Plant Physiol. (1969) 44, 871-880

Hormonal Control of Cell Proliferation and Xylem Differentiation in Cultured Tissues of Glycine max var. Biloxi[1]

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Received January 28, 1969.

Abstract. **The relationship between tracheary element differentiation, cell proliferation and growth hormones was examined in agar-grown soybean callus. The time course of cell division and tracheary element formation in tissues grown on a medium containing 5 $\times$ 10^{-7} M kinetin and 10^{-5} M NAA was determined by means of a maceration technique. After a slight lag period, a logarithmic increase in cell number was observed through the twelfth day of the culture period. Cell numbers increased at a considerably slower rate after the twelfth day. The rate of tracheary element formation varied with the rate of cell proliferation. Tracheary elements increased logarithmically during the log phase of growth. As the rate of cell division decreased after the twelfth day of culture, the rate of tracheary element formation also decreased. In the presence of 10^{-5} M NAA, cell number increased as the kinetin concentration was increased between 10^{-9} and 10^{-6} M. However, tracheary element formation was not initiated unless the kinetin concentration was 5 $\times$ 10^{-8} M or above. When the Biloxi callus was subcultured repeatedly on media containing 10^{-8} M kinetin, a tracheary element-free population of cells was obtained. This undifferentiated tissue produced tracheary elements upon transfer to a medium containing 5 $\times$ 10^{-7} M kinetin. In the presence of 5 $\times$ 10^{-7} M kinetin, NAA stimulated cell proliferation between 10^{-7} and 10^{-5} M, but no tracheary elements were formed without auxin, or with 10^{-7} M NAA. Neither NAA nor kinetin at any concentration tested stimulated tracheary element formation in the absence of an effective level of the other hormone. However, 2,4-D at 10^{-7} or 10^{-6} M promoted both cell proliferation and tracheary element differentiation in the absence of an exogenous cytokinin.**

Auxin and sucrose are of considerable importance in the initiation of xylem differentiation. Auxin has been shown to be a limiting factor in wound xylem differentiation (10, 11), primary vascular differentiation (12, 28), secondary xylem differentiation (4, 29), and in tracheary element formation in cultured callus tissues (13, 30, 31). Studies in which sucrose has been shown to be necessary for xylem differentiation have been confined, for the most part, to callus tissues cultured *in vitro* (14, 19, 30, 31). However, Fosket and Roberts (6) found sucrose to be required for wound xylem formation and the work of Solberg and Higinbotham (21) suggests that sucrose may be a limiting factor for primary xylem differentiation in etiolated plants. Jeffs and Northcote (14) showed that sucrose does not simply provide an energy source for the differentiating cells, but that it has a role in the actual initiation of differentiation.

A number of studies have attempted to elucidate the relationship between cytokinins and xylem differentiation. Some investigators have reported the stimulation of xylem differentiation by cytokinins (1, 16, 22). Gautheret (8) found kinetin ineffective in promoting xylem element formation in Jerusalem artichoke tissue and Fosket and Roberts (6) found that kinetin inhibited wound xylem differentiation in cultured *Coleus* stem segments. These apparent conflicts may simply result from differences in the capacity of these tissues to produce their own cytokinins. Nevertheless, none of the studies cited above clearly demonstrates that a cytokinin was directly limiting xylem differentiation.

One of the principal effects of cytokinins is the stimulation of cell division in concert with auxin (3, 18). Recently Fosket (5) reported that mitotic activity was necessary for wound xylem differentiation in *Coleus*. If cell division should prove to be a general prerequisite for the initiation of xylem differentiation, then cytokinins could limit xylogenesis indirectly through their role in the regulation of mitosis. In order to demonstrate a direct effect of a cytokinin on xylem differentiation, it would appear to be necessary to show that the hormone does not simply stimulate cell division and thus increase the number of cells which may initiate differentiation. Since Torrey (26) reported that kinetin promoted tracheary element formation in suspension cultures of soybean, this tissue should be a favorable material for an investigation of the relationship between cytokinin, cell division, and xylem differentiation. This is particularly true since

[1] This research was supported by an NSF post-doctoral fellowship to D. E. Fosket and by research grant RG-08145 from the National Institutes of Health, United States Public Health Service, to J. G. Torrey.

Miller (17) showed that soybean callus has an absolute requirement for exogenous cytokinin for growth.

Materials and Methods

Seeds of *Glycine max* var. Biloxi were germinated in vermiculite under a 16-hr photoperiod. Five days after planting, the cotyledons were excised, surface sterilized with 5 % sodium hypochlorite, and cut into segments of various sizes. The cotyledonary segments were placed in 125 ml Erlenmeyer flasks containing 50 ml of a modification of Miller's soybean callus medium (17) with 100 mg/l *myo*-inositol added. Also, 10^{-6} M 2,4-D was substituted for IAA. The explants were cultured at 24° and received 16 hr warm-white fluorescent light per day. Within 3 weeks, the explants produced a leathery, convoluted, sheet-like callus which contained green patches. When the callus tissue was well established, it was subcultured to a second modification of Miller's medium containing 10^{-5} M α-naphthaleneacetic acid as the sole auxin, 5×10^{-7} M kinetin, and 100 mg/l *myo*-inositol. This medium was designated SCF. After the third subculture on SCF medium, the light-grown tissue began to brown severely. A reduced growth rate was associated with the browning. However, the callus gave satisfactory growth on the same medium, without browning, if cultured in darkness. The experiments described in this paper were conducted entirely with dark-grown Biloxi callus tissue. Eventually a strain of the callus was isolated which grew well on SCF medium in the light, but this tissue lacked tracheids and was not used for the present study.

In the experiments described below, small, uniform blocks of tissue, averaging 3 to 7 mg fresh weight, were cut from 21-day-old dark-grown callus. Four blocks of tissue were placed in each 125 ml Erlenmeyer flask, each containing 50 ml of agar medium. The tissue pieces were harvested at regular intervals during culture or at the end of the 21-day culture period.

Cell and tracheid determinations were made by a modification of the Brown and Rickless tissue maceration technique (2). Each callus piece was placed in a 4-dram vial containing 1 ml of a 5 % HCl—5 % chromic acid macerating fluid. After 2 days at room temperature, the fluid and cells were drawn repeatedly into a 5 ml hypodermic syringe equipped with a No. 19 needle. The total number of drops of fluid per vial was determined. Four drops were placed individually on glass slides and covered with a 22-mm square cover slip. The number of cells and tracheids in each drop were counted under the microscope and the cell and tracheid numbers per callus piece calculated. With large callus pieces, the volume of macerating fluid was increased.

Histological sections of Biloxi callus grown on SCF medium were prepared. The tissue was fixed in FAA, dehydrated by passage through an ethanol-tert-butyl alcohol series and embedded in paraffin, as outlined by Johansen (15). The paraffin-embedded material was cut at 10 μ with a rotary microtome, the sections mounted on glass slides, and the tissue was stained with safranin and fast green.

Results

The Biloxi callus grew in the dark as a hard, white, compact mass. No organ initiation was observed during any of the 3-week culture periods, or under any of the experimental conditions. An analysis of histological sections prepared from SCF-grown tissue suggested that centers of cell division activity appeared at random within the callus mass. As mitotic activity continued, the meristematic regions became organized into structures with a nest-like or strand-like appearance (Fig. 1). The majority of the tracheary elements were observed within the meristematic nests and strands, although some isolated tracheary elements, or small groups of tracheary elements, were also found. This type of callus organization is not unique, having been described repeatedly (*cf.* 7). Upon maceration, the majority of the tracheary elements were found to possess perforation plates (Fig. 2).

The growth kinetics of the agar-grown Biloxi callus were determined over a 21-day period. As is illustrated in Fig. 3, the growth of the Biloxi callus on SCF medium describes a typical sigmoid curve when the log of the cell number is plotted against time. After a slight lag period during the first 3 days, a logarithmic increase in cell number was observed over the subsequent 9 days, with a doubling time of approximately 60 hr. The growth rate was decidedly slower after the twelfth day of culture. The cell number increased by a factor of approximately 2 between the twelfth and the twenty-first day after inoculation.

Tracheary elements were differentiated in all phases of callus growth (Fig. 3). While the total cell population was increasing logarithmically, the number of tracheary elements increased correspondingly. As the growth rate declined after the twelfth day of culture, the rate of tracheary element increase also declined. Thus it appeared that tracheary element formation was directly correlated with cell formation.

The relationship between cytokinin concentration, cell division, and tracheary element differentiation was examined. The mean values for the cell and tracheid numbers observed with the different kinetin levels are given in table I.

The inocula used for this experiment consisted of tissue pieces containing approximately 200,000 cells, of which 16,000, or 8 %, were tracheary elements. After 21 days' growth in the presence of kinetin, cell numbers were found to increase in proportion to the amount of kinetin added to the

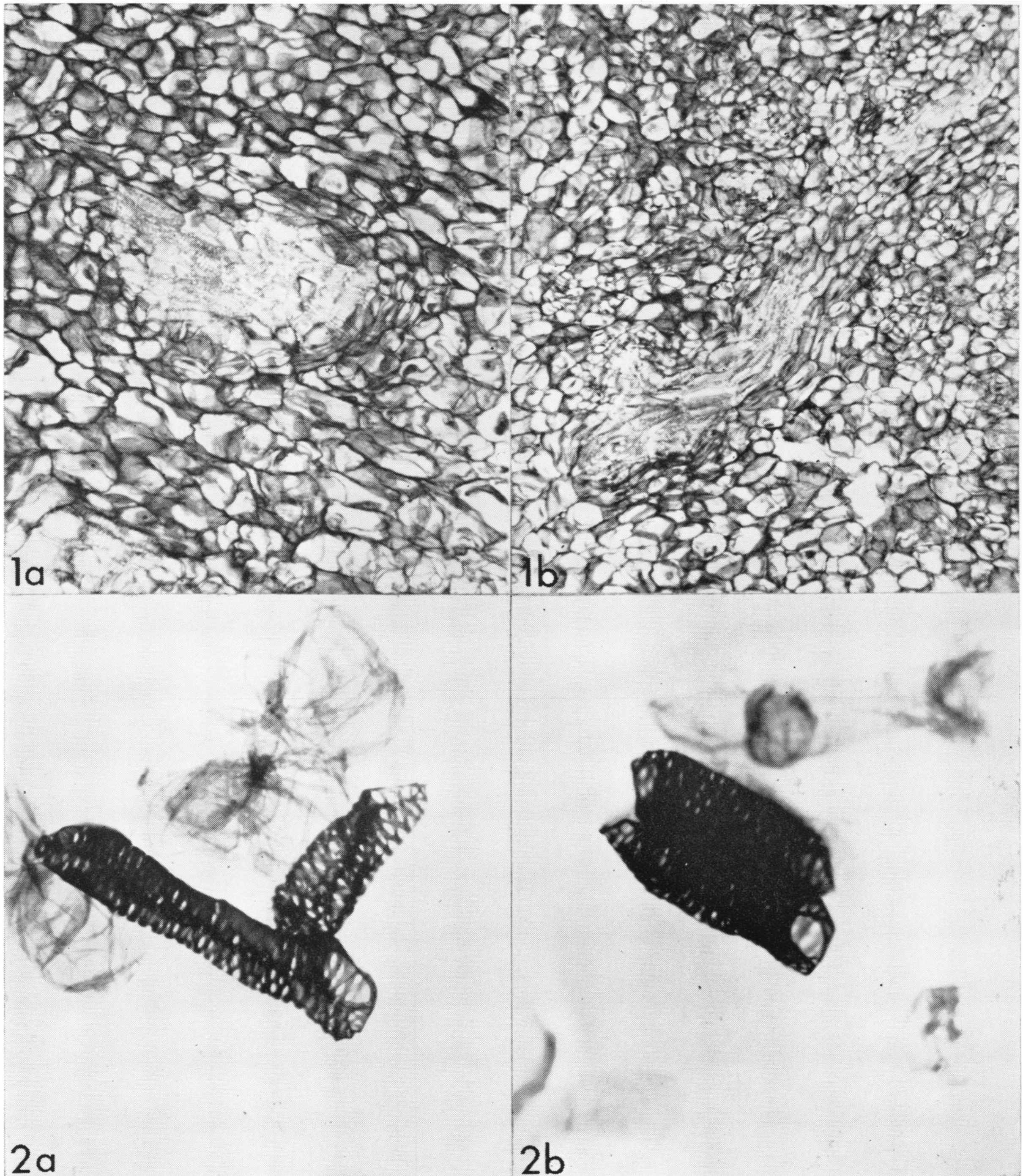

FIG. 1 a and b. Histological sections of Biloxi callus tissue. A nest of tracheary elements, partially surrounded by a cambial-like layer of dividing cells, is shown in 1a, while 1b illustrates a strand-like array of tracheary elements.

FIG. 2 a and b. Photomicrographs of tracheary elements obtained from macerated Biloxi callus tissue. The 2 elements shown in 2a have perforation plates near the ends of these cells toward the right. In 2b, perforation plates are present at both ends of the tracheary elements.

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Table I. *The Response of Biloxi Callus to Exogenous Kinetin in the Presence of Auxin*

Biloxi callus tissue was grown on SCF media containing 10^{-5} M NAA and no kinetin, or kinetin at various levels from 10^{-9} M to 10^{-6} M. The fresh weight, cell number and tracheary element number were determined for each callus mass after 3 weeks' growth. The means of these parameters are given, together with the standard errors.

	Inoculum	No kinetin	10^{-9} M kinetin	5×10^{-9} M kinetin
Fresh wt. (mg)	7.03 ± 0.45	31.63 ± 4.27	23.78 ± 1.56	29.18 ± 2.81
Cells × 10^5	2.05 ± 0.35	3.38 ± 0.43	4.37 ± 0.23	4.66 ± 1.00
Tracheids × 10^4	1.63 ± 0.67	7.70 ± 0.66	1.44 ± 0.25	1.78 ± 0.47
% tracheids	8.11 %	5.03 %	3.30 %	3.82 %
	10^{-8} M kinetin	5×10^{-8} M kinetin	10^{-7} M kinetin	10^{-6} M kinetin
Fresh wt. (mg)	38.14 ± 3.77	86.25 ± 8.01	136.68 ± 9.49	206.24 ± 16.38
Cells × 10^5	6.15 ± 0.42	13.98 ± 2.18	22.44 ± 1.99	43.98 ± 4.40
Tracheids × 10^4	1.94 ± 0.19	4.52 ± 0.81	13.19 ± 2.49	30.00 ± 2.60
% tracheids	3.15 %	3.23 %	5.88 %	6.82 %

Table II. *The Behavior of Biloxi Callus After Successive Subculture Periods on Media Containing Low Levels of Kinetin*

Biloxi callus was grown for 3 weeks on standard SCF medium with 5×10^{-7} M kinetin. The SCF-grown tissue was then subcultured to SCF with 10^{-8} M kinetin, grown for 8 weeks, and subcultured to freshly prepared SCF with 10^{-8} M kinetin for an additional 4 weeks.

	SCF + 5×10^{-7} M kinetin, 3 weeks' growth	SCF + 10^{-8} M kinetin, 1st subculture, 8 weeks' growth	SCF + 10^{-8} M kinetin 2nd subculture, 4 weeks' growth
Cells × 10^5	29.2	22.7	5.40
Tracheids × 10^3	251.8	17.5	0.01
% tracheids	8.6 %	0.7 %	0.02 %

Table III. *The Effect of Kinetin on the Proliferation and Differentiation of Biloxi Callus in the Absence of Exogenous Auxin*

Biloxi callus was grown for 21 days on SCF media lacking NAA, but containing the indicated amount of kinetin. The fresh weight, total cell number, and tracheary element number were determined for each callus piece. The means of these parameters are given together with the standard errors.

	Inoculum	No auxin No kinetin	10^{-7} M kinetin	10^{-6} M kinetin	10^{-5} M kinetin
Fresh wt. (mg)	2.85 ± 0.26	6.99 ± 0.72	20.69 ± 3.54	106.78 ± 4.96	86.55 ± 4.77
Cells × 10^5	1.78 ± 0.10	2.82 ± 0.39	4.12 ± 0.90	18.82 ± 1.52	13.9 ± 4.05
Tracheids × 10^4	1.67 ± 0.08	2.12 ± 0.22	2.42 ± 0.43	2.06 ± 0.38	1.85 ± 0.42
% tracheids	9.38 %	7.52 %	5.87 %	1.09 %	1.33 %

Table IV. *The Effect of NAA on Proliferation and Differentiation of Biloxi Callus in the Absence of Exogenous Kinetin*

Biloxi callus was grown for 21 days on SCF media lacking kinetin, but containing NAA as indicated below. The fresh weight, total cell number, and tracheid number were determined for each callus piece and their means are given together with the standard errors.

	No auxin No kinetin	10^{-8} M NAA	10^{-7} M NAA	10^{-6} M NAA	10^{-5} M NAA
Fresh wt. (mg)	7.82 ± 0.61	8.86 ± 1.02	7.19 ± 0.66	8.35 ± 1.43	19.99 ± 3.39
Cells × 10^5	1.88 ± 0.29	1.98 ± 0.49	1.52 ± 0.34	1.60 ± 0.30	3.54 ± 0.69
Tracheids × 10^4	1.32 ± 0.57	1.29 ± 0.10	0.94 ± 0.19	1.12 ± 0.48	1.12 ± 0.47
% tracheids	7.02 %	6.51 %	6.18 %	7.00 %	3.16 %

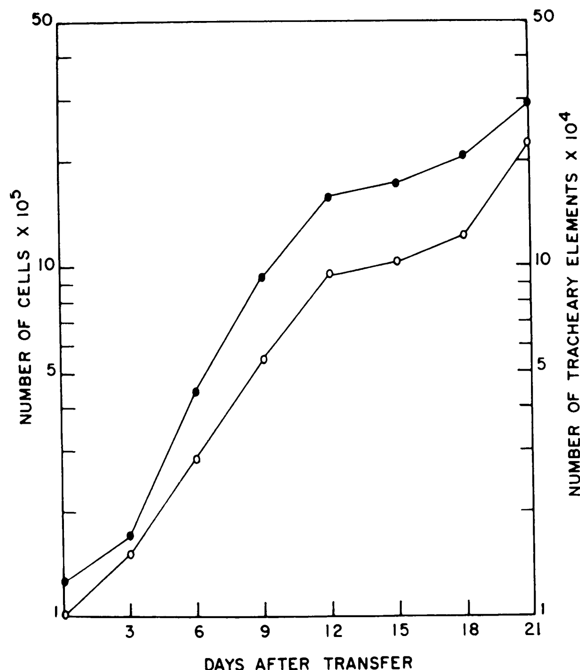

FIG. 3. The growth curve of agar-grown Biloxi callus tissue. Uniform blocks of Biloxi callus were used to inoculate flasks of standard SCF medium. Every third day, 8 such blocks were sacrificed and the cell and tracheid numbers were determined for each callus piece. The logarithm of the mean number of cells or tracheids is plotted against time. ● = cells; ○ = tracheids.

medium. When the inoculum cell number was subtracted from the final cell number, a logarithmic increase in cells was observed with increasing kinetin concentration between 10^{-9} and 10^{-6} M. Tracheary element differentiation did not parallel the cell division response to increasing kinetin concentration. Kinetin at 10^{-9}, 5×10^{-9}, or 10^{-8} M was ineffective in promoting tracheary element formation, although these kinetin concentrations led to increases in cell numbers. No statistically significant increase in tracheary elements was observed unless the kinetin concentration was raised to at least 5×10^{-8} M. Increasing the amount of kinetin above this level not only permitted tracheary element formation, but increased the percentage of tracheary elements as well. At 5×10^{-8} M kinetin, 3 % of the cells formed were tracheary elements, while almost 7 % of the cells differentiated as tracheary elements when the callus was grown on medium containing 10^{-6} M kinetin.

Since the data of table I show that the cytokinin concentration limits cell division and tracheary element differentiation independently, it should be possible to cultivate Biloxi callus on a medium containing a low level of kinetin and obtain a cell population essentially free of tracheary elements. This proved to be the case, as is shown by the data in table II. Biloxi callus was subcultured from standard SCF medium to a medium of the same composition, but containing 10^{-8} M kinetin. A substantial amount of growth occurred over an 8-week period, without a significant increase in the total number of tracheids. As a result, the percentage of tracheary elements was greatly reduced. After the second subculture on to medium containing 10^{-8} M kinetin, the callus tissue was essentially undifferentiated.

To determine if the undifferentiated callus retained the potential for tracheary element formation, small pieces of the tissue were subcultured from the medium containing 10^{-8} M kinetin to the standard SCF medium (0.1 mg/l, or approximately 5×10^{-7} M kinetin). As is shown in Fig. 4, a logarithmic increase in cell number occurred after a pronounced lag period. No tracheary elements were observed until after the sixth day of culture. Tracheary element numbers continued to increase between the ninth and twelfth day of culture. It should be emphasized that, although 5×10^{-7} M kinetin permitted xylem differentiation in the previously undifferentiated tissue, no tracheary elements were formed until after cell division had been initiated. Judging from their size and shape, the tracheary elements were formed from the recently divided cells.

While kinetin was necessary for xylem differentiation, it did not promote xylogenesis in the absence

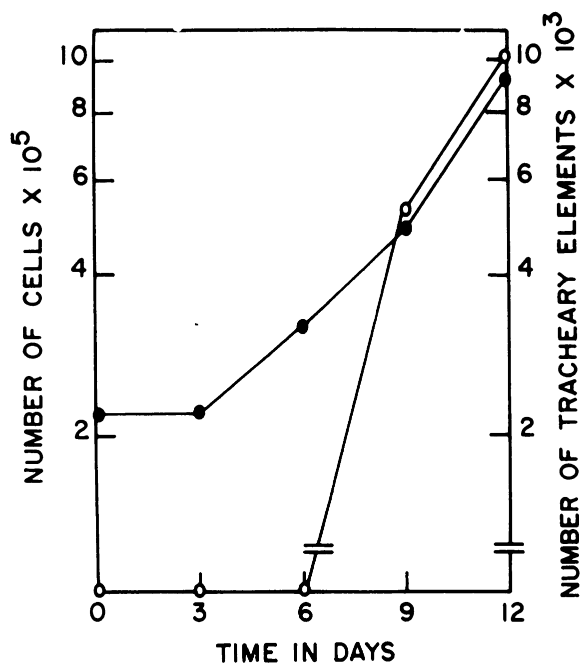

FIG. 4. The response of a tracheary element-free population of Biloxi cells to levels of kinetin supporting differentiation. Biloxi callus tissue lacking tracheary elements after 2 passages on SCF + 10^{-8} M kinetin medium was subcultured to standard SCF medium (5×10^{-7} M kinetin). Tissues were harvested every 3rd day to determine cell and tracheid numbers. The log of the mean number of cells or tracheids was plotted against time. ● = cells; ○ = tracheids.

of an effective level of auxin. It is apparent from table III that all levels of kinetin tested in the absence of exogenous auxin supported a significant amount of growth, whether measured as an increase in fresh weight or cell number. Maximum growth was obtained with 10^{-6} M kinetin, while higher levels of kinetin (10^{-5} M) were supra-optimal. Although kinetin definitely supported growth in the absence of exogenous auxin, it did not effect xylem differentiation. None of the values obtained for tracheary element numbers was significantly different from the initial value. Note that the values for percent tracheary elements drop in proportion to the increase in cell number.

The response of the Biloxi callus to different levels of auxin was also determined. The growth response of the tissue to increasing levels of NAA, shown in Fig. 5, was not as striking as that observed with increasing amounts of kinetin. Maximum total cell numbers were obtained with 10^{-5} M NAA. This represented a 3.5 fold increase in cell number as compared to the lowest level of NAA tested. By contrast, the optimum kinetin concentration (10^{-6} M) led to the formation of approximately 20 times as many cells as the lowest kinetin concentration tested (initial cell numbers subtracted from the final cell numbers). Substantial growth was observed with no added auxin, amounting to approximately a 7-fold increase in cell number, as compared to the cell number observed in the inoculum. While this might suggest that the tissue was not dependent upon exogenous auxin for growth, it was subsequently found that the growth observed in the absence of added auxin was most probably due to the utilization of auxin taken up by the tissue during the previous culture period on auxin-containing medium. No growth occurred when the tissue produced on medium lacking auxin was subcultured to freshly prepared medium containing no auxin.

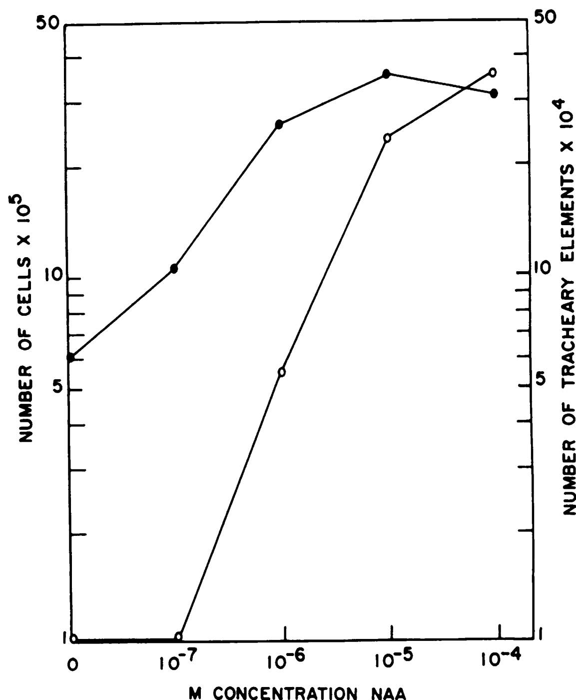

FIG. 5. The response of Biloxi callus tissue to various levels of NAA. Uniform segments of SCF-grown Biloxi callus were subcultured to media containing NAA at various concentrations. After 3 weeks' growth, cell and tracheid numbers were determined for each callus piece. The mean number of cells or tracheids in the inoculum was subtracted from the final number of cells or tracheids and the log of this value was plotted against the concentration of NAA. ● = cells; ○ = tracheids.

Despite the comparative lack of sensitivity of the Biloxi callus growth to exogenous auxin, tracheary element differentiation was greatly influenced by the amount of auxin provided to the tissues (Fig. 5). No tracheary elements were formed in the absence of NAA, or with 10^{-7} M NAA. Between 10^{-7} and 10^{-5} M, NAA promoted tracheary element formation more strongly than growth. At 10^{-6} M NAA, only 3.4 % of the cells were tracheids, whereas 6.5 % of the cells differentiated as tracheids with 10^{-5} M NAA. While 10^{-4} M NAA inhibited both growth and differentiation, growth was affected more strongly and 11 % of the cells differentiated as tracheary elements.

NAA did not support tracheary element differentiation in the absence of exogenous kinetin. At 10^{-6} M or less NAA had no significant effect on growth as measured by either fresh weight or cell numbers (table IV). However, 10^{-5} M NAA was found to support a significant increase in fresh weight, as compared to tissue cultured on a medium lacking both auxin and cytokinin. An increase in cell number was observed with 10^{-5} M NAA, but this increase was not statistically significant. In none of the treatments was there any significant change in the tracheid number, as compared to the no auxin—no kinetin controls.

When 2,4-D was substituted for NAA, somewhat different results were observed. In the presence of 0.1 mg/l kinetin, the response of the Biloxi callus to 2,4-D was similar to that produced by NAA (compare Fig. 6 with Fig. 5). At 10^{-5} M, however, 2,4-D was decidedly inhibitory to both growth and differentiation, whereas 10^{-4} M NAA was only slightly inhibitory. As with NAA, increasing levels of 2,4-D affected growth and xylogenesis differently. With 10^{-7} M 2,4-D, only 3 % of the cells differentiated as tracheary elements, but with 10^{-6} M 2,4-D, 12 % of the total cells were tracheary elements.

In the absence of exogenous cytokinin, 2,4-D was found to promote both cell multiplication and differentiation. This effect was particularly striking at a concentration of 10^{-6} M, where 2,4-D supported an almost 10-fold increase in cell number. A substantial amount of xylem differentiation was also observed with 10^{-6} M 2,4-D: 7.3 % of the cells differentiated as tracheary elements. At a concentration of 10^{-5} M, 2,4-D alone was decidedly inhib-

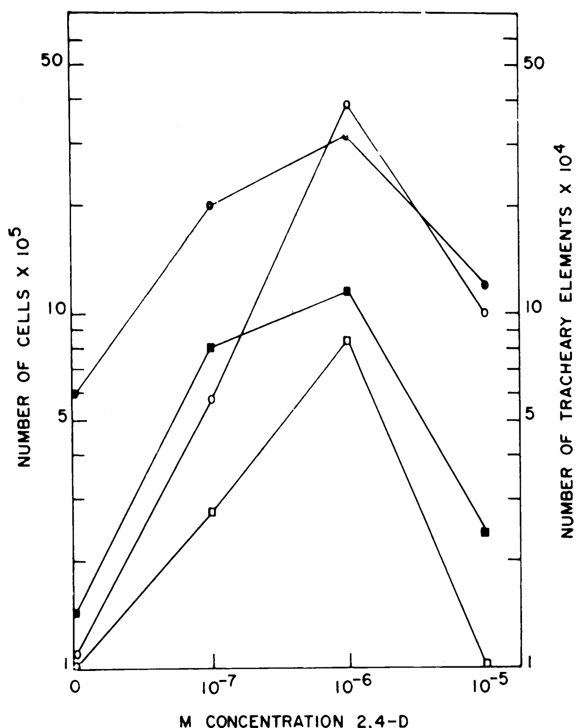

FIG. 6. The effect of 2,4-D in the presence or absence of kinetin upon cell proliferation and differentiation in Biloxi callus tissue. Uniform segments of SCF-grown Biloxi callus were subcultured to media with or without kinetin (0.1 mg/l or approximately 5×10^{-7} M), and containing the indicated amount of 2,4-D. After 21 days on the experimental media, cell and tracheid numbers were determined. The log of the mean number of cells or tracheids was plotted against the 2,4-D concentration. ● = cells, + kinetin; ○ = tracheids, + kinetin; ■ = cells, — kinetin; □ = tracheids, — kinetin.

tory to both cell proliferation and differentiation. In fact, in the absence of kinetin, 10^{-5} M 2,4-D completely blocked differentiation.

The appearance of the tissue produced on media containing 2,4-D but lacking cytokinin was not basically different from the tissue grown on the standard SCF medium. However, it tended to be more friable. This was particularly true of the tissue produced in the presence of 10^{-5} M 2,4-D. Cytokinin appeared to antagonize the effect of 2,4-D on tissue friability. With 10^{-5} M 2,4-D alone, the tissue was very watery and translucent, but with the same concentration of 2,4-D in the presence of 5×10^{-7} M kinetin, the tissue was still moderately firm and an opaque white in color.

Discussion

The relationship between cell division and xylem differentiation is complex. Under *in vitro* conditions, continued cell division may lead to a loss of tracheary elements from a tissue that actively formed these cells in the intact plant. For example, Geissbuhler and Skoog (9) reported that a callus tissue derived from the cambium of jack pine exhibited fewer tracheary elements with each subculture. The tissue lacked tracheary elements after the eighth passage. Under other conditions, cultured tissues retain the ability to form tracheary elements, even though they may not exhibit other types of differentiation. Torrey (25) reported that the prolonged culture of pea callus did not alter the ability of this tissue to form tracheary elements, although it gradually lost the potential for organ initiation.

Cell division is not necessarily antagonistic to xylem differentiation in the intact plant. During secondary xylem formation, active cell division by the cambium is directly involved in the increase of the xylem element population. Torrey (24) proposed that xylem differentiation usually follows a recent cell division. Fosket (5) has shown that cell division is necessary for wound xylem differentiation in cultured *Coleus* stem segments. He suggested that xylogenesis is initiated during some phase of the mitotic cycle. This is analogous to certain examples of cytodifferentiation in animal tissues where cell division has been shown to precede and to be required for the differentiative event (23, 27). Stockdale and Topper (23) proposed that the hormonal milieu in which cell division occurs is the critical factor in the formation of a specific type of differentiated cell. The findings of the present study would support the hypothesis that cell division is necessary for the initiation of xylogenesis and further that mitosis must occur in a particular hormonal environment for the derivative cells to appear as tracheary elements.

Interactions between growth hormones have been shown to be highly significant factors in the regulation of plant development. This fact is best illustrated by the work of Skoog and Miller (20) where it was demonstrated that organogenesis in cultured tobacco tissues was controlled by the relative levels of auxin and cytokinin. It has been suggested (26) that similar hormonal interactions are important in the initiation of specific types of cellular differentiation. The results of the present study substantiate this idea with respect to xylem differentiation. Neither NAA alone nor kinetin alone was effective in initiating tracheary element differentiation in Biloxi callus tissue. An effective level of both substances was required for the formation of tracheary elements.

In addition to the control of organogenesis by the interaction of auxin and cytokinin (20), Das *et al.* (3) have shown that a similar interaction regulates cell division in the same tissue. The relationship between the initiation of a specific type of organ formation and cell division is not clear. The results of the present study not only suggest that tracheary element differentiation is initiated during cell division, but that the balance between auxin and cyto-

kinin controls cell proliferation as well as tracheary element formation in Biloxi callus tissue. Because of the problem of auxin carry-over, we did not determine the actual limiting auxin concentration for cell division. However, since cell proliferation could not be maintained in the absence of exogenous auxin, the Biloxi callus appeared to require a certain limiting amount of auxin for cell division. The present study clearly showed that kinetin could limit cell division in this tissue. In either case the amount of NAA or kinetin necessary to initiate cell division, in the presence of adequate levels of the other hormone, was considerably below the level required for the initiation of tracheary element formation.

Although 2,4-D was found to stimulate tracheary element formation in the absence of exogenous cytokinin, this observation may actually support the hypothesis that an auxin and a cytokinin are both necessary for cytodifferentiation. From the data presented in Fig. 6, it can be seen that 2,4-D alone can support both cell division and xylogenesis in the Biloxi callus. Witham (32) observed a similar phenomenon in callus tissue of *Glycine max* var. Acme. Both the fresh weight and cell numbers of this tissue were found to increase in response to high levels of 2,4-D in the absence of a cytokinin. Witham suggested that 2,4-D may be a weak cytokinin as well as an auxin, or that 2,4-D may cause the tissue to produce its own cytokinin. Whatever the explanation, the fact remains that 2,4-D was shown to stimulate cell division in a cytokinin-requiring tissue. Thus, the present findings cannot be considered to argue against the idea that the initiation of xylogenesis requires an interaction between auxin and cytokinin. It would appear that tracheary element formation occurs after a cell has undergone division in an environment which contains an effective level of an auxin and a cytokinin.

Acknowledgments

The authors thank Lydia Goetze for her valuable technical assistance and Gustav Strain for the original isolation of the Biloxi callus tissue.

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