

John R. Jablonski and Folke Skoog

202

Cell Enlargement and Cell Division in Excised
Tobacco Pith Tissue

Reprinted from
Physiologia Plantarum, vol. 7 p. 16-24, 1954

Cell Enlargement and Cell Division in Excised Tobacco Pith Tissue¹

By

JOHN R. JABLONSKI² and FOLKE SKOOG.

Department of Botany University of Wisconsin Madison, Wisconsin
(Received July 17, 1953)

Introduction

In recent years tissue culture techniques have been employed to advance in studies of hormone or growth factor requirements in plants as well as in studies of certain aspects of the functions and interrelationships of such factors which cannot be readily determined in intact plants. However, the experience with excised organs and fragments consisting of complex tissues and also with callus cultures as experimental material often has been that various cell types or regions of the tissue respond differently to different substances and to different concentrations of a single substance. Difficulties have arisen therefore in interpreting the effects of the applied substances, particularly in relating the effects of treatments to specific changes in growth and tissue composition.

It has been found that when tobacco stem segments, consisting of cortical, vascular, and pith tissues, are cultured on White's medium with various growth substances added, the pith cells undergo some proliferation as well as enlargement on media containing indoleacetic acid (IAA), naphthalene-acetic acid, or other substances with auxin activity (Skoog and Tsui, 8).

¹ This work was supported in part by the University Research Committee with funds from the Wisconsin Alumni Research Foundation and in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

² Present address: Dept. of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh 13, Pennsylvania.

In contrast with this behavior, pith parenchyma sections freed vascular elements fail to grow appreciably on the same control medium but in the presence of suitable concentration of IAA the pith cells respond with an enormous cell enlargement entirely unaccompanied by cell division.

In view of this differential response of the pith cells in the presence or absence of vascular elements and the unusual magnitude of the cell enlargement in response to IAA, a detailed study of the growth of tobacco tissue *in vitro* has been carried out, the results of which are reported in this paper.

Materials and Methods

Young rapidly growing internodes of 2–3 ft. tall tobacco plants (*Nicotiana glauca*, var. Wis. no. 38), grown in seven inch pots in the greenhouse with supplementary illumination were used as stock material for the preparation of stem segment and pith section cultures. Culture media and methods for preparing sterile cultures were essentially those described by Skoog and Tsui (8). The basic nutrient medium contained the following substances (mg./l.): Ca(NO₃)₂ 100, KNO₃ 80, MgSO₄ 35, KCl 65, K₂O 0.5, MnSO₄·H₂O 2.5, ZnSO₄ 1.5, H₃PO₄ 1.6, KH₂PO₄ 37.5; glycine 2, thiamine hydrochloride 0.1, nicotinic acid 0.5, and pyridoxine hydrochloride 1.0, well as 20 g./l. sucrose and 10 g./l. agar. The pH of the solution was adjusted initially to about 5.0 with 0.1 N HCl or NaOH. The vitamins, pure from Nutritional Biochemicals Corp., were used without purification. Glycine and indole-3-acetic acid (Eastman Kodak Co.) were recrystallized before use.

Pith tissue sections free from vascular tissue and of uniform diameter were prepared as indicated in Figure 1. Stem segments were cut with a sharp cutting tool into one centimeter long cylinders. The cylinders were then longitudinally with a second cutting tool, into blocks which were then with a scalpel into rectangular pith parenchyma sections of diameter 10×4×1 mm. These pith sections were cultured on the surface of 50 ml. nutrient agar medium in 125 ml. Erlenmeyer flasks. Two to four sections were planted in each flask in individual experiments, and the cultures were grown in cabinets in a central hallway of the building where conditions although not controlled, remained fairly uniform. The temperature was 25° C., and continuous, weak diffuse illumination was provided by fluorescent ceiling lights. Growth was measured in terms of changes in weight and dry weight of pooled samples of from six to twenty sections each treatment. The fresh weight determinations were carried out by blotting off the surfaces and weighing the samples in glass stop

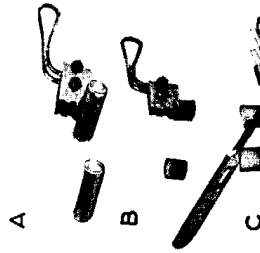


Figure 1. Steps in the preparation of tobacco pith sections.

weighing bottles. The dry weights were determined after keeping the sections for 48 hours in a forced air oven at 65° C. In general, measurements of fresh and dry weights were reproducible within 10 per cent with samples of ten sections each, but only results based on much greater differences than this between treatments will be considered in this report.

Results

Cell Enlargement

Results of an experiment with serial concentrations, from 0 to 20 mg./l., of IAA added to the medium are presented in Figure 2. During the first two to three days in culture, all sections generally remain visibly unchanged, but some growth occurs as indicated by increases in weights of the sections and occasionally in size of individual surface cells. The sections with 0.02–2.0 mg./l. IAA treatments then begin rapid expansion, the exact starting time, duration, and final extent depending on the concentration of IAA supplied, and apparently also on the growth vigor or other seasonal variations in the plant material. The optimal concentration varies slightly from one experiment to the next but generally is 2–3 mg./l. Higher concentrations of IAA, from 4 to 20 mg./l. result in progressively less increase and finally complete inhibition of growth. The progress of cell expansion for optimal treatments with IAA is shown in Figure 3. The enlargement of the individual cells involves marked elongation followed by further increase in volume to produce practically isodiametric cells. The cells in the upper surface layers expand first, and as they round up into spheres are peeled away from the tissue underneath. The process continues for 10–14 days, i.e., until all or nearly all the tissue has been transformed into a loose mass of practically

CELL ENLARGEMENT AND CELL DIVISION

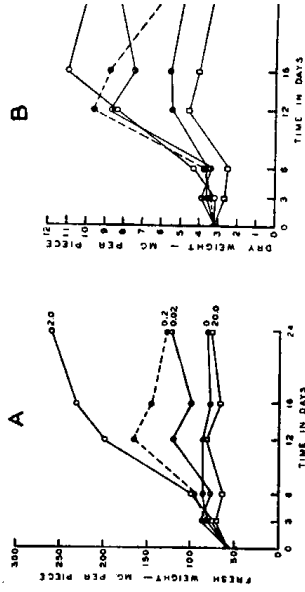


Figure 2. Effect of IAA on cell enlargement in pith sections with age of culti point is the average value from two experiments with 6–17 segments used for determination.

Concentrations of IAA 0, 0.02, 0.2, 2.0, and 20.0 mg./l. of nutrient medium. weight, (B) dry weight.

separate giant cells. Many cells undergo about a tenfold increase in but the optimal average increase for all cells is ca. 6 times.

It may be seen from comparison of Figures 2 a and b, that there is a three to fourfold increase in dry weight, the increase in fresh (water uptake) is proportionally still greater. These general feature IAA effect have been reproduced consistently in numerous experiments a two year period.

A complete mineral medium was used above. However, experim Newcomb (unpublished) have shown that the rapid cell enlargement place also on media with all nutrients removed except the sugar a



Figure 3. Photomicrographs of pith fragments showing stages in IAA induced cell enlargement (A) after 6 days; (B) after 16 days on a culture medium with 2.0 mg./l. IAA

Table 1. Effect of IAA (2.0 mg./l.) on dry weight, total sugar and reducing sugar content of tobacco pith sections. Expt. started Oct. 7, 1950 and harvested Oct. 30, 1950.¹

Treatment and age of culture	Average dry wt., mg./per section	% dry weight	Total sugars		Reducing sugars	
			% of fresh wt.	% of dry wt.	% of fresh wt.	% of dry wt.
Control.....	2	5.71	4.05	69.9	1.55	26.7
(0 days).....	2	5.82				
Control.....	2	5.87	3.93	67.8	1.39	24.0
(23 days).....	5.6	5.64	2.58	47.6	1.78	32.9
IAA.....	4.8	5.20	2.67	49.3	1.72	31.7
(23 days).....	15.7	4.25	2.36	54.5	1.49	31.4
	17.4	4.40	2.72	62.3	1.43	35.0

¹ Data by Robinson (1950).

² Values obtained from a composite sample of pith sections.

In fact, although the sugar is often required, occasionally only the IAA is essential. The expansion process, therefore cannot be osmotically controlled through salt uptake. Nor is the effect of sugar primarily osmotic, for analyses carried out by Robinson (7) shows that in spite of increases in total dry weight and sugar content, there is no increase in percentage sugar content during the early stages but a gradual drop from an initial 69 to 48 per cent of the dry weight in the controls and to ca. 59 per cent in the IAA treated sections at the end of the growth period (Table 1). Possibly the water is taken up by an active non-osmotic mechanism, as has been proposed for potato tuber tissue (Hackett and Thimann, 4; Hackett, 3), but as increases in cell wall materials, particularly pectic substances occur prior to and concomitant with cell expansion of the pith tissue (Wilson and Skoog, 9) and pending conclusive evidence to the contrary, the water uptake may be a secondary phenomenon of growth accompanying the increase in cell surface and regulated by existing osmotic properties of the tissue.

Extensive anatomical examination of sectioned material has revealed no evidence of cell division in control or rapidly enlarging, IAA treated pith sections. However, Sterling and Mallott (unpublished) found marked enlargement of the nuclei in expanding cells treated with IAA. A detailed cytological study of the pith sections with and without IAA treatments for optimal cell enlargement is reported by Naylor et al. (6).

Proliferation and Organ Differentiation

In attempts to obtain cell division in pith sections free from vascular tissues, pure substances which are known to stimulate plant growth, such as adenine,

CELL ENLARGEMENT AND CELL DIVISION

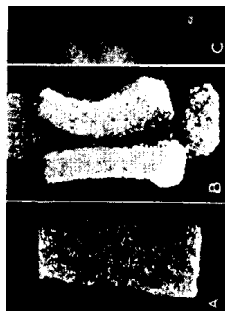


Figure 4. Callus formation at basipetal ends of pith sections placed in contact with vascular stem segments. (a) control pith section on medium; (b) pith section with 2 shorter vascular segments placed on top; (c) pith section placed on top of vascular stem segments. Age of cultures 2—3 weeks.

various vitamins and amino acids, were tested in several concentrated negative results. Similarly traumatic acid,¹ which promotes cell division in bean pod parenchyma (English et al., 2) was inactive when applied to tobacco pith sections in concentrations of 1, 10, and 100 µg./l.

Next attempts were made to obtain cell division in pure pith sections by placing them in physical contact with vascular tissue of stem segments in various ways. Replacing the cut pith sections in contact with their corresponding vascular tissue failed to induce cell division in the pith, because the vascular tissue tended to curve and thus to lose contact with the pith. However, the pith tissues were planted on the agar surface and shorter vascular segments were placed on their upper surfaces, cell division was obtained at the basipetal ends of a few pith sections in each experiment (Fig. 4) and with the inverse arrangement, i.e., with the pith sections on top and the water and nutrient flow to the pith was passing through the vascular segments, induction of cell division was obtained in 95—100 per cent of the pith sections in all experiments (4 b), whereas control sections placed on the medium failed to divide (4 a).

When pith sections were planted in the center of the agar surface and were surrounded by, but not in contact with, a large number of vascular segments, cell divisions were also obtained at the basipetal ends of pith sections. The minimum effective ratio of stem segments to pith sections was ca. 8:1 in these experiments. These results demonstrate that the material from the vascular tissue is diffusible through agar, and show that the induction of cell division in pith sections on top of vascular tissues was not due merely to the arrangement whereby the ends of pith sections were projecting, free from contact with the medium or other vascular tissue. This fact was also demonstrated by placing pith sections one on

¹ The traumatic acid was kindly furnished by Prof. Haagen-Smit, California Institute of Technology, Pasadena, California through Donal D. W. Skoog.

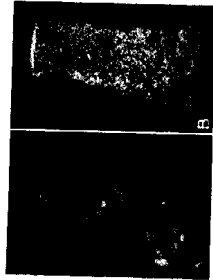


Figure 5. Effects of (a) coconut meat extract (equivalent to 150 g./l. coconut meat) and (b) malt extract (10 %) included in the medium on growth and differentiation of tobacco pith sections. Age of cultures (a) 30 days (b) 9 days.

another or by mounting them on porous gypsum blocks which were placed on the agar surface. In the latter case, cell division did not occur. In the former case, occasionally one of the two sections, generally the one on top, exhibited some cell division at the basipetal surface, but invariably much less so than pith sections mounted on vascular tissue in the same experiment. As might be expected, the pith sections themselves seem to contain small amounts of the active material but generally less than required under the conditions of these experiments to produce visible callus growth.

As a preliminary step toward isolating the active material, Seitzfiltered aqueous extracts were prepared from stem internodes, with epidermis, cortex, and most of the pith removed. About 0.1 ml. of extract was applied to each pith section by puncturing its surface with a hypodermic syringe. After a nine day growth period, callus formation was observed at the basipetal ends of the pith sections so treated. However, this effect of tissue extracts was not obtained consistently in subsequent experiments.

Turning to other sources, it was found that when a coconut-meat concentrate (Mauney et al., 5) corresponding to 15 per cent by weight coconut-meat was included in the culture medium, very active cell division occurred in the pith sections particularly at their basipetal ends but also over their entire upper surfaces (Figure 5 a). In fact, on this medium the tissue can be maintained indefinitely in subcultures, and is capable of differentiation to produce vascular strands and organs such as buds. A malt extract (10 per cent malt per 100 ml. medium (Figure 5 b)) could be substituted for the coconut extract (cf. Blakeslee and Satina, 1).

Discussion

The above results show that tobacco pith tissue may be cultured under conditions which permit either enormous enlargement of the cells in response to IAA without the occurrence of cell division or rapid proliferation

of the cells in response to an additional growth factor. However, in nuclear material are also associated with cell enlargement.

The nature of the material which induces division of the pith cells known. Presumably it is not identical with a coconut factor which stimulates the growth of carrot tissues, because more highly purified fractions of factor, which are active on carrot tissue, were inactive on tobacco pith. Nor is it traumatic acid, the so-called wound hormone, which stimulates cell division in bean pod parenchyma. It should be noted that the substance evidently exerts its effect primarily at the point of application whereas the active material for pith cell division is transported through tissue and exerts its effect first at the basipetal end of the sections. In fact, however, need not necessarily indicate that the substance is transported, because the distribution of growth may be determined by distribution of auxin. Low concentrations of IAA enhance the effect of active material and were included in the medium in most experiments. Results of attempts to characterize the active material by solvent fractionation and chromatographic techniques are as yet inconclusive.

The marked and differential growth responses of the pith tissue culture as described above make it an excellent material for studies of changes in respiration, enzyme activities, and tissue composition associated specifically with enlargement and/or proliferation of plant cells. The results of studies are reported elsewhere.

Summary

Tobacco pith tissue (*Nicotiana tabacum*, Variety Wisconsin no. 38) to grow on a modified White's nutrient agar medium, but undergoes marked enlargement resulting in a ca. tenfold increase in size of cells when optimal concentrations (ca. 2–3 mg./l.) of indoleacetic acid (IAA) are added to the medium. Higher and lower concentrations elicit less response, and 20 mg./l. IAA are often completely inhibitory.

No cell divisions have been found in pure pith tissue either in the absence of added IAA or in connection with the cell enlargement in response to optimal concentrations of IAA even though there is an increase in nuclear material in the expanding cells. Cell divisions do occur, however, in tissues with attached vascular strands, or in severed pith tissue placed in contact with vascular tissues.

The material active in inducing cell division is obtained in water extract from vascular tissue and various plant products such as coconut malt. It is diffusible through agar, but is not replaceable by traumatic

In its presence callus formation and organ differentiation as well as continued *in vitro* growth of the pith tissue are obtained.

The marked differential growth responses of pith tissue to IAA and various plant extracts make it an excellent material for studies of metabolic and compositional changes associated specifically with the process of enlargement or proliferation of plant cells.

References

1. Blakeslee, A. F. & Satina, S.: New hybrids from incompatible crosses in *Datura* through culture of excised embryos on malt media. — *Science* 99: 331. 1944.
2. English, J., Jr., Bonner, J. & Haagen-Smit, A. J.: The wound hormones of plants. IV. Structure and synthesis of traumatin. — *Jour. Amer. Chem. Soc.* 61: 3434. 1939.
3. Hackett, D. P.: The osmotic change during auxin-induced water uptake by potato tissue. — *Plant Physiol.* 27: 279. 1952.
4. — & Thimann, K. V.: The action of inhibitors on water uptake by potato tissue. — *Plant Physiol.* 25: 648. 1950.
5. Mauney, J. R., Hillman, W. S., Miller, C. O., Skoog, F., Clayton, R. A., & Strong, F. M. Bioassay, purification and properties of a growth factor from coconut. — *Physiol. Plant.* 5: 485. 1952.
6. Naylor, J., G. Sander & F. Skoog.: Mitosis and cell enlargement without cell division in excised tobacco pith tissue. — *Physiol. Plant.* 7: 25. 1954.
7. Robinson, B. J.: A relationship between indoleacetic acid effects upon growth and carbohydrate concentrations in tobacco stem tissues cultured *in vitro*. — M. S. Thesis, Univ. of Wis. 1951.
8. Skoog, F. & Tsui, C.: Chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro*. — *Amer. J. Bot.* 35: 782. 1948.
9. Wilson, C. M. & F. Skoog.: Indoleacetic acid induced changes in uronide fractions and growth of excised tobacco pith tissue. — *Physiol. Plant.* 7. 1954 (in press).