The Study of Plant Tissue Fluids. A Critical Review.*

By B. N. Sastri and M. Sreenivasaya.
(*Indian Institute of Science, Bangalore.*)

Preservation of Tissue Fluids.

THE tissue fluids thus obtained cannot be preserved even at low temperatures (0°C) without appreciable changes occurring in the fluid, the most prominent change being the precipitation of proteins through gradual coagulation. Gooke[38] has shown that, in the case of tissues, low temperatures induce an intramolecular change in the tissue proteins which leads to their denaturation; this circumstance has been correlated with winter-hardiness in plants. The coagulum adsorbs many essential constituents, particularly enzymes and viruses. Duggar[39] has found that the mosaic of tomato is not seed borne and opines that the seed proteins adsorb the virus. The failure to transmit many of the viruses through artificial injection of sap, as in the case of the spike-disease of sandal, is probably due to the circumstance that the virus gets adsorbed by the associated proteins during the preparation of the sap. In such cases, it is necessary to carry out the extraction with various buffers and the one which elutes the essential constituent chosen.

A similar procedure should be adopted for the study of enzymes on account of their analogous behaviour. The protein coagulation is influenced by tannins which in the case of certain enzymes like diastases are known to exert an inhibiting or inactivating influence. The effect of tannins and allied substances on the virulence of plant viruses has not been investigated. The extraction of sap from tannin-bearing tissues for certain enzyme studies, is best accomplished by adding hide powder during mincing and grinding. In their studies on the diastatic activity of the diseased and healthy sandal leaves, the authors have adopted this procedure. For the investigation of special constituents, certain modifications in technique have therefore to be introduced, and it is difficult to recommend any one general procedure for universal adoption.

For a study of the infectivity of viruliferous tissue fluids or for determining the resistance offered by tissue fluids to the growth of certain pathogens, the fluid should be obtained under aseptic conditions as it does not permit of heat sterilization.

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[4] Singh, B. N., and Singh, R. B., "The Relative Efficiency of Leaf Water-Content and Absorption in Transpiration." (*In course of publication.*)

* Continued from *Curr. Sci.*, 1934, **3**, 8.

[38] Gooke, *C. A.*, 1907, **1**, 196.

[39] Duggar, *J. Bact.*, 1930, **19**, 20.

[5] Singh, B. N., and Chakravarty, S. C., "Effect of Ultra-violet Rays on the Permeability of Protoplasm of *Trapa bispinosa* to Ions." (*In course of publication.*)

[6] Singh, B. N., and Sheshagiri, P. V. V., "Effect of Ultra-violet Rays on the Permeability of Protoplasm of Storage Tissues to Ions." (*In course of publication.*)

A convenient method for obtaining sterile fluids has been described by Ranker.[40]

METHODS OF INVESTIGATION AND THEIR CHOICE.

Tissue fluid studies may be classified into three broad divisions: (1) Physico-chemical; (2) Chemical, and (3) Biochemical. Physico-chemical methods include determinations of density, refractive index, depression of freezing point, electrical conductivity, hydrogen-ion-concentration, buffer values, optical activity, oxidation-reduction potential, temperature of coagulation, etc. Chemical methods are employed for an estimation of total solids, ash and ash constituents, total and amino nitrogens, carbohydrates and sugar alcohols, volatile and gaseous constituents like hydrogen cyanide, aldehydes and carbon dioxide, chlorophyll and other pigments, glycosides, alkaloids, tannins, fats and lipoids. Biochemical and biological methods are invoked for a study of the enzyme make-up of a fluid or a determination of the infectivity of a virus-bearing sap, or for an estimation of certain constituents like sucrose, maltose, inulin, glycosides, which are attacked by specific enzymes.

The choice and adoption of a particular method depends upon the nature of the problem, the accuracy required and the rapidity and frequency with which the samples have to be analysed. Generally, for the investigation of a given problem, one is rarely called upon to estimate all the constituents or conduct all physico-chemical measurements.

Physico-chemical methods are, in general, more elegant, less cumbersome, possess greater ease of reproducibility, require smaller quantities of samples and are conducted with greater rapidity and accuracy. In many of the measurements like the determination of the depression of the freezing point and electrical conductivity, the chemical composition of the tissue fluid is not affected, and the same aliquot of the fluid usually suffices for a few of the other determinations, thus effecting great economy in the use of research material. Wherever possible physico-chemical methods should be preferred to chemical methods.

Chemical methods, indispensable for the determination of certain constituents, require appreciable amounts of tissue fluid for analysis. The quantity required depends upon the concentration of the constituent in question and the sensitiveness of the method employed for its determination. Micro methods, if adopted, economise the material, and their application to the study of tissue fluids offers a wide and fruitful line of research. Animal physiologists have attained great distinction in this line, many of whose methods can be adopted in these studies.

Biochemical methods are characterised by the great specificity of their reactions which render the estimation of certain constituents infallible, and in certain cases they are the only methods available. An estimation of a glucoside, maltose and sucrose in a mixture, for example, is best accomplished with the aid of suitable enzyme reagents free from interfering enzymes.

PHYSICO-CHEMICAL MEASUREMENTS AND THEIR SIGNIFICANCE.

A. *Density.*—Density, determined pyknometrically, has been found useful in evaluating the solid content of the tissue fluids of a plant at various phases of its growth. The measurement does not influence the composition of the fluid in any manner and requires 5—10 c.c. of the fluid.

Density measurements have revealed that rapid growth is accompanied by lower concentrations while a dormant condition induces higher concentration of solutes in sap;[37,41,42] so does production of fruit.[43] Density is influenced by soil moisture[44,45] and by other factors of habitat of the plant.[41,45] Aquatic plants generally have a sap of low density. An idea of the water requirement of a crop or plant can be obtained by a determination of the density of its tissue fluids; the density is inversely proportional to their water requirements.[44] No relationship seems to have been observed between winter hardiness and density.[9,12] Determinations of density form a routine procedure in the chemical control of certain industries such as sugar, fruit syrups, etc.

Sap concentration is affected by the onset of diseases. Corn stalks affected by smut,[46] tissues from spiked sandal plants,[47] and

[40] Ranker, *Phytopathology*, 1930, **20**, 569.

[41] Reed, *J. Agr. Res.*, 1921, **21**, 81.

[42] Reed and Halma, *J. Agr. Res.*, 1926, **32**, 1177.

[43] Webber, *C. A.*, 1921, **15**, 3131.

[44] Eaton, *Amer. J. Bot.*, 1927, **14**, 212.

[45] Bates, *J. Agr. Res.*, 1923, **24**, 131.

[46] Karrer, *Amer. J. Bot.*, 1926, **13**, 286.

[47] Iyengar, *J. Indian Inst. Sci.*, 1929, **12A**, 295.

tumourous growths comprising proliferous tissue[48] yield fluids of a lower concentration than the corresponding healthy tissues.

A fluid of higher density is obtained from shoots attacked by lac insects.[33]

B. *Refractive Index.*—Refractive index[49] offers a quick method of evaluating the total solids in a given sap and requires just a drop of the fluid, and has been extensively employed both in physiological research and in commercial practice, in estimating the sucrose content of cane juices.

C. *Depression of the Freezing Point,* Δ—*Osmotic Pressure.*—Depression of the freezing point is proportional to the total concentration of the osmotically active constituents of the sap, and gives a measure of its osmotic pressure. Dixon and his co-workers have employed this method in connection with their studies on the ascent of sap in plants.[50] The method has been adopted for elucidating the osmotic relations between hosts and parasites,[18,51] for understanding the nature of draught resistance[52] and winter-hardiness,[6,53,54,55] for a study of the changes accompanying the vegetative growth, fruiting and maturation of crops,[56,57,58,59] for obtaining an insight into the response of plants to changes of environment[2,60,61,62,63,64,65,66,67] and treatment, and for a study of pathological changes induced in the plant with the onset of physiological and parasitic diseases.[68,69]

About 5 c.c. of the tissue fluid suffice for a determination by Beckmann's cryoscopic method, but by Dixon's thermo-electric method,[70] the quantity can be reduced to 2 c.c. Still smaller quantities, (0·1 or 0·2 c.c.) can be employed for the determination by the "melting point method"; the liquid is placed in a capillary tube attached to a Beckmann's thermometer, and the freezing point determined by the disappearance of the liquid meniscus observed with a reading telescope.[71]

Factors which increase the concentration of crystalloids in the tissue fluids of an organism, are those which contribute towards a greater depression of the freezing point. Increased photosynthetic activity under the stimulus of light or artificial illumination, results in a concentration of sugars[72]; increased absorption of soil nutrients results in a corresponding enhancement of the inorganic constituents in the tissue fluids, particularly in the roots.[73] Conditions which favour transpiration also lead to a concentration of crystalloidal constituents of the sap and increase its osmotic pressure.

Minced tissue is often employed for the depression of the freezing point but the method yields higher values.[74]

Among the other methods of determining osmotic pressure are (1) the plasmolytic method, and (2) the drop method of Barger.[75] The relative merits of the plasmolytic and cryscopic methods have been discussed by Atkins.[76]

Mention may also be made of another term O_g—Osmotic value often employed and is defined as the molal concentration of cane sugar, which is necessary to effect

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48 Harris and Gortner, *Biochem. Bull.*, 1915, **4**, 52.

49 Gortner and Hoffman, *Bot. Gaz.*, 1922, **74**, 308.

50 Dixon, *Transpiration and Ascent of Saps in Plants* (Macmillan & Co., Ltd.), 1914.

51 Harris, *C. A*, 1919, **13**, 2898.

52 Harris and Henry, *Hawaiin Planters Rec.*, 1930, **34**, 167.

53 Sutherst, *Chem. News*, 1901, **84**, 234.

54 Gail, *Bot. Gaz.*, 1926, **81**, 434.

55 Maximov, Krasnosalskaia—Maximova, *Physiol. Abs.*, 1919, **4**, 413.

56 Dixon and Atkins, *Sci. Proc. Roy. Dub. Soc.*, 1912, **13**, 219.

57 Gail and Cone, *Bot. Gaz.*, 1930, **88**, 437.

58 Lutman, *Amer. J. Bot.*, 1919, **6**, 181.

59 Harris, Gortner and Laurence, *Physiol. Abs.*, **2**, 436.

60 Drabble and Drabble, *Biochem. J.*, 1907, **2**, 117.

61 Harris, Laurence and Gortner, *Physiol. Rev.*, 1916, **2**, 1.

62 Fitting, *C. A.*, 1912, **6**, 766.

63 Hector, *C. A.*, 1928, **22**, 3723.

64 Blagoveshchenskii, Bogobyubova and Chernova, *C. A.*, 1928, **22**, 2590.

65 Arrhenius, *C. A.*, 1920, **14**, 2358.

66 Harris, *Amer. J. Bot.*, 1918, **5**, 490.

67 Harris and Laurence, *Bot. Gaz.*, 1917, **64**, 285.

68 Sprecker, *Physiol. Abs.*, **2**, 649.

69 Iyengar, *J. Indian Inst. Sci.*, 1928, **11A**, 103.

70 Dixon and Atkins, *Sci. Proc. Roy. Dub. Soc.*, 1910, **12**, 275.

71 Drucker and Schreiner, *Biol. Zbl.*, 1913, **33**, 99.

72 Dixon and Atkins, *Sci. Proc. Roy. Dub. Soc.*, 1916, **15**, 51.

73 McCool and Millar, *Soil Sci.*, 1920, **9**, 217.

74 Bouyoucos and McCool, *J. Amer. Soc. Agr.*, 1916, **8**, 50.

75 Barger, *J. C. S.*, 1904, **85**, 286.

76 Atkins, *Sci. Progress*, 1917, **11**, 562.

incipient plasmolysis.[77,78] The variation of Og serves as an index of the physiological activities of the tissue.

D. *Electrical Conductivity, K.*—The electrical conductivity of a sap is a measure of the total electrolytes present in a sap.[79] The depression of freezing point accounts for the crystalloids—both electrolytes, and non-electrolytes. From a knowledge of these two values, the concentration of non-electrolytes can be calculated and expressed in terms of cane sugar.

Conductivity measurements could be made with very small quantities of liquids—about 1 c.c. There is no particular advantage in using larger quantities. The choice of the conductivity cell depends upon the conductivity of the liquids; generally speaking for the investigation of physiological fluids, Arrhenius-Ostwald conductivity cell has been found convenient. The cell is provided with three sets of dip electrodes, differing from one another in the distance between the electrodes. In the case of tissue fluids with low conductivity, the one with the electrodes close to each other is employed.

Poisoning of the electrodes with the proteins of the sap is a common experience. To obtain reliable results, frequent platinising of the electrodes is necessary.

Conductivity determinations coupled with those of the depression of the freezing point, give useful information regarding the origin of osmotic forces in plants which may arise from two or more independent sources. Absorption of soil nutrients[80] and metabolic processes favouring the formation of organic acids, contribute towards conductivity, while an increase in photosynthetic sugars merely enhances the concentration of non-electrolyte crystalloids.

Conductivity measurements are affected by the viscosity[81] and in the case of mucilaginous saps, the effect may be appreciable. The suppression of dissociation due to the mutual action of salts and acids[79] also affects the results for the total electrolyte content through conductivity measurement. A rough relationship has been found to exist between conductivity and inorganic constituents of saps as determined by ash content, and for most purposes the conductivity may be taken as a rough estimate of the ash content of the tissue fluids.

E. *H-ion Concentration and Buffering Capacity of Tissue Fluids.*—There is a vast amount of literature on this aspect of plant physiology. The importance of hydrogen-ions as a regulating mechanism for many of the vital processes of plants, has long been recognised and a large number of methods have been developed for its determination. The usual methods consist in measuring the potential of a hydrogen electrode dipping in the sap against the standard calomel electrode. The hydrogen electrode can be replaced by quinhydrone, antimony or other electrodes. An elegant glass electrode suitable for working with a few drops of tissue fluid has been described.[81] The quantity of fluid necessary for the determination depends upon the apparatus employed; 3-5 c.c. are required for Clark's electrode.[82] Haas[83] describes an electrode vessel suitable for work with 3 to 4 drops of plant juice. With the modified micro-electrode of Bodine and Fink,[84] the quantity may be reduced to 0·25 c.c. to 0·01 c.c. depending upon the size of the electrode vessel. The latter can function either as a hydrogen or quinhydrone electrode. Further refinement has been secured by Taylor and Whittaker[85] who succeeded in constructing a micro-non-polarisable electrode which can be used for determining the hydrogen-ion concentration of living cells and tissues, with great accuracy. The capillary electrode devised by Robertson and Smith[86] is suitable for measurement of pH at a point *in situ* of the plant tissue, and can therefore be employed for surveying the pH values at various points of an organ, and also in such of those cases, where the tissues during pulping suffer chemical transformations leading to an alteration in the pH values.

Hydrogen-ion concentration in the cell sap without expression from the cell has been

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77 Beck, *Plant Physiology*, 1928, **3**, 413.

78 Beck, *Ibid.*, 1931, **6**, 315.

79 Haynes, *Biochem. J.*, 1919, **13**, 111.

80 Hoagland, *Bot. Gaz.*, 1919, **18**, 297.

81 MacInnes and Doll, *J. Gen. Physiol.*, 1929, **12**, 805.

82 Clevenger, *Soil Science*, 1919, **8**, 217.

83 Haas, *Soil Science*, 1920, **9**, 341.

84 Brunstetter and Magoon, *Plant Physiol.*, 1930, **5**, 249.

85 Taylor and Whittaker, *Protoplasma*, 1928, **3**, 1.

86 Robertson and Smith, *J. Soc. Chem. Ind.*, 1930, **49**, 120.

measured both electrometrically and colorimetrically. Such *in vivo* studies of the properties of the cell sap represent a great improvement over the *in vitro* study of saps and there is a fruitful field in this line for developing the *in vivo* technique for elucidating the nature of the cell sap. Mention may also be made of the Range Indicator Method by Small and his co-workers[87,88,89] which has proved invaluable in studying the reaction of individual cells and their variations due to seasonal factors.

For rapid work, particularly field work, colorimetric methods are available, where a series of indicators, each measuring a different range of hydrogen-ion concentration, are employed. For coloured solutions the method is unsuitable. Further the colorimetric method is affected by the 'salt' and 'protein' errors and does not yield accurate results. Arland[90] has carried out comparative studies of the potentiometric and colorimetric methods. Domontvich[91] finds fair agreement between hydrogen and quinhydrone electrodes.

The simple quinhydrone electrode is unsuitable to saps not because of the 'salt' error which is negligible with the concentration of salts obtained in the tissue fluids, but is due to the presence of sugars and phosphates. Billmann and Katagiri[92] have shown that the use of the hydroquinhydrone electrode gives reliable values.

The concentration and reaction of the nutrient solution in which the plant thrives does not greatly influence the hydrogen-ion concentration of the sap[93] but the root sap responds to the soil acidity and builds up a buffering mechanism to withstand the adverse effects of soil reaction. The buffering capacity is influenced by ions—potassium and phosphorus enhancing and calcium and chlorine lowering the capacity.[21] A marked diurnal variation[94] in the hydrogen-ion concentration has been observed, not to speak of the seasonal and regional fluctuations.[87] The external factors appear to influence only the outer cells of the plants and the inner cells enjoy a greater uniformity with respect to the hydrogen-ion concentration. There is a gradient in the pH from the top to the root.[95] Application of fertilizers like potash and phosphate tends to increase and nitrogenous manures decrease the pH.[96]

The effect of CO_2 produced as a result of respiration, on the buffering of the living cells deserves extended investigation. Instances are known where the CO_2 content may be as high as 20–30 per cent.[97] The CO_2 balance in a plant sap is comparatively little investigated. Our knowledge on the subject is mainly due to the work of Ingold,[98] Willman and Brown,[99] Small[100] and others. The extent to which CO_2 exists in the dissolved condition is important in the photosynthetic activity of the plants.

The methods of identification of plant acids have been investigated by Czapeck.[101] More recently Iyengar[102] has examined the acids in the tissues of healthy and spiked sandal plants. The acidity changes are particularly marked when the physiological state of the plant is upset by the entry of parasites through adverse environmental conditions. Reduced root activity due to phylloxera attack on vines lead to high acidity of plant saps.[14] The stem weevil attack of cotton leads to an increase in alkalinity.[103] In cases of tumour growth and proliferous tissue there is a decrease in hydrogen-ion concentration.[104] Disease resistance in grape vines[14] is characterised by a high acidity, a low sugar content, and a poor oxidase activity. In the case of the tobacco root rot it has been found that low soil acidity favours the incidence of disease. The subject has been investigated by Morgan and others.[105]

87 Rear and Small, *Protoplasma*, 1927, **2**, 428.

88 Ingold and Small, *Protoplasma*, 1928, **3**, 458.

89 Small, "Hydrogen-ion concentration in plant cells and tissues," *Verlag von Gubruder Borntraeger, Berlin*, 1929.

90 Arland, *C. A.*, 1925, **19**, 2225.

91 Domontvich, *C. A.*, 1927, **21**, 3217.

92 Billmann and Katagiri, *Biochem. J.*, 1927, **21**, 441.

93 Arrhenius, *Physiol. Abs.*, 1922, **8**, 375.

94 Truog and Meacham, *Soil Science*, 1919, **7**, 469.

95 Gustafson, *Amer. J. Bot.*, 1924, **11**, 1.

96 Miyake and Arachi, *J. Biochem.* (Japan), 1924, **4**, 317.

97 Magness, *Bot. Gaz.*, 1920, p. 308.

98 Ingold, *Protoplasma*, 1930, **9**, 456.

99 Willman and Brown, *Plant Physiology*, 1930, **5**, 535.

100 Small, *New Phytologist*, 1920, **50**, 19.

101 Czapeck, *Biochemie der Pflanzen*, 1923, **3**, 99.

102 Iyengar, *J. Indian Inst. Sci.*, 1933, **16A**, 139.

103 Lakshmana Rao, *Madras Agr. Dept. Year Book*, 1926, p. 65.

104 Harvey, *Amer. J. Bot.*, 1920, **7**, 211.

105 Morgan, Anderson and Dorsey, *Connecticut Agr. Exptl. Stn. Bull.*, 1929, No. 306.

The buffering capacity of a particular fluid is connected with its chemical make-up, which consists mainly of organic acids, their salts, and phosphates.[108-109] This has been amply demonstrated in a number of plant juices. In fact, the salt content of a juice can be determined with a fair degree of accuracy through a determination of the pH and titratable acidity and referring to a table showing the relationship between titratable acidity and pH of related artificial buffer mixtures.[110] Proteins play a negligible rôle in the buffering systems. This is not surprising as a consideration of the basis of the buffering capacity will show that it is an ionic phenomenon and the proteins possess a large molecular weight.

The buffer index β is equal to $\frac{dB}{dpH}$ where dB is the gramme-equivalent concentration *per litre* of added base and dpH is the observed change in the pH when the quantity dB is added to the solution when buffer index is being determined. The introduction of the conception of the buffer index[111] has largely served to clarify our conceptions regarding buffers.

The midrib and petiole sap of a leaf has less buffer effect compared with that derived from the rest of the leaf. In the intact cell, the cell wall itself resists the change in acidity of the cell contents due to external H^+ or OH^- and thus acts as a buffer. This was demonstrated in the case of *Nitella* by Hoagland and Davis.[112]

Buffers offer a fine mechanism through which the hydrogen-ion concentration is maintained at a particular optimum. On account of this mechanism, the reaction of plant cells is not affected by fluctuations in the reaction of soil medium, by the violent metabolic disturbances caused by the invasion of parasitic organisms or changes of environment. In fact the buffering capacity has been considered to be a measure of disease resistance.

Glycerine, sugars and alcohols increase the dissociation of acids and therefore act as negative buffers,[113] and an abnormal accumulation of these products always renders the plant susceptible to fungus infection.

Other Physico-Chemical Measurements.—There is little literature regarding a study of viscosity or surface tension of plant saps. A determination of the viscosity of saps would provide useful data for obtaining an idea of the hydrophillic colloid content and perhaps throw some light on the ascent of sap in plants.

The optical activity of a plant sap is not usually determined since the concentration of the optically active constituents in the sap are generally small except in special instances like cane juice, when this method is extremely valuable in assaying the sucrose content. Further, the method is not directly applicable to the great majority of plant tissue fluids which, being coloured, have to be treated with clarifying agents before a determination can be carried out. The method is no doubt a valuable one which merits a wider employment and should be extended to a determination of the exaltation in optical activity after addition of borates or arsenates. This will differentiate hydroxy compounds, *e.g.*, sugar alcohols like mannitol and organic acids like tartaric. If an appreciable exaltation is obtained, the presence of such or related compounds should be suspected.

Physico-Chemical Data: Their Interrelationships and Interpretations.—The depression of the freezing point is a measure of the total concentration of the osmotically active substances in solution, which include, not only the crystalloids in true solution but also the colloidal micelle with different osmotic valencies. The conductivity measurements give an idea of the total concentration of the electrolytes and the colloidal micelle carrying ionic charges. The non-electrolytes like sugars and the colloidal micellæ which do not carry any effective charge but which are osmotically active do not contribute towards the electrical conductivity of the solution. If therefore we have the depression of the freezing point, Δ, and the electrical conductivity K, we can obtain a ratio of the two values $\frac{K}{\Delta}$ which indicates changes in relative concentration of ions in relation to total solutes. This ratio has been determined in a great number

106 Martin, *Protoplasma*, 1928, **3**, 273.

107 Martin, *Ibid.*, 1928, **3**, 282.

108 Ingold, *Ibid.*, 1929, **6**, 51.

109 Ingold, *Ibid.*, 1930, **9**, 447.

110 Haynes and Brown, *Biochem. J.*, 1928, **22**, 947.

111 Van Slyke, *J. Biol. Chem.*, 1922, **52**, 525.

112 Hoagland and Davis, T., *Gen. Physiol.*, 1923, **5**, 629.

113 Lenthardt, *Kolloidchem. Beihefte.*, 1927, **25**, 1.

of investigations. In the case of the sandal leaves, the ratio is lower in spiked sap than in normal material; on the other hand, the sap derived from shoots attacked by lac insects has been found to possess a higher ratio.

The average molecular weight of the solutes can be computed from (1) the total solids of the sap (C) and (2) depression of the freezing point, Δ.

$$M = C. \frac{K}{\Delta}$$

where K=1000 × molecular lowering for a given solvent.

It has been found that host plants of lac reputed to yield thick encrustations of lac, contain a sap whose solutes have a high average molecular weight, indicating the existence of high molecular compounds favouring the production of resin. The tissue fluids of the sandal leaf in the diseased condition contain solutes whose average molecular weight is low and indicate a disintegration of the high molecular proteins. It should be possible to make a further differentiation between the mean molecular weight of the electrolytes and that of the non-electrolytes in the sap, by taking into consideration the data for electrical conductivity. An attempt in this direction may lead to significant results.