freely on media with the carcinogens used. Indole acetic acid (5 mg./l.) at the concentration used in these studies hastens the formation of cauline initials from excised fibrous roots of the carrot. Their development occurs when the influence of the growth substance becomes attenuated or when freed from the IAA by transfer to media lacking it.

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TISSUE CULTURE OF MONOCOTYLEDONS[1]

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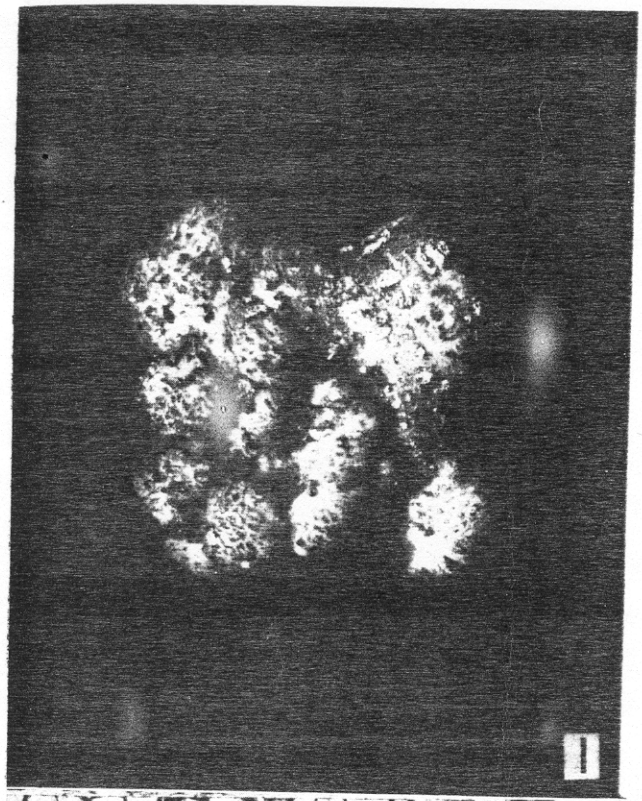
DURING THE last 10 years, plant tissue culture has undergone considerable progress. The results which were at first obtained by White (1939), Gautheret (1939), and Nobécourt (1939) with tissues of tumors or of fleshy organs of tuberous plants have been extended to a large number of herbaceous or woody dicotyledons distributed through more than a dozen different families. However, in spite of a certain number of attempts, no one has been successful until now in obtaining a callus or root tissue culture of any monocotyledon. However, Loo (1945) was successful in growing stem cultures *in vitro* from apical meristems of *Asparagus officinalis*.

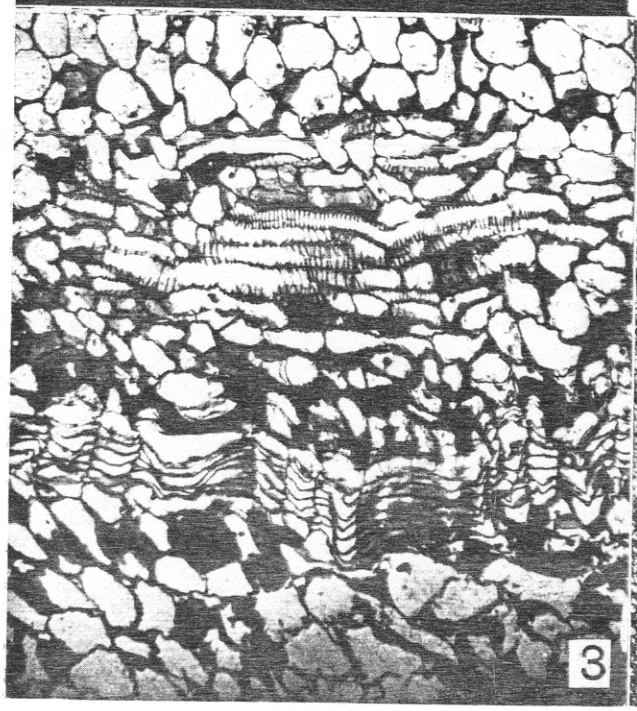
Recently we have attempted to resolve this problem. Our studies were initiated upon a tropical member of the *Araceae* which produces a very large tuber, *viz.*, *Amorphophallus* (*Hydrosme*) *Rivieri* Dur. This plant, which originates from Indo-China, has a very peculiar mode of development. The stem is reduced to an underground tuber weighing several pounds. Growth begins in the spring by the development of the inflorescence which is composed of a long spadix enclosed by a spathe. As soon as the inflorescence has wilted, the tuber produces a single leaf which is the only leaf produced all summer (Chao-Nien Sun, 1948).

In order to obtain tissue cultures of this plant, we first sterilized the tuber for 20 min. in a 5 per cent solution of calcium hypochlorite (Pittchlor). Then we removed cubes aseptically, each with a dimension of 0.5 cm. and a weight of approximately 250 mg. These fragments were planted in April 1949, on Gautheret's medium (Gautheret, 1942) to which were added several B-vitamins at the following concentrations by weight: thiamin, 10^{-6}; nicotinic acid, 10^{-6}; calcium d-pantothenate, 10^{-6}; biotin, 10^{-8}; i-inositol, 10^{-4}; pyridoxin, 10^{-6}; folic acid, 10^{-5}; and also naphthaleneacetic acid at the concentrations of 10^{-6}, 10^{-7}, and 10^{-8}. All the explants proliferated uniformly forming voluminous calluses. Moreover, bud initials appeared on certain cultures, and both roots and buds on still others (fig. 2). Roots, however, were more abundant on tissues cultivated in the presence of high concentrations of auxin. A month later in May 1949, the growing explant had attained a weight of approximately 2 g. Fragments of these were then subcultured on the same basal medium including the same vitamins and naphthaleneacetic acid at the concentrations by weight of 5×10^{-7} and $5 \times$

[1] Received for publication May 5, 1950.

This work was supported in part by a grant-in-aid from the American Cancer Society upon a recommendation of the Committee on Growth, and in part under a grant from the Massachusetts Division of the American Cancer Society, Inc.





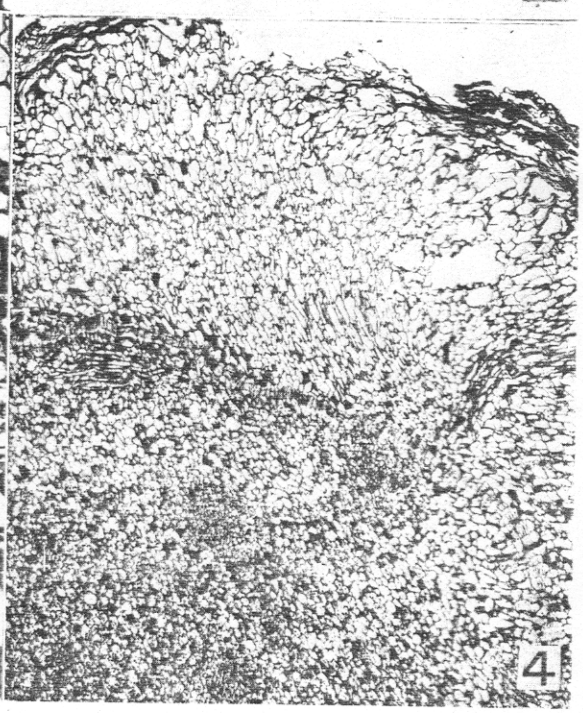


Fig. 1–4. Callus of *Amorphophallus Rivieri* and *Sauromatum guttatum* grown *in vitro*.—Fig. 1. Tissue culture of *Amorphophallus Rivieri* Dur. 6 weeks old. Note irregular surface resulting from periderm produced by a regular cork cambium. ×2.6—Fig. 2. Bud and roots produced by callus of *Amorphophallus* cultivated *in vitro*. ×1—Fig. 3. Section of callus culture of *Sauromatum guttatum*. Note cork cambium and nest of tracheids. ×100—Fig. 4. Section of callus culture of *Amorphophallus*. Note cork cambium forming a continuous periderm. ×6.

10^{-8}. Growth continued but at a slower rate. The average growth of each explant was approximately 1500 mg. in 2 months. We noticed at the same time that some of these cultures had stopped growing and were dying; this seemed to indicate a depletion of one or several growth substances indispensable to the tissue development. We then subcultured a part of these on the same basal medium devoid of vitamins but including 15 per cent coconut milk (van Overbeek *et al.*, 1941, 1942; van Overbeek *et al.*, 1944; Caplin and Steward, 1948). The milk was obtained from green coconuts[2] which had not yet attained their full development and which measured approximately 5 in. in diameter in the husk. The milk, sterilized by filtration and water-clear, was included in the agar medium which had been autoclaved and was still fluid at a temperature of 50°C.

On this new medium all cultures developed very rapidly, showing an increase in weight of about 1.5 g. in a month and a half. They have been subcultured since then regularly at 6 week intervals on the same medium. Their growth was maintained at a rapid rate and we are now assured of obtaining their proliferation for potentially unlimited periods. However, it should be pointed out that growth of this callus of *Amorphophallus* upon a medium containing the same concentration of ripe coconut milk is much less active, as measured by increase in weight. In fact the milk obtained from green coconuts gradually loses its stimulating effect upon the growth of this callus after it has been stored in sterile condition for 3 or more months.

Part of these cultures were maintained on the initial medium containing vitamins and naphthaleneacetic acid at various concentrations. The proliferation in this case was slow and mortality very high so that we are not certain that we can maintain the strain without coconut milk.

Amorphophallus tissues form very compact and irregularly-surfaced colonies (fig. 1). They are made up of homogenous, large-celled parenchyma, rich in starch, within which appear a few clusters of irregular xylem elements and also scattered bundles of raphides (fig. 4).

A cork cambium develops in the periphery and produces a sort of bark which surrounds the whole growth. Their suberized surface gives them a brownish look.

We should point out that we have obtained comparable results with another member of the *Araceae*, *viz. Sauromatum guttatum* Schott (fig. 3).

[2] The authors wish to express their appreciation to Mr. Hugh M. Matheson and Mr. Allen Williams of Miami, Florida, who have provided them repeatedly with coconuts.

SUMMARY

It has been possible to obtain the proliferation *in vitro* of tissue of monocotyledons for potentially indefinite periods. Our studies seem to indicate that these tissues require certain growth substances, as yet undetermined, which are found in the milk of immature coconuts.

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