

role in blood regeneration. It is true that, as in Alcock's experiments, in normal animals tryptophane deficiency produces no marked anæmia, but this must be explained on the assumption that blood composition is maintained constant by utilization of tryptophane derived from tissue wastage as is proved by the fact that although animals on a tryptophane-deficient diet present a nearly normal blood picture, they lose weight continuously during the experimental period.

TABLE II

	Hydrolysed casein + tryptophane			Hydrolysed casein		
	Days		%	Days		%
	0	12		0	12	
R.B.C. in millions per c.mm.	3.04	6.45	112.1	3.06	4.75	55.24
Hæmoglobin in gm. per 100 mm.	8.34	13.7	63.52	8.39	11.77	40.28

	R.B.C.	Hæmoglobin
Standard error of difference	17.9	6.77
Value of <i>t</i>	4.66	11.95

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University Biochemical
Laboratory, Madras,
November 8, 1943.

K. M. YESHODA.

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THE INDUCED OXIDATION OF OXALIC ACID BY DICHROMATE WITH FERROUS SULPHATE AS INDUCTOR

DURING the course of some other work we discovered that the presence of oxalate interferes with the titration of ferrous sulphate with dichromate by giving rise to a consumption of dichromate far in excess of the amount required for the oxidation of the ferrous iron present. This excess consumption of dichromate cannot be explained as due to any primary reaction between oxalate and dichromate, for it is known from the work of Dhar¹ that oxalic acid is only slowly oxidised by dichromate at ordinary temperatures.

Further, we have observed that, in the absence of ferrous sulphate, oxalate at the concentrations employed in our experiments does not consume any dichromate, the deep blue colour indicative of the end point with the diphenyl amine reagent being produced by the addition of a single drop of dichromate. Detailed experiments carried out by us have led to the conclusion that the observed interference of oxalate is due to the fact that the rapid reaction between ferrous sulphate and dichromate induces the reaction between oxalate and dichromate.

The following table incorporates some of the typical results:—

10 c.c. N/20 Fe SO₄ + 5 c.c. 4N H₂SO₄ + 2.5 c.c. H₃PO₄ (1.75 Spgr) + 0.5 c.c. 0.1% diphenylamine + X c.c. oxalate + water to make up the volume to 50 c.c.

Concentration of oxalate	Amount of dichromate 0.0529 N (in c.c.)	Induction factor (F) F = $\frac{\text{No. of moles of oxalate oxidised}}{\text{No. of moles of FeSO}_4 \text{ oxidised}}$
Nil	9.45	
0.01N	12.05	0.14
0.03N	14.15	0.25
0.05N	14.80	0.28

From the results given in the above table, it will be seen that the induction factor increases with increasing concentration of oxalate the concentration of ferrous sulphate and hydrogen ion being kept constant.

We have also found that ferrous sulphate induces the reaction between dichromate and tartaric, citric, and malic acids but not succinic acid.

Andhra University,
November 17, 1943.

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AN IMPROVED METHOD OF LOCATING TANNINS IN PLANT SECTIONS

COMMON TESTS FOR TANNINS

VARIOUS methods¹ have been described for locating tannins in plant tissues, but they all suffer from one disadvantage or the other. Vinson² fixes and stains tannins *in situ* by exposing whole organs to vapour of amyl or ethyl nitrite. He recommends a 20 per cent. alcoholic solution of ethyl nitrite, but, owing to its low boiling point (16° C.) it volatilizes so rapidly at the temperatures prevailing in this country, that in practice it becomes difficult to get satisfactory results. Amyl nitrite on the other hand though less volatile, is disagreeable to use.

Methylene blue (1:500,000) followed by saturated aqueous picric acid, has also been

recommended for staining tannins in the living state³ but the test is not very specific as other tissues besides tannins are also stained.

One of the most commonly used methods for detecting tannins in plant sections is to draw a drop of 10 per cent. ferric chloride under the cover glass by means of filter paper.⁴ The tannins are stained dark green, blue or black. The method is very useful especially when there is a high accumulation of tannins as in galls or near wounds or infected tissue. But as the test is not very sensitive it cannot be successfully used when the concentration of tannin in the tissues is very low (1-10 p.p.m.).

Recently Dastur⁵ has prepared standard slides of sections of cotton leaves, showing different degrees and extent of tannin accumulation, but unfortunately he has not described the technique employed.

ADVANTAGES OF FOLIN DENIS REAGENT (F.D.R.)

The author while working on the causes of discolouration of jute during retting tried the above methods for locating the tannins in the stem sections but without any success. It was then, that the idea of using Folin Denis Reagent suggested itself. F.D.R. has previously been used with a great amount of success as delicate test for microquantities of tannins as 1 p.p.m.⁶; but the possibility of its being used as a new stain in histological work has never been explored. The author after repeated trials has been able to develop a standard technique by which F.D.R. can successfully be used as a delicate and reproducible test for locating the tannins in plant sections.

The method is simple and presents great advantages over those described before. In the first place it is very specific, no other tissues besides those containing tannins being stained. In order to verify its specific nature, sections of different plant materials were separately stained with standard lignin and cellulose stains, viz., phloroglucin, Maule, anilin sulphate, Schultze's solutions, iodine followed by sulphuric acid and also F.D.R., and examined under the microscope. It was found that tissues which gave a positive reaction either for lignin or for cellulose were in many cases not stained by F.D.R. For instance the xylem or wood though mostly lignified was not stained blue with F.D.R.; so also the pith and rays which are predominantly cellulosic in character, showing thereby that F.D.R. is neither a lignin nor a cellulose stain. Cellulose and lignified tissues are stained by F.D.R. only in the presence of tannin. Thus cotton fibres and filter-paper pulp do not show any colour when stained with F.D.R. but the same materials when treated with F.D.R. after a preliminary steeping in tannin (10 p.p.m.) give a distinct blue colouration.

The method is also more sensitive than any of those described earlier and can be used with very good results even when the concentration of tannins in the tissues is as low as 1 p.p.m. Staining is also permanent. It has been successfully tried for number of plants like flax, tea, jute, etc., and is described below.

THE F.D.R. METHOD FOR DETECTING TANNINS IN PLANT SECTION

Preparation of reagents.—Only two solutions are required and they are prepared as follows:—

A. 2.25 gms. of phosphotungstic acid, 0.5 gm. of phosphomolybdic acid and 1.5 c.c. of syrupy phosphoric acid are taken in a small conical flask to which are then added 17.5 c.c. of distilled water. The mixture is boiled over a water-bath under reflux for two hours, allowed to cool and then made up to 25 c.c. by adding the required quantity of distilled water. The solution deteriorates on exposure to strong light, and it is advisable to keep it in an amber coloured bottle preferably wrapped in black paper.

B. A saturated aqueous solution of sodium carbonate is prepared by boiling 70-75 gms. of anhydrous sodium carbonate with 100 c.c. of distilled water till dissolved. The solution is allowed to cool and settle down and the clear liquid from the top is then decanted and if necessary filtered before use.

Procedure.—As the stain is aqueous the sections have to be brought down to distilled water before applying the stain. The sections are covered with just sufficient quantity of solution A for about 15-30 minutes though with fresh green sections even 5 minutes were found to be quite sufficient. The solution is then drained off and the sections neutralized with a few drops of solution B. The staining is complete when the effervescence due to escape of carbon dioxide ceases. A blue colour is obtained with the formation of a cloudy white precipitate. No attempt should be made to remove the white precipitate by washing the sections in water as the blue stain is readily soluble in water. As soon as neutralization is effected the sections are quickly passed through 30, 50, 70, 85, 95 per cent. and absolute alcohol, cleared in xylol and mounted in balsam. Tissues containing tannins are stained a deep, steel-blue. The staining is permanent and the preparations keep well.

MATERIAL STUDIED

The author has tried the method with uniformly good results for a number of plants. In jute, only the fibre bundles of the phloem are stained the tannins being located in the cell walls, contrary to the findings of Nodder.⁷ According to Kundu,⁸ in jute, the dark deposits which occur abundantly in the cortex and rays are tannins, but they do not take any stain with F.D.R. which clearly shows that they are not tannins. Their chemical nature, however, appears to be in doubt.

A list of plant materials where the presence of tannins has been demonstrated by the above method is given below.

STEM.—*Corchorus capsularis*, *C. olitorius*, *Hibiscus sabdariffa*, *H. rosasinensis*, *H. esculentus*, *Crotalaria juncea*, *Linum usitatissimum*, *Cajanus indicus*, *Mangifera indica*, *Ricinus communis* Rosa sp.

TWIGS.—*Camellia Thea*, *Gossypium* sp., *Sesbania grandiflora*.

LEAVES.—*Agave sisalana* *Bromelia* sp.
FRUIT.—*Musa* sp., *Gossypium* sp.
Jute Agricultural Research
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Indian Central Jute Committee,
October 4, 1943.

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A NEW ASCOMYCETOUS FUNGUS ON SELAGINELLA

THE fungi, so far recorded on *Selaginella*, are *Pythium Debaryanum* and *Synchytrium Selaginellae* among the Phycomycetes; *Taphrina Selaginellae*, *Acrospermum urceolatum* and *Leptosphaeria helvetica* among the Ascomycetes; the Deuteromycete *Phyllosticta Selaginellae*, and two smuts *Melanotanium Selaginellae* and the Indian specimen *Entyloma polysporium*.¹ According to some² the smut recorded from India³ as *Entyloma* bears greater resemblance to the genus *Melanotanium*. Recently Sydow⁴ has described *Melanotanium oreophilum* as a new species on two Indian *Selaginellae*.

The present specimen is an Ascomycete belonging to the order Sphaeriales and the family Sphaeriaceae. The minute perithecia are globose and black and are found superficially in groups at the tips of vegetative shoots or sporangiferous spikes. They are black, smooth, hard, devoid of hairs and lack long beaks. However, an ostiole, situated on a minute papilla, is clearly visible under a lens. The ascospores, which are eight in number in each ascus, are oblong to spindle-shaped, hyaline and bi-celled. Paraphyses are present.

The fungus thus agrees with the description given^{5,6} for the genus *Melanopsamma* to which it belongs. Studies based on microtome sections as well as measurements of spores, asci, etc., show that it is a new species. An interesting feature of this fungus is that its hyphae grow down to a considerable extent, along the vascular bundle, from the tip, but do not affect other parts of the stem such as endodermis, cortex, etc., or the leaves. A full description of the fungus together with the naming of the species will be given elsewhere. It may be noted here that this is also the first record of *Melanopsamma* on any host in India. Butler and Bisby⁷ and Mundkur⁸ do not mention anything about this genus.

The present fungus was found growing on living *Selaginella chrysocaulos* in the Lloyd

Botanical Gardens, Darjeeling, in the month of September.

Department of Botany,
University of Allahabad,
October 5, 1943.

A. K. MITRA.

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CASES OF ANTIPODAL POLYEMBRYONY IN ALANGIUM LAMARCKII Thw.

A DETAILED review on polyembryony has already been published by Webber.² In most of the cases multiple embryos are formed by nucellar budding and these are sporophytic in nature. Development of additional embryos from the antipodals are comparatively of rare occurrence. Cases of antipodals developing further into adventitious embryos are reported by Ernst¹ in *Allium* and Woodworth³ doubtfully refers to such cases in *Amus rugosa*.

In the course of the studies on the Gametogenesis and Embryogeny in *Alangium Lamarckii* Thw., the writer noticed in many cases, the antipodals developing into immature embryos. In normal cases the antipodal nuclei degenerate just prior to fertilization. But in some cases they become cellular. Such a cell

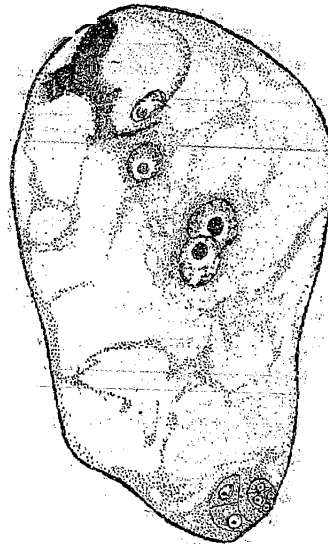


FIG. 1
Embryo-sac of *Alangium Lamarckii* showing antipodal polyembryony. $\times 700$.