## Extensive Endoplasmic Reticulum: a Distinguishing Feature of Auxin-Producing Plant Cells

The growth of higher plant cells is dependent upon the hormone auxin (indole 3-acetic acid). In intact plants the growing tips and cambia are believed to be the source of this hormone; in tissue culture most plant cells require the addition of indole 3-acetic acid to the medium for growth. Under certain conditions plant cells that usually depend upon meristematic cells for auxin produce this hormone themselves. For example, cells of bacteria-free crown gall tissue are able to synthesize their own required auxin and grow best on auxin-free medium. Similarly, tissue from the hybrid tumor formed by *Nicotiana glauca* × *N. landsdorfii* is able to grow on hormone-free medium, despite the fact that both of the parental tissues require exogenous auxin for growth in vitro[1]. A third example is the case referred to as 'habituation' or 'anergia', a situation where plant cells that at one time required auxin in the medium for their growth in vitro spontaneously produce this hormone[2].

Previous fine structure studies on crown gall cells[3–6] have demonstrated that these auxin-producing cells are distinguished by extensive endoplasmic reticulum, as are cells from habituated cultures of *Vinca* and *Acacia*[7]. Moreover, cells stimulated to active proliferation by the exogenous application of auxin do not become richer in endoplasmic reticulum[4,5]. This report suggests that abundant endoplasmic reticulum appears to be a common substructural characteristic of auxin-producing cells.

Tissues of *N. glauca*, *N. langsdorfii* and their hybrid were grown in culture, the first two on auxin-supplemented media, the last on auxin-free medium. Tissues were fixed in 3% glutaraldehyde in cacodylate buffer (pH 6.8), postfixed in 2% osmium, dehydrated, and embedded in Epon 812. Sections were cut, stained with lead[8] and examined with a Zeiss EM9S electron microscope. For further comparison, cells from tumors incited by bacteria and dependent on their continuing presence for a source of auxin were examined. These tumors were obtained on *Kalanchoe* by infecting stem punctures with a virulent strain of *Pseudomonas savastanoi*; tissue from these tumors was fixed and processed in the manner described above.

The cells from the hybrid tumor tissue (Figure 1) can be seen to be considerably richer in endoplasmic reticulum than those from either parent (Figures 2 and 3). The abundant endoplasmic reticulum in the hybrid cells is similar in amount to that seen in crown gall cells[5], and is largely of the ribosome-studded or rough type. In contrast, cells

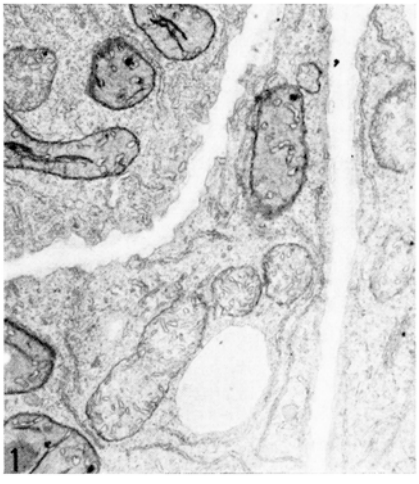


Fig. 1. *Nicotiana* hybrid tumor cells. Note extensive endoplasmic reticulum. ×10,500.

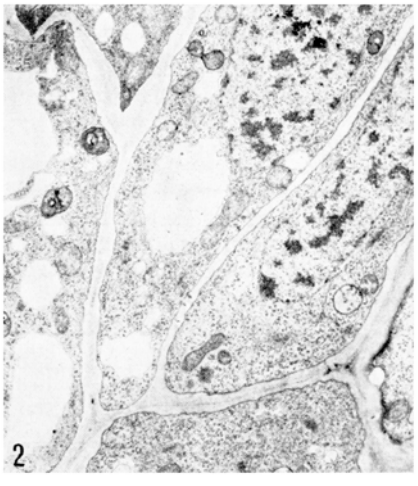


Fig. 2. *N. langsdorfii* cells. ×4153.

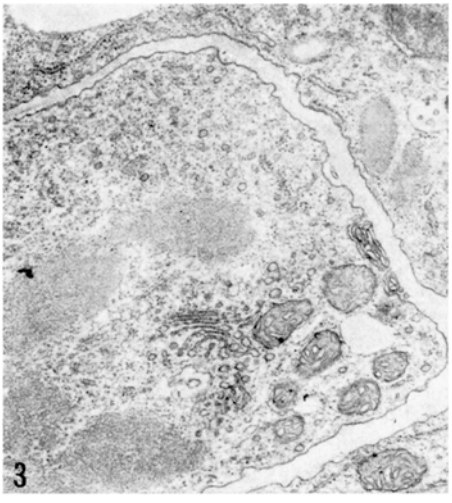


Fig. 3. *N. glauca* cells. ×12,858.

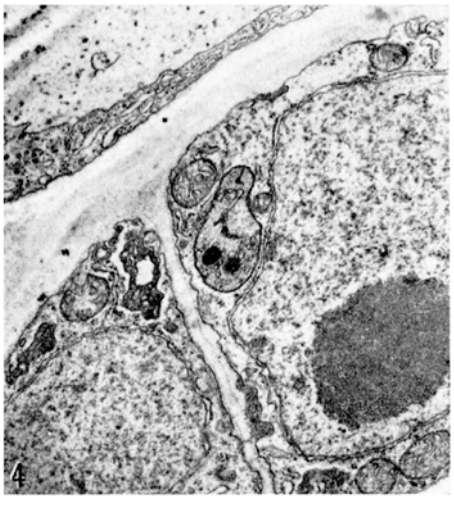


Fig. 4. Tumor cells from *P. savastanoi*-induced tumor in *Kalanchoe*. ×9,286.

from the *Kalanchoe* tumors, although capable of rapid growth in the presence of the inciting bacteria, have little endoplasmic reticulum (Figure 4), and closely resemble *Kalanchoe* cells stimulated to rapid growth by exogenously applied auxin[5].

Other workers have demonstrated that meristematic cells, which are believed to actively secrete auxin, are similary rich in endoplasmic reticulum[9,10]. These observations support the hypothesis that extensive endoplasmic reticulum is characteristic of auxin-producing cells and is not a cellular response to auxin.

The important role of rough endoplasmic reticulum in protein synthesis in plant and animal cells has been extensively documented. An abundance of endoplasmic reticulum in protein-secreting plant cells has also been reported[11]. This suggests that protein synthesis and auxin synthesis may have a common subcellular site[12].

*Résumé.* Le cytoplasme des cellules végétales capables de synthétiser l'auxine est caractérisé par l'abondance du réticulum endoplasmique. Les cellules dont la croissance est stimulée par de hautes concentrations de cette hormone contiennent beaucoup moins de réticulum endoplasmique. D'où l'hypothèse que le lieu de synthèse de l'auxine (dans les cellules capables de cette synthèse) est peut-être le réticulum endoplasmique.

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## Inhibition by Ergocornine and 2-Br-α-Ergocryptin of Spontaneous Mammary Tumor Appearance in Mice

Hyperplastic nodules (HN) are represented as the preneoplastic state of spontaneous mammary tumor development in mice[1]. The authors previously reported that ergocornine and 2-Br-α-ergokryptin (CB-154) suppress the pituitary prolactin secretion and inhibit the development and growth of mammary HN in mice[2]. Furthermore, they have demonstrated that these ergot alkaloids inhibit the prolactin secretion not only from the in situ but also the grafted pituitaries[3]. The present experiment was carried out in order to investigate whether or not ergocornine and CB-154 suppress the appearance of spontaneous mammary tumors in mice when administered continuously for a long period as pellet.

*Materials and methods.* Animals used were 3- to 5-month-old virgin mice of C3H/He strain bred in the authors' laboratory. They were divided into 3 groups. Groups I and II were implanted subcutaneously with pellets of ergocornine methanesulfonate and CB-154, respectively. Each ergot alkaloid was thoroughly mixed with cholesterol in the ratio of 1:4, and 50 mg of the mixture was pelleted in the size of 5 mm in diameter and 2 mm in thickness. Group III as the control received the same size of pellet of cholesterol only.

Approximately 4 weeks after the pellet implantation, all mice were given 1 pituitary isograft each under the right kidney capsule expecting the enhancement of mammary tumor development[4]. Four months after the first pellet implantation, each group received one additional pellet of the same drug. All mice were examined for palpable mammary tumors once a week throughout the experiment. The mice with palpable tumors were killed

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Body and organ weights and mammary tumor incidence in each group

| Group | Treatment | No. of mice | Body weight (g) | | Change (%) (%) | Anterior pituitary weight (mg) | Ovarian weight (mg) | Mammary tumor incidence (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | Final | | | | |
| I | Ergocornine | 10 | 23.0 ± 0.7[a] | 32.4 ± 0.9 | 43 ± 6 | 1.98 ± 0.10 | 11.1 ± 1.9 | 20.0[b] (2/10)[d] |
| II | CB-154 | 10 | 22.1 ± 0.5 | 33.1 ± 1.5 | 50 ± 8 | 2.11 ± 0.12 | 8.7 ± 0.7[c] | 10.0[c] (1/10) |
| III | Control | 19 | 22.3 ± 0.6 | 32.1 ± 0.6 | 43 ± 5 | 2.30 ± 0.09 | 14.8 ± 1.2 | 73.7 (14/19) |

[a] Mean ± standard error of the mean. [b] Significant against control $P < 0.02$. [c] Significant against control $P < 0.01$. [d] No. of mice with tumors/total No. of mice examined.