

The results of a typical experiment are indicated in the accompanying graph.

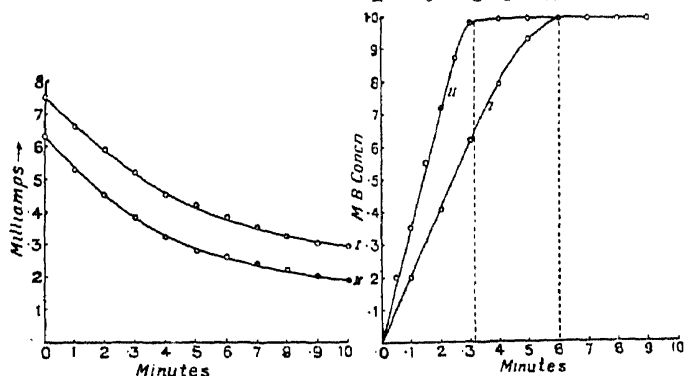


Fig. 1.

Fig. 2.

Fig. 1. Calibration curves. (i) 0.5 c.c. and (ii) 1.0 c.c. enzyme, 5 c.c. phosphate buffer, pH 7.6, 1 c.c. 0.02 per cent. methylene blue and water to make up 10 c.c.

Fig. 2. Dehydrogenating action of succinic dehydrogenase. (i) 0.5 c.c. and (ii) 1.0 c.c. enzyme, 5 c.c. of phosphate buffer, pH 7.6, 1 c.c. M/10 sodium succinate, 1 c.c. 0.02 per cent. methylene blue, and water to make up 10 c.c.

The method will be found applicable to the study of systems involving changes in intensity of colour and in turbidity.⁴ By employing appropriate colour filters greater accuracy will be rendered possible.

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¹ Pister, *Z. Physiol. Chem.*, 1937, 246, 248.

² Evelyn, *J. Biol. Chem.*, 1936, 115, 63.

³ Malherbe, *Biochem. J.*, 1937, 31, 300.

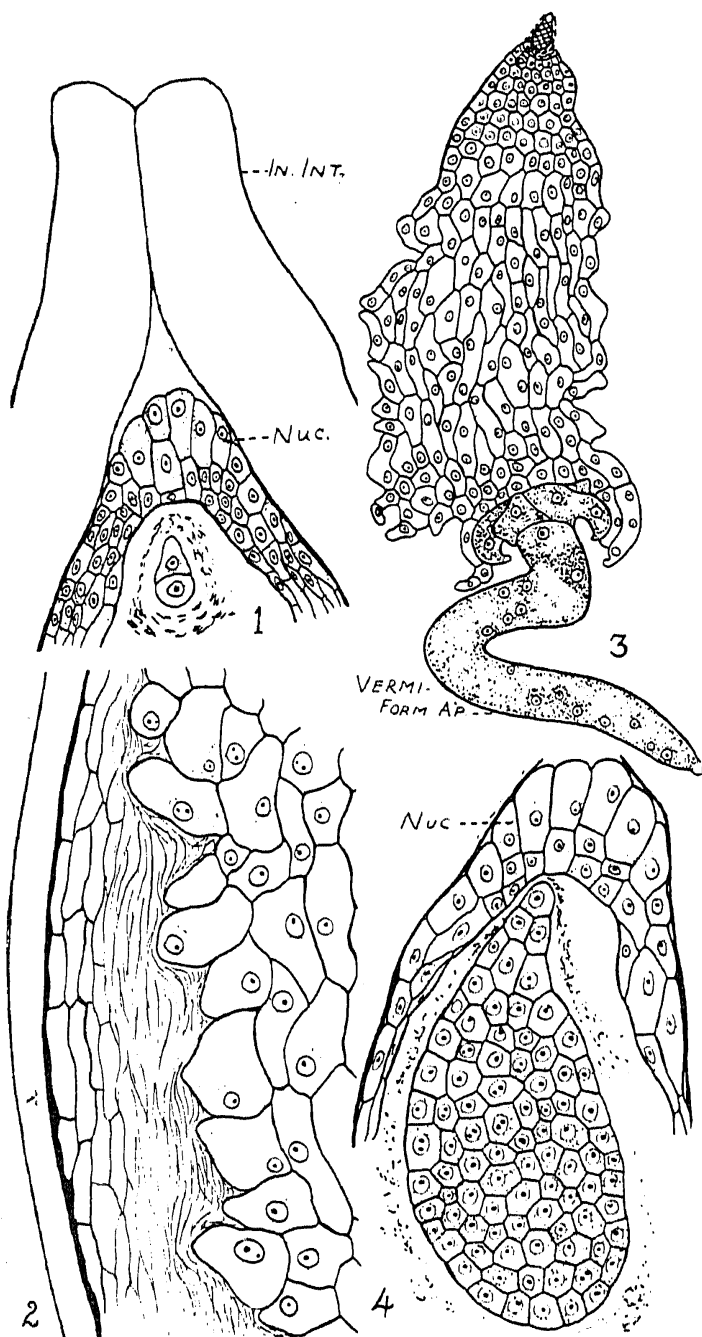
⁴ Sreenivasaya, Unpublished work. The method was employed for studying the kinetics of the peptic hydrolysis of isolated proteins; and later, extended to the study of the peptic hydrolysis of casein in its natural and artificial environments.

The Endosperm in *Grevillea robusta* Cunn.

In the literature on the Proteaceæ, the papers by Ballantine (1909)¹, Messeri (1928)² and Brough (1933)³ stand out conspicuously as important recent contributions. The most complete of these papers is by Brough who, in a study of *Grevillea robusta* Cunn. gives an exhaustive account of floral organogeny, pollination and seed morphology and discusses the systematic position of the family. He also describes significant phases in sporogenesis, development of the gametophytes, endosperm and embryo and states that the haploid number of chromosomes is ten.

The following account of the same species is intended to describe certain features in the life-history which are either not mentioned by Brough or appear to be at variance with his observations. The tip of the nucellus fits into the micropyle as a conical projection and is almost intact with its glandular cells (Fig. 1) till after the formation of the embryo. On the other hand, Brough's Figures 73 and 80, particularly the former, suggest that the tip of the nucellus has disorganised and consequently the endosperm is slightly projecting into the micropyle in Fig. 73. The endosperm does not enter the micropyle at the stage depicted in Brough's figure and even later when the nucellar tip is disorganised, the endosperm is also broken down by the embryo at the region of the micropyle. The cells of the nucellar tip have dense contents and no doubt serve to nourish the growing embryo for some time, after which they disorganise and the embryo therefore becomes lodged in the micropyle.

In the formation of the endosperm, while cell formation is complete only in the upper half of the embryo-sac, it proceeds more or less sluggishly in the lower region as stated by Brough. The present author has been able to discover that the lowermost region remains cœnocytic and grows as a large *vermiform appendage* of the endosperm, penetrating through the cells at the base of the nucellus and breaking them down, where a large mass of cytoplasm is therefore formed. In addition, the marginal cells at the base of the endosperm grow in the form of processes and attack the cells of the nucellus all along the sides of the embryo-sac (Fig. 2). A large cavity is thus later formed all round the embryo-sac by the disorganisation of the nucellus, which persists only as a very thin layer within the integument in the developing seed. The entire mass of endosperm, which in the early stages of embryo formation is hanging loose within this cavity, can therefore be easily picked up with the aid of a dissecting needle and mounted whole for examination. Such a preparation reveals remarkably clearly the complete mass of endosperm with its marginal cells in the form of processes (simulating the marginal cells of the foot of the sporophyte in *Anthoceros*) and the very curious coiled *vermiform appendage* (Fig. 3) which is described here for the first time. On the other hand, a



Figs. 1-4.

Figs. 1 & 2. $\times 200$; Fig. 3. $\times 280$; Fig. 4, very highly magnified from a whole mount.

mere examination of serial microtome sections does not give a complete picture of the peculiarly developed endosperm and its interesting features may therefore be overlooked.

In the many celled embryo, the proximal region appears to be more or less wedge-shaped and the cells are larger with less dense contents than those in the more distal region (Fig. 4). The change in the shape of the embryo, as described by Brough, is not attained. His statement of the absence of

a suspensor is however fully borne out by the present investigation.

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- ¹ Ballantine, A. J., *Ann. Bot.*, 1909, **22**, 161-62.
² Messeri, A., *Nuovo Giorn. Bot. Ital.*, 1928, **34**, 1037-42.
³ Brough, P., *Proc. Linn. Soc. New South Wales*, 1933, **58**, 33-73.

Rottlerin—Part III.

THE difficulty of explaining the nitrogen trioxide addition product of methyl ether of rottlerin has been emphasised in Part II. It now appears that if the formula for rottlerin is taken to be $C_{31}H_{30}O_8$, then the methyl ether m.p. 144° described before, becomes a penta-methyl ether of the composition $C_{36}H_{40}O_8$ and the hydrogen peroxide oxidation product (m.p. 128°) becomes $C_{36}H_{40}O_9$. The analytical value for this oxide was found to be C, 70.06; H, 7.2, and (C, 69.87, H, 7.1 in duplicate) whilst $C_{36}H_{40}O_9$ requires C, 70.01 and H, 6.5; and $C_{36}H_{42}O_9$ requires C, 69.9; H, 6.8%. By the action of sodium nitrite either with acetic or butyric acid, the same product m.p. 207° (decomp.) is formed. Therefore, in the formation of this compound an acetic acid residue could not have been fixed by the molecule. If simple N_2O_3 be fixed by the molecule at one of the double bonds and the methyl ether be taken as $C_{36}H_{40}O_8$, then the additive product becomes $C_{36}H_{40}O_{11}N_2$ requiring C, 63.9, H, 5.92 and N, 4.14, whilst values found were C, 63.87; H, 5.9 and N, 4.2%. The dihydro derivative m.p. 162° requires 63.7; H, 6.2; N, 4.1 on the basis of formula $C_{36}H_{42}O_{11}N_2$ whilst the values found were C, 63.44; H, 6.23 and N, 4.0%. The *iso* and the dihydro *iso* bodies necessarily will also be accommodated by this formula.

The M.W. of tetrahydro rottlerin has been found to be 507 whilst $C_{31}H_{34}O_8$ requires 534. The methyl ether (m.p. 144°) has given M.W. in a recent determination as 572, whilst $C_{36}H_{40}O_8$ requires 600.

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