leaf also. Just before use the leaves were rinsed first in tap and then in distilled water and were dried between sheets of filter paper. They were then killed by dipping for a few seconds in ether according to the method of Chibnall,³ and, after the removal of excess ether by evaporation, transferred to a screw press (tincture press) in which the sap was expressed. In all cases moderately cloudy saps, orange to reddish in color and nearly free of chlorophyll, were obtained. Immediately after expression the sap was treated with an equal quantity of alcohol slightly acidified with acetic acid (or in two cases strongly acidified by the solution of dry HCl gas). A small portion of untreated sap was retained in each case for the determination of acidity, pH, and, where HCl-alcohol was used, for chloride ion.

In the treated saps after 24 hours in the ice box a heavy grey-white precipitate had formed. This was removed by centrifugalizing at 4000 R.P.M. for 20 to 30 minutes. The decanted sap was perfectly clear, but still highly colored. Microscopically the precipitate was found to consist of a small amount of fibrous material, a great deal of poorly crystallized material, probably organic, and, except where the strongly acidified alcohol was used, a few well defined crystals. These might have been calcium or magnesium oxalate or phosphate. Analyses were then made according to the following procedures.

Phosphate.—This was determined by the ammonium phosphomolybdate method of Woy,⁴ the precipitate being subsequently purified according to the recommendations of Treadwell.⁵ The sample was first dried on the water bath to remove alcohol, then treated several times with concentrated nitric acid to destroy organic matter. After removal of the excess acid by evaporation just to dryness, the residue was taken up in water, and the precipitation was carried out in the prescribed manner.

Calcium and Magnesium.—Calcium and magnesium were found to be present in the two cases where the sap was treated with the HCl-alcohol mixture.

Preliminary experiments indicated that when the sap was made alkaline with ammonia a flocculent precipitate of organic matter was thrown out. Accordingly it was considered advisable to get rid of organic matter first. The sap was dried on the water bath, and in the oven at 130°, and finally ignited at low red heat until a fairly white ash was secured. The ash was extracted with hot dilute HCl to ensure the solution of oxochlorides and of $Mg_2P_2O_7$. After filtration the

³ Chibnall, A. C., *J. Biol. Chem.*, 1923, **55**, 333.

⁴ Woy, R., *Chem.-Zig.*, 1897, **21**, 442.

⁵ Treadwell, F. P., and Hall, W. T., *Analytical chemistry*. II. Quantitative analysis, New York, John Wiley and Son, 6th edition, 1924, 383.

calcium was precipitated in the filtrate by means of ammonium oxalate in the presence of ammonia and NH_4Cl . A visible precipitate was produced but after ignition the amount of CaO was too little to weigh on an ordinary balance. Magnesium was precipitated in the filtrate from the calcium determination as MgNH_4PO_4 and ignited to $\text{Mg}_2\text{P}_2\text{O}_7$, according to the method of Schmitz.⁶

Sodium and Potassium.—These were determined together as the sulfates, and potassium as potassium chlorplatinate according to the Lindo-Gladding procedure,⁷ the sodium being then calculated by difference. The sample was dried and ignited just below red heat. In order to avoid the loss of KCl ignition was carried only far enough to decompose the organic compounds. The black residue was broken up and extracted with hot water. Where phosphate was present it was removed by the familiar basic ferric acetate method, and magnesium (in the two cases where it was present) was removed by means of the Schaffgottsche reagent.⁸ The filtrate from these separations containing sodium, potassium, and ammonium sulfate and chloride was evaporated to dryness, and the residue ignited below red heat to remove ammonium. It was then taken up in water treated with a few drops of concentrated sulfuric acid. The solution was then evaporated first on the water bath and then on the sand bath, and finally ignited at low red heat to decompose the acid sulfates. After weighing the salts were dissolved and the potassium was determined in the solution as described above.

Sulfate.—Sulfate was determined in the alcohol mixture directly by precipitation as barium sulfate. The precipitate was filtered through paper and ignited to get rid of occluded organic matter.

Chloride.—In order to remove organic materials which might reduce silver nitrate the sample was ignited below red heat until all the organic matter was decomposed. The black residue was extracted, filtered, and the chloride was determined gravimetrically in the filtrate as silver chloride.

Acidity.—The acidity of the sap was determined by titrating with CO_2 -free sodium hydroxide to the change point of phenolphthalein.

The pH was determined in two cases by the glass electrode.⁹

Nitrate.—Tests were made for nitrate by means of nitron and diphenylamine but only doubtful traces were found.

Organic Acids.—Unfortunately the literature contains no satisfactory procedures for the determination of the mixtures of organic anions in small quantities of material. Vickery and Pucher¹⁰ have devised methods for the anions

⁶ Schmitz, B., *Z. anal. Chem.*, 1906, **45**, 512.

⁷ See Treadwell, F. P., and Hall, W. T., *Analytical chemistry*. II. Quantitative analysis, New York, John Wiley and Son, 6th edition, 1924, 79.

⁸ See Treadwell, F. P., and Hall, W. T., *Analytical chemistry*. II. Quantitative analysis, New York, John Wiley and Son, 6th edition, 1924, 62.

⁹ These determinations were kindly made by S. E. Hill.

¹⁰ Vickery, H. B., and Pucher, G. W., *Bull. Conn. Agric. Exp. Station*, No. 323, 1931.

present in tobacco leaves. These methods depend on precipitating the anions as barium salts, decomposing the salts by sulfuric acid, extracting them into ether, and esterifying, and fractionally distilling the esters, and are better adapted to large samples.

In view of these facts it was deemed inadvisable to attempt at this time any thorough investigation of the acids present in our plants. In all cases oxalic acid was identified by means of the fairly characteristic calcium oxalate crystals.¹¹

RESULTS

The results of the analyses are given in Table I.

Evidently the leaf saps were mixtures derived from three possible sources, the vacuoles, the protoplasm, and the intercellular spaces. However, we are chiefly interested in the ionogenic constituents of the first.

Chibnall⁸ has shown that the method of extracting the leaf sap by treating the leaves with ether and then pressing results in the expulsion of the vacuole sap but not of the protoplasm, the cells being flattened but not apparently ruptured.¹²

The admixture of the intercellular fluid with sap from the vacuoles cannot be avoided but it does not seem important in this work since it would not be expected to increase the ratio of cations to anions, which is the essential point involved in the present investigation. The intercellular fluid probably consists almost entirely of inorganic compounds, being mostly the external solution (somewhat concentrated by evaporation) plus CO₂ and perhaps some other materials given out by the cells. Its content of solutes is very small as compared with the sap¹³ and the amount mixed with the sap cannot be large since the intercellular spaces are mostly filled with gases.

In the discussion which follows it will be assumed that the substances reported were present in the clarified saps as ions or parts of dissolved ionogenic substances. This assumption, which we believe to be valid, will be discussed more fully in Part 2.

¹¹ Behrens, H., and Kley, C., *Organische mikrochemische Analyse*, Leipsic, Leopold Voss, 2nd edition, 1922, 332.

¹² In the experiments here described the saps were chlorophyll-free, which indicates that they did not contain much protoplasm.

¹³ In this connection see the work of Shedd, O. M., and Kastle, J. H., *J. Am. Chem. Soc.*, 1912, **34**, 1415, on the liquid exuding from cut branches of the grape.

TABLE I
Analyses of Plant Leaf Saps

Plant	Equivalent concentrations										Cations Anions	Acidity*	pH
	K ⁺	Na ⁺	Ca ⁺⁺	Mg ⁺⁺	H ₂ PO ₄ ⁻	SO ₄ ⁻⁻	Cl ⁻	Total cations	Total anions				
<i>Rheum rhabonticum</i> , L.	0.0702	0.0130	None	None	0.0038	Trace	0.0203	0.0832	0.0241	3.5	0.0471	4.49	
	0.0722	0.0104	None	None	0.0024	0.0022	0.0187	0.0826	0.0233	3.5	0.0775		
	0.0871	0.0046	Trace	0.0433	0.0039	0.0020	0.0199	0.1350	0.0258	5.3	0.0577		
<i>Rumex acetosella</i> , L.	0.0330	0.0091	None	None	Trace	0.0018	0.0066	0.0421	0.0084	5.0	0.0975	3.32	
	0.0174	0.0046	Trace	0.0101	0.0063	0.0045	0.0074	0.0321	0.0182	1.8	0.0988		
<i>Oxalis</i> (90 per cent <i>Oxalis corniculata</i> , L. + 10 per cent <i>Oxalis filipes</i> , Small.)	0.0735	0.0060	None	None	0.0057	0.0032	0.0117	0.0795	0.0206	3.9	0.1007	1.2†	

* Determined by titration to the change point of phenolphthalein.

† Smith, E. F., and Quirk, A. J., *Phytopathology*, 1926, 16, 491.

In Table I the equivalent concentration of the phosphate ion has been calculated on the assumption that in the saps we have studied phosphoric acid is monobasic. The pH of rhubarb sap is about 4.5 (Table I),¹⁴ and pK_2 for phosphoric acid is 6.9, so that according to the Henderson-Hasselbalch equation only about 0.4 per cent of the total phosphate can be present as HPO_4^{--} . Naturally in the saps of *Rumex* and *Oxalis*, which have still greater hydrogen ion activities, the proportion of bivalent phosphate will be less. It may be pointed out that even if the phosphate ion is assumed to be trivalent it does not alter the major conclusion which may be drawn from the analyses.

The data show that in these saps the cation equivalents exceed the anion equivalents, the smallest ratio of cation to anion being 1.8 and the largest 5.3. The average is 3.8.¹⁵ The excess of cations is, of course, paired with various organic anions.

As to the nature of these anions, it has already been stated that the quantitative separation of the complicated mixture of organic anions derived from plant sources has not yet been accomplished satisfactorily and no attempt has been made to solve this problem in the present case.

In *Rheum rhaponticum*, L., according to Wehmer,¹⁶ benzoic and gallic acids have been detected, and in a number of closely related species, oxalic and malic acids. There is no doubt that the last two are the most important. According to Bau¹⁷ from 0.05 to 0.5 per cent of oxalic acid exists in fresh young leaves of rhubarb. Maue¹⁸ reports 0.358 per cent oxalic acid in fresh rhubarb leaves and Tsakalotos¹⁹ 0.46 per cent in fresh leaves of *Rheum undulatum*. In *Rumex* and *Oxalis* oxalic acid is said to be abundant²⁰ and this is confirmed by our qualitative tests.

Probably there are small amounts of proteins present in all saps, but at the low pH's encountered in the plants studied by us it is probable that they were acting as cations. In this connection it is interesting that Chibnall and Grover¹⁴

¹⁴ Chibnall, A. C., and Grover, C. E., *Biochem. J.*, London, 1926, **20**, 108.

¹⁵ If phosphate is taken as bivalent the ratio of cations to anions in the second sample of *Rumex* is still 1.30.

¹⁶ Wehmer, C., in Klein, A., *Handbuch der Pflanzenanalyse*, Vienna, Julius Springer, 1932, 496.

¹⁷ Bau, A., *Z. tech. Biol.*, 1921, **8**, 151.

¹⁸ Maue, G., *Z. Nahr.-Genussm.*, 1920, **40**, 345.

¹⁹ Tsakalotos, A. E., *Schweiz. Apoth.-Ztg.*, 1919, **57**, 303.

²⁰ Czapek, F., *Biochemie der Pflanzen*, Jena, Gustav Fischer, 3rd edition, 1922-25, 69.

found that no soluble cytoplasmic protein could be extracted from *Rumex* and *Rheum* because the sap was below the isoelectric points of the proteins.

It will be observed that calcium and magnesium were present in but two samples of sap. This is probably due to the preliminary treatment of the sap after expression. With the exception of the two samples mentioned the sap was treated at once with an equal volume of alcohol and a few drops of glacial acetic acid, and chilled for about 24 hours. Under these circumstances there separated out not only a heavy organic precipitate, but also crystalline material which contained traces of calcium and much magnesium and which may have been either calcium and magnesium oxalate or other organic salts or phosphates or all of these. Assuming that these substances were originally retained in solution in the sap due to its low pH their deposition before analysis does not vitiate our conclusion as to the excess of cations. In the exceptional cases the salts probably did not deposit because of the very low pH due to the added HCl.

The small amount of calcium in the sap recalls the situation in *Valonia*.²¹ Recent experiments on *Nitella* support the suggestion²² that the chief function of calcium may be to prevent certain organic substances from leaching out of the cell surface and in that case calcium would not be necessary in the vacuole since it contains these organic substances.

These results are in harmony with those of Teakle²³ who finds an excess of inorganic cations over anions in the sap expressed from wheat plants.

It is clear that the excess of cations over anions can be explained on the assumption that the cations have entered as hydrates. This suggestion has already been made on other grounds²⁴ and has been applied to models.²⁵

²¹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1922-23, **5**, 225. Here the figure for calcium in parts per thousand is 10 times larger than it should be. The method is such that the molar concentration given may be too large.

²² Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 967. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87.

²³ Teakle, L. J. H., *Plant Physiol.*, 1929, **4**, 211.

²⁴ Osterhout, W. J. V., *Science*, 1912, **36**, 571; *Proc. Soc. Exp. Biol. and Med.*, 1926-27, **24**, 234; *Bull. Nat. Research Council, No. 69*, 1929, 170; *J. Gen. Physiol.*, 1930-31, **14**, 285; *Biol. Rev.*, 1931, **6**, 369; *Ergebn. Physiol.*, 1933, **35**, 967. Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1933-34, **17**, 727.

²⁵ Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, 1931-32, **15**, 667. Osterhout, W. J. V., *J. Gen. Physiol.*, 1932-33, **16**, 529. Osterhout, W. J. V., and Kamerling, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 507. Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, 1933-34, **17**, 445, 469.

It is, of course, possible that cations enter in exchange for H^+ produced in the cell but it seems improbable that this plays an important rôle.²⁶ There are, however, other possibilities which will be discussed in the second part of this paper.

SUMMARY

The inorganic constituents of the sap of *Rheum* (rhubarb), *Rumex* (field sorrel), and *Oxalis* (wood sorrel) show a great preponderance of cations over anions, as would be expected if the cations entered chiefly as hydrates (other possibilities will be discussed in Part 2).

²⁶ Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 994.