of accuracy. Whenever a problem is referred to them they put the model (generally to a scale of 25 : 1) in a sand tank and run the experiments for a certain length of time and photograph a few streamlines with colouring dyes. This photograph with a few certain other data of the experiment is analysed in a very ingenious way[7] and they know how the prototype is going to behave under similar conditions. Of course the porosity of the soil below the prototype forms an important factor in this behaviour. In river constructions particularly it is known that the soil medium is stratified. This stratification alters the course of streamlines and pressure gradients in a very marked way. This has also been tackled by the Vienna School. How to take account of the heterogeneity of the soil has been considered by Prof. Terzaghi and forms an interesting chapter in his book "Erdbaumechanick". So between Forchheimer, Schaffernak and Terzaghi they claim to have solved this problem of seepage and safety of dams more or less completely and they claim very close agreement. It will be seen that their whole claim rests on the assumption of Forchheimer which if not supported by experiments the whole superstructure falls.

The above is a very short account of my experience during my last visit to the principal hydraulic laboratories of the West. I am afraid some of the points require amplification and it is hoped to deal with them more at length in some future issue.

[7] "Versuchstechnische Lösung von Grundwasserproblemen". Prof. F. Schaffernak and Dr. R. Dachler. *Die Wasserwirtschaft Jahrgang*, 1931, heft 1 and 3.

The Study of Plant Tissue Fluids. A Critical Review.

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PLANT physiologists have interested themselves in the study of saps which represent the nearest phytological analogue of blood. The great success achieved by physiologists in the field of clinical chemistry, has stimulated the investigation of tissue fluids which regulate all the principal metabolic processes in plants. A study of the sap should give a closer insight into the metabolic changes in the plant than that obtained by an investigation of the whole tissue which includes the static reserves and the physiologically inert structural units comprising the plant organism.

Tissue fluid studies have proved useful in elucidating the nature of the physiological changes accompanying the (*a*) various phases of growth, (*b*) changes of environment and season, (*c*) manuring, and (*d*) onset of diseases. Valuable information has been obtained with regard to the suitability of a particular soil for the cultivation of a given crop. The fact that the fluid of the tea leaf has a pH value of 4·3—4·5, is an indication that the crop prefers an acid soil and instances are known where liming of tea soils and attendant reduction in acidity, has proved detrimental to tea growth.[1] The analysis of indigenous plants has provided significant indication regarding the suitability or otherwise of the area for the introduction of related exotics.[2] Tissue fluid studies have been employed with more or less success for obtaining information regarding the biogenics of essential constituents of plants; establishing varietal differences of crops [3-7] for elucidating the nature of drought [8-13]

[1] Cooper, *Ind. Tea Ass. Sci. Dept. Quart. J.*, 1925, pt. iv, p. 130.

[2] Harris *et al*, *J. Agr. Res.*, 1924, **27**, 893.

[3] Balls, *Proc. Camb. Phil. Soc.*, 1914, **17**, 467.

[4] Harris, Gortner and Lawrence, *J. Phys. Chem.*, 1921, **25**, 122.

[5] Harris, Gortner and Lawrence, *J. Gen. Physiol.*, 1921, **3**, 343.

[6] Harris and Popenoe, *J. Agr. Res.*, 1916, **7**, 261.

[7] Newton and Martin, *Can. J. Agr. Res.*, 1930, **3**, 336.

[8] Gortner, Hiffman and Newton, *C.A.*, 1924, **18**, 2543.

[9] Pantanelli, *C.A.*, 1919, **13**, 1602.

[10] Harvey, *J. Agr. Res.*, 1918, **15**, 83.

[11] Dexter, Tottingham and Graber, *Plant Physiol.*, 1932, **7**, 63.

[12] Salmon and Fleming, *J. Agr. Res.*, 1918, **13**, 497.

[13] Maximov, *Protoplasma*, 1929, **7**, 259.

and disease[14-17] resistance, for revealing the nature of relationship between hosts and parasites,[18] for determining the nutritional requirements of plants,[19-23] for evaluating the vitaminic content of potable juices and more recently, in a study of the plant viruses.[24,25]

THE CHOICE OF TISSUE FOR ANALYSIS.

In the above studies, choice of tissue is an important consideration which determines the success of the investigation. In a study of the functional disorder brought about by disease as reflected in the composition of the tissue fluid, the most sensitive tissue suffering the maximum metabolic disturbance has to be chosen for the study. The leaves are usually the most sensitive of plant organs, and show the changes in a pronounced manner.

The choice of tissue for study will also depend upon the nature of the problem. Biogenetic studies of constituents involve mostly the investigation of those tissues where the constituents tend to accumulate. A study of the physico-chemical relationship between the host and parasite involves an examination of their root systems if one is dealing with a root parasite like *Santalum Album* Linn.

In a study of the changes induced by environmental conditions, the most susceptible tissue has to be chosen, as otherwise, the difference may not be significant. In developing methods for diagnosing a disease, for example, the tissue that has to be chosen for examination should be the one which has suffered the most drastic change. In the study of the spike disease of sandal, the most significant changes are to be found in the leaf.[26]

The scope of our choice of tissue is unfortunately limited by the special anatomical structure of plant organs which renders an easy differentiation and separation into specific tissues extremely difficult and tedious, with the result that one invariably encounters a mixture of two or more kinds of organised tissue fluids, instead of one specific fluid. This circumstance leads to several complications not only in securing a truly representative tissue fluid but also in the subsequent interpretation of results and renders certain types of investigations extremely difficult. Attempts have, however, been made through which it has been possible to obtain a single type of tissue fluid in a predominant proportion.[27]

It is, however, possible to differentiate broadly the leaf tissue fluid from the one derived either from stem or root, and such a distinction is in most cases fortunately sufficient.

FACTORS INFLUENCING THE COMPOSITION OF SAP.

The expression of sap from tissues in a state which represents its true condition in the cell is the ideal which the biochemist has in view. This is a difficult ideal to attain for the simple reason that the sap of the individual cells widely vary both in composition and concentration.

It is possible that in the course of the preliminary operation of tissue disintegration and mincing preceding the expression of sap, alterations in the composition of the sap are brought about by (*a*) mutual precipitation of constituents, (*b*) adsorption by the insoluble tissues of the plant, (*c*) contamination with the contents of intracellular, special or dead cells, and (*d*) the bio-chemical processes accompanying the process of extraction. The sap thus obtained therefore represents only a mixture, with an average composition of saps derived from the numerous cells of varying types. It is therefore clear that one can obtain only a mixture, perhaps representing an average composition of sap derived from the numerous cells of a particular type.

COLLECTING, SAMPLING AND PRESERVATION OF TISSUES FOR STUDY.

The large diurnal variations in the composition of the tissue fluids indicate that the collection of the samples should be carried out at a specified period of the day.

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[14] Degli Atti, *C.A.*, 1920, **14**, 2010.
[15] Hurd, *J. Agr. Res.*, 1924, **27**, 725.
[16] Hurd, *J. Agr. Res.*, 1923, **23**, 373.
[17] Ranker, *J. Agr. Res.*, 1930, **41**, 613.
[18] Harris and Lawrence, *Am. J. Bot.*, 1916, **3**, 437.
[19] Gilbert and Harden, *J. Agr. Res.*, 1927, **35**, 185.
[20] Pettinger, *J. Agr. Res.*, 1931, **43**, 95.
[21] Böning and Boning, *Biochem. Z.*, 1932, **247**, 35.
[22] Gilbert and Smith, *Soil Sci.*, 1929, **27**, 459.
[23] Gilbert, McLeon and Adams, *Plant Physiol.*, 1927, **2**, 139.
[24] Nelson and Breese, *J. Agr. Res.*, 1930, **41**, 749.
[25] Matz, *J. Agr. Res.*, 1933, **46**, 821.
[26] Sreenivasaya and Sastri, *J. Indian Inst. Sci.*, 1929, **12A**, 230.
[27] Mason and Maskel, *Ann. Bot.*, 1928, **42**, 190.

The plant from which samples are to be collected should enjoy similar ecological conditions since variations in shading and sunshine affect the composition of the sap. When the object of the investigation is to compare the compositions of the tissue fluids derived from the healthy and spiked sandal leaves or those from attacked and free lac hosts, the samples should be obtained from plants having similar conditions of environment. In spite of all the care bestowed in the selection of samples, individual differences due to unknown factors still persist from plant to plant. In such cases, "random sampling" from a large number of apparently similar individuals is the best course to be adopted. Wherever permissible such studies should be extended over a protracted period and the results subjected to statistical analysis. It is also necessary to carry out a preliminary study of the limits of variation of the factor for which the study is undertaken for given area, by analysing a large number of samples taken at random. The effects of a treatment imposed or a variation under study, will become significant if the limits of variation after the treatment are distinctly large.

Among other factors which affect the composition of the tissue fluid and which should accordingly be taken into consideration while collecting samples may be mentioned the height or insertion of the tissue from the ground. degree of suberisation in the case of stems, and freedom of tissue from adhering foreign matter which should be removed by careful brushing and washing with water. If the laboratory is situated at a distance from the seat of sampling, the tissues must be transported quickly in an ice-chest so as to minimise changes.

METHODS OF EXTRACTION—A COMPARATIVE STUDY.

A review of the methods can be obtained elsewhere.[28-29] Generally speaking, the methods in vogue are (*a*) pressure extraction after rendering cell walls permeable to the sap by a suitable preliminary treatment, and (*b*) centrifugal methods. The first method is the one largely employed, the preliminary treatment consisting in exposure of the tissue to toluene or chloroform vapour or to intense cold by surrounding the tissue in liquid air, solid CO_2 and various cryoscopic mixtures like solid CO_2 and acetone.

A pressure method due to Dixon[30] and later used by Chibnall and Groover[31] consists in enclosing the tissue in a cylinder with the cut ends exposed. Compressed air is admitted into the cylinder, and when the pressure reaches 20–22 atm. to the square inch, the juice flows out at the cut ends. Preliminary treatment of the tissue with plasmolysing materials is found to be necessary.

Methods such as the flushing out of the sap under reduced pressure by means of water, have also been used. This method offers great possibilities but has received comparatively very little attention.

Comparative studies of the various freezing agents have been made. According to the work of Meyer[32] there is little or no difference in osmotic pressure between the samples of fluids expressed after freezing the tissue by liquid air, solid CO_2 and in salt mixture. Comparative studies by us between liquid air and toluene treatments with several types of plant material, revealed no significant differences. Whatever be the treatment, leaf tissues offer little difficulty; with stem, however, a preliminary mincing of the tissue was found essential[33] to secure concordant results with respect to concentration and yield of tissue fluid. Sayre and Morris[34,35] have studied the effect of size of samples on the yield of the tissue fluid and found that larger percentages of sap were removed from the smaller samples, under uniform pressures and times of draining. From these considerations it is clear that the following factors have to be standardised: (*a*) Extent of tissue disintegration particularly with the stem tissue, (*b*) quantity of sample, (*c*) pressure of extraction, and (*d*) time of extraction. The method which has been in use in our laboratory and has given satisfactory results has been described by us elsewhere.[36] We have replaced liquid air by

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[28] Meyer, *Plant Physiol.*, 1929, **4**, 103.

[29] Sayre and Morris, *Plant Physiol.*, 1931, **6**, 139.

[30] Dixon, *Sci. Proc. Roy. Dub. Soc.*, 1924, **17**, 263.

[31] Chibnall and Groover, *Ann. Bot.*, 1920, **40**, 491.

[32] Meyer, *Am. J. Bot.*, 1928, **15**, 449.

[33] Krishna, *Thesis for the M.Sc. of the Bombay Univ.*, 1929.

[34] Sayre and Morris, *Plant Physiol.*, 1931, **6**, 139.

[35] Sayre and Morris, *Ibid.*, 1932, **7**, 261.

[36] Sreenivasaya and Sastri, *J. Indian Inst. Sci.*, 1928, **11A**, 24.

toluene treatment; the minced material is placed in a well-stoppered bottle, toluene added, and the bottle immersed in a freezing mixture of ice and salt for 10-12 hours. This modification has been found to yield comparable and concordant results.[37] The fluid obtained at 1 ton pressure to the sq. inch is centrifuged at 3000 R.P.M. for 15 mins. to free it from all debris and the clear centrifugate used for subsequent analysis.

Artificial Culture of the Male Gametophyte of *Ephedra foliata* Boiss and *Ephedra Gerardiana* Wall. and a Study of the Number and Morphology of their Chromosomes.

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EPHEDRA FOLIATA Boiss (*E. peduncularis* Boiss) is a native of the Punjab plain. Plants of *E. Gerardiana* Wall. (*E. vulgaris* Hook. f.) were raised in the Government College Botanic Garden, Lahore, from seeds brought by Prof. Kashyap from Zanskar (about 12,000 ft. above the sea) and sown in October 1928. The plants produced flowers for the first time in 1933. In its natural habitat at high altitudes the flowers of *E. Gerardiana* appear during June and July and the seeds are set in the later half of August. These ripen by the end of September before the onset of the severe winter which brings the period of vegetative activity to a close and the plants enter the dormant phase of life to resume their activity during the next summer. The conditions are different in the Punjab plain. The period of vegetative activity is during the spring months of March and April. The plants of *E. Gerardiana* bear flowers during this period —from the middle of March to the middle of April—this period coinciding with the flowering period of the native *E. foliata*. After this the seeds are set which ripen about the middle of May. The plants then enter the resting period because of the strong heat of the plains.

The spindle-shaped pollen grain at maturity possesses a sculptured exine with ridges running longitudinally from pole to pole. Remains of two evanescent vegetative cells on one side, a stalk nucleus embedded in the peripheral part of the cytoplasm of the naked body cell in the centre and a rather large tube nucleus at the other end completes the structure of the pollen grain at the time when it is shed.

The pollen grains were germinated on the mucilaginous secretion that oozes out of the ripe ovules, placed on glass slides kept in a moist chamber. It is possible to germinate the pollen grain of one species on the mucilage secretion of the other. The secretion absorbs water from the surrounding atmosphere of the glass chamber and gets diluted. The grains gradually absorb the nutritive medium, swell in size, and at the same time prophasic changes start in the body nucleus. On the pollen grain becoming highly turgid the exine ruptures by two splits starting on opposite sides from the tube nucleus and of the grain and extending to about the middle of its length. This throws out the grain bounded by the intine with a jerk from the inside of the outer coat which immediately undergoes torsion. Thus liberated the grain increases to about double its former length. By this time the body nucleus is in the mid-prophase or early metaphase stage. All the further changes take place outside the exine in the medium. The total time for the complete division of the body nucleus and the organisation of the two male nuclei on the opposite poles is about five hours from the time the pollen grains are put into the secretion.

The pollen tubes are given out after about six to eight hours. From one to as many as four or more tubes may be given out from different sides of a grain, sometimes in a most irregular manner. More commonly only a single tube is given out usually laterally from just near the tube nucleus end of the grain but it may grow out from the mid-lateral position, or as a direct continuation of its tube nucleus end. When a number of tubes are given out, only one develops further, and the others remain

[37] Narasimhacharya and Sastri, *J. Indian Inst. Sci.*, 1931, 14A, 2.