FERN CALLUS TISSUE CULTURE[1]

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THE FIRST INDEFINITE plant callus tissue cultures were obtained by White (1939), Gautheret (1939), and Nobécourt (1939), from tumorous tissue or from fleshy organs composed for the most part of undifferentiated parenchyma. Subsequently it proved feasible to cultivate tissues obtained by the proliferation *in vitro* of cambium and phloem parenchyma of various herbaceous and woody dicotyledons, and more recently it has been found possible to obtain tissue cultures from monocotyledons (Morel and Wetmore, 1950).

Vascular cryptogams, and specifically ferns, on general grounds would not seem to be very favorable for tissue culture studies. They are, in fact, devoid of secondary activity and are almost entirely made up of highly differentiated tissues seemingly unlikely to proliferate masses of meristematic parenchyma, which tissue is conducive to growth *in vitro*. General observations, however, led to the belief that this problem was not impossible to solve. For example, in the course of his investigations on regeneration in ferns, Goebel (1907) and his students (Beyerle, 1932) observed several times growth of undifferentiated formations on the petiole and blade of sporeling leaves which he had removed from the plant and maintained on damp soil. These outgrowths were analogous to callus of dicotyledons. The early observations of Farlow (1874), de Bary (1878), Heim (1846), and others more recently showed that a similar type of formation may also appear on the prothalli of certain species of ferns. However, in these cases the proliferation was usually followed by the appearance of apogamous sporelings. It was such proliferations as these that we employed to obtain callus cultures of fern.

Spores of *Osmunda cinnamomea* L. were planted in March 1948, on a medium devised by Knudson (1925) in the course of his investigations on orchid embryo cultures. They germinated in a few days and developed rapidly forming thin heart-shaped prothalli. After 2 months of culture, we observed on five of these prothalli the spontaneous appearance of undifferentiated calluses. These calluses appeared on the upper surface and were formed of a mass of greenish cells containing fewer chloroplastids than those of the remainder of the prothallus. They developed slowly and by June 1949, were transferred to the same medium, upon which their growth continued very slowly. After 8 months, the largest had attained a diameter of only 1 cm., while three of them had died. The living cultures were then transferred to Knudson's medium to which were added certain B-vitamins at the following concentrations by weight: thiamin, 10^{-6}; i-inositol, 10^{-4}; pyridoxin, 10^{-6}; calcium d-pantothenate, 10^{-6}; biotin, 10^{-8}; nicotinic acid, 10^{-6}. The effect of these substances was spectacular. In less than a month the callus had grown considerably more than during 8 months on the original medium. The diameters of each of the subcultured fragments measured more than 15 mm. We propose now to attempt to determine which of these substances are indispensable for the optimum growth of *Osmunda* tissue, the cultures of which now seem stable and continue to grow actively.

These cultures form tissues which appear to be of granular and friable masses (fig. 1). They are composed of parenchyma made up of large cells, in the center of which meristematic islets are formed which grow independently one from another forming a multitude of small nodules (fig. 2). In the older parts of the callus certain cells are transformed into tracheids (fig. 3).

The existence of tracheids in prothallial tissue of ferns has been pointed out many times. (See review of literature by Steil, 1939). In all cases, so far as the authors can determine, this phenomenon has sooner or later been attributed to apogamy. The evidence provided, except in the studies of Farmer and Digby (1907), has not been supported by chromosomal studies. In the unusual occurrence of tracheids in the prothallus of *Psilotum* reported by Holloway (1939), Manton (1942) has established the chromosomal complement of the gametophyte as "not fewer than 100" whereas that of the sporophyte proved to be "more than 170" chromosomes. Manton concluded that the gametophytes of *P. triquetrum* SW. [=*P. nudum* (L.) Beauv.], which formed the basis for Holloway's report were diploid. The single sporophyte from the same locale proved to be tetraploid.

In smears of callus tissue of *Osmunda cinnamomea*, stained with aceto-carmine,[2] the cells possess twenty-two chromosomes (fig. 5). By contrast, smears of root-tips show the sporophyte number to be forty-four (fig. 4). These numbers are in accord with the observations on this species by Yamanouchi (1910), de Litardière (1921), and Okuno (1936).

It is clear from the present study and from that of Holloway (1939) on *Psilotum* that tracheids can occur in prothallial tissue without the presence of

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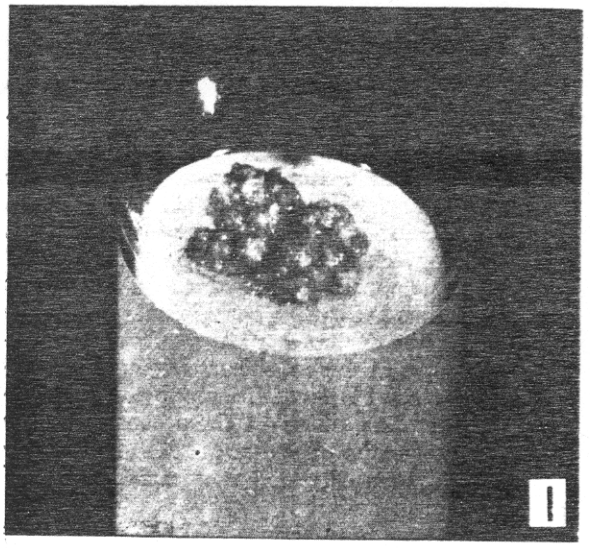

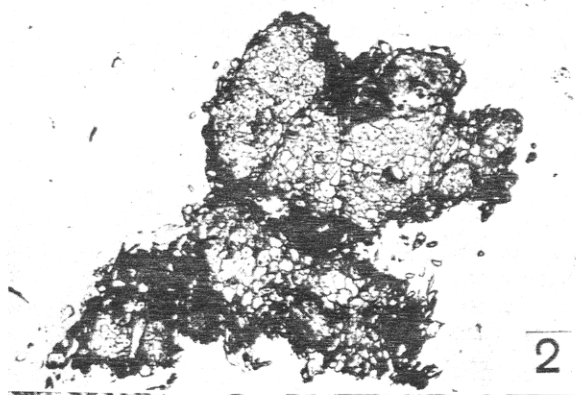

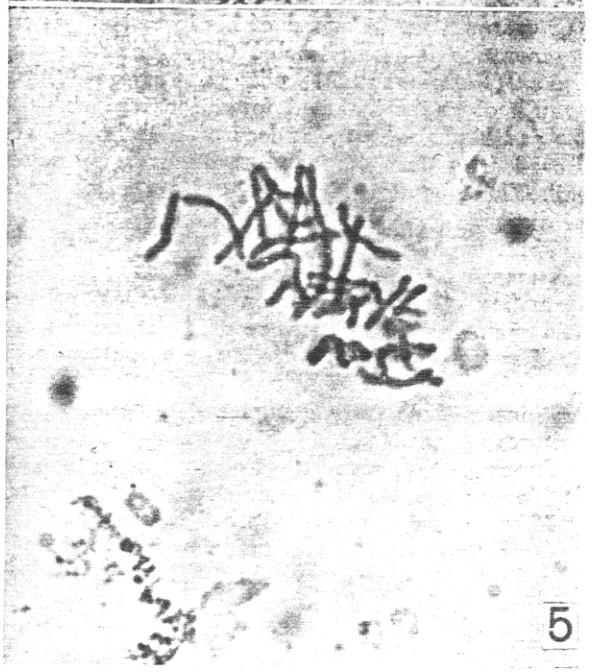

Fig. 4–5. Aceto-carmine preparations of *Osmunda cinnamomea*.—Fig. 4. Root-tip preparation showing forty-four chromosomes. ×800.—Fig. 5. Preparation from callus culture of prothallial tissue showing twenty-two chromosomes. ×1000.

Fig. 1–3. Callus of *Osmunda cinnamomea*.—Fig. 1. Culture of *O. cinnamomea* callus, 1 month old, on Knudson's medium supplemented by B-vitamin mixture. ×2.—Fig. 2. Section of callus culture of this species showing general parenchymatous organization. ×40.—Fig. 3. Section of part of callus culture of the same species showing nest of tracheids. ×230.

apogamy. The authors therefore suggest that the existence of tracheids in the independent gametophytes of vascular cryptogams is therefore not by itself sufficient evidence that apogamy must be an accompanying phenomenon.

We have tried to induce experimentally the development of these calluses on *Osmunda* prothalli. In order to do this we planted spores of this fern on a medium containing strong concentrations of naphthaleneacetic acid (10^{-5} and 10^{-6} by weight). On this type of medium most of the spores, instead of germinating to form normal heart-shaped prothalli, developed into undifferentiated masses of colorless tissue. Nevertheless, these formations grew very little and when we tried to subculture them on a medium devoid of auxin they either ceased to grow or re-formed normal chlorophyllous prothalli.

We then attempted to produce small lesions on adult prothalli and to do this we cultivated colonies of prothalli in the Knudson's medium containing 2 per cent dextrose but not solidified by agar. The cultures were placed on a shaking machine and agitated for two months. Previous observations (Hurel-Py, 1942) have shown that, on other ferns, the dextrose at this concentration inhibits the formation of sporophytes. But in this case, in a liquid medium and with constant agitation, sporophytes were formed in a large number on the adult prothalli. At the same time in one case an undifferentiated callus appeared which was analogous to those previously observed.

SUMMARY

Spores of *Osmunda cinnamomea* L. germinated on Knudson's medium developed into the usual heart-shaped, thin, green prothalli. Five produced green calluses on the upper surfaces after 2 months. These calluses grew slowly or died on this medium. When transferred to the same medium supplemented by B-vitamins in appropriate concentrations the calluses grew rapidly and continue to do so. The calluses were of nodular masses of large-celled parenchyma in the center of each of which is an islet of meristematic tissue. Certain of the older parenchyma cells form tracheids. Chromosomal studies show the prothallial callus to be haploid. It is suggested that the presence of tracheary tissue in the independent prothalli of vascular cryptogams is therefore not by itself sufficient evidence that apogamy is an accompanying phenomenon. Only one callus has so far been experimentally induced on prothalli of *Osmunda*.

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LITERATURE CITED

BARY, A. DE. 1878. Ueber apogame Farne und die Erscheinung der Apogamie im Allgemeinen. Bot. Zeit. 36: 449-487.

BEYERLE, R. 1932. Untersuchungen über die Regeneration von Farnprimärblättern. Planta 16: 622-665.

FARLOW, W. G. 1874. Ueber ungeschlechtliche Erzeugung von Keimpflänzchen an Farn-Prothallien. Bot. Zeit. 32: 180-183.

FARMER, J. B., AND L. DIGBY. 1907. Studies in apospory and apogamy in ferns. Ann. Bot. 21: 161-199.

GAUTHERET, R. J. 1939. Sur la possibilité de realiser la culture indéfinie des tissus de tubercule de carotte. Comp. Rend. l'Acad. Sci., Paris 208: 218.

GOEBEL, K. 1907. Experimentelle morphologische Mitteilungen. I. Sitzber. Akad. Wiss. München, Math-Phys. Kl. 37: 114-136.

HEIM, C. 1896. Untersuchungen über Farnprothallien. Flora 82: 329-372.

HOLLOWAY, J. E. 1939. The gametophyte, embryo, and young rhizome of *Psilotum triquetrum* Swartz. Ann. Bot. 3: 313-336.

HUREL-PY, GERMAINE. 1942. Étude de quelques milieux permettant la culture illimitée d'un prothalle d'*Asplenium*. Compt. Rend. l'Acad. Sci., Paris 214: 571-573.

KNUDSON, L. 1925. Physiological studies of the symbiotic germination of orchid seeds. Bot. Gaz. 79: 345-379.

LITARDIÈRE, R. DE. 1921. Recherches sur l'élément chromosomique dans la caryocinèse somatique des Filicinées. Cellule 31: 255-473.

MANTON, IRENE. 1942. A note on the cytology of *Psilotum* with special reference to vascular prothalli from Rangitoto Island. Ann. Bot. 6: 283-292.

MOREL, G., AND R. H. WETMORE. 1951. Tissue culure of monocotyledons. Amer. Jour. Bot. 37: 138-140.

NOBÉCOURT, P. 1939. Sur la perennité et l'augmentation de volume des cultures des tissus végétaux. Comp. Rend. Soc. Biol. (Paris) 130: 1270.

OKUNO, S. 1936. Chromosome numbers in some sporophyll-bearing ferns. Bot. Mag. Tokyo 50: 332-337.

STEIL, W. M. 1939. Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. 5: 433-453.

WHITE, P. R. 1939. Potentially unlimited growth of excised plant callus in artificial nutrient. Amer. Jour. Bot. 26: 54-64.

YAMANOUCHI, S. 1910. Chromosomes in *Osmunda*. Bot. Gaz. 49: 1-12.