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4. This work was supported in part by research grant C-1786(c) from the National Institutes of Health, U.S. Public Health Service.

28 November 1956

Effects of Zinc and Amino Acid on Cell Division in Ustilago

Grimm and Allen (*1*), studying cytochrome synthesis in *Ustilago sphaerogena,* reported that when zinc was added to the liquid growth medium used for this organism, a culture of single ovid cells was produced and cytochrome formation was promoted. Without zinc, the cells were filamentous in form. Apparently zinc was involved somehow in cellular processes related to cell division.

In studies in our laboratory, an opposite effect of zinc was observed. The strain of *Ustilago sphaerogena* (*2*) used in this work produced long clumped cells when zinc was added to the basic medium. Thus, zinc, whether it promotes or hinders division, appears to be involved in reactions intimately related to the processes by which, as a culture of *Ustilago* grows, cells divide to form additional, small, uniform cells.

It was also observed in our studies that differences in cell form occurred in cultures that were grown on different amino acids or ribonucleic acid as nitrogen sources. Apparently these acids influence other cellular reactions involved in cell-division processes, and cell form may vary from short, rod-shaped cells to long, tangled, mycelium-like cells.

In our experiments, cultures were grown at 23° ± 1°C in 125- or 250-ml erlenmeyer flasks on a reciprocal shaker. Medium A (*1*), without zinc or thiamine, was used as the basic medium. Concentrations of hydrogen ion in the several media were similar and remained fairly constant (see also *3*). Cells were examined microscopically, and, in lieu of a completely quantitative method, were assigned percentage-wise to the following classes. Class 1: long, clumped, mycelial-type cells. Class 3: long, single cells (over 70 μ). Class 5: intermediate-sized, single cells (approx 35 to 70 μ). Class 7: short, single, rod-shaped cells approximately 20 μ in length. The sum of the products of class numbers multiplied by the percentage of each class in a culture provided a form index. An index of 250 or less describes a culture consisting mostly of long cells; an index of 550 or more a culture consisting primarily of short cells. Cultures of short cells or long, clumped cells were easy to rate; cultures of a mixed type, because of the estimated percentages, were not as accurately described. Cells of all classes except short rods occurred both in branched and in straight form.

Table 1 lists some of the form indices obtained. The zinc effect occurred when zinc was added to the basic medium or to media in which an amino acid substituted as a nitrogen source for the ammonium acetate of the basic medium. The effect occurred with addition of only a small amount of zinc, and it was similar following tenfold or still greater zinc additions. The long cells appeared in zinc-supplemented cultures only after more than 18 hours of growth (the log phase of growth was reached at 2 to 4 hours and ended at 36 to 40 hours in control cultures). Growth, in terms of dry weight, with added zinc or on an amino acid was quite similar to growth on the basic medium. The dry-weight figure shown for proline (Table 1) is low; in other experiments, in which a complete survey of amino acids was made (*3*), this acid produced a dry weight only slightly less than that produced by the basic medium.

Table 1. Cell form in cultures of *Ustilago sphaerogena* grown on various nitrogen sources and with or without added zinc. The starting cell concentration was 1.4×10^5 cells/ml. An index of 250 or under describes long cells; one of 550 or over describes short cells. The control was cultured in 0.3-percent ammonium acetate.

Nitrogen source	Zinc added (ppm)	Cell index		Dry wt. (percentage of control) at 46 hr
		18 hr	46 hr	
Ammonium acetate (0.3%)	0	680	700	100
Ammonium acetate (0.3%)	0.2	685	205	118
Ammonium acetate (0.3%)	1.0	690	260	99
Ammonium acetate (0.3%)	5.0	680	200	101
Glycine (0.3%)	0		210	93
Glutamic acid (0.3%)	0		420	124
Glutamic acid (0.3%)	1.0		180	105
Proline (0.3%)	0		700	71
Proline (0.3%)	1.0		120	100
Ribonucleic acid (0.3%)	0		130	89

Because growth was not markedly changed, it appears that the cellular reactions involved in these form changes are specifically related to processes of division. Although speculation concerning what reactions are affected does not seem appropriate at this time, these observations add to the list of agents which may be used as tools in studying cell-division processes. Such studies, in addition to their intrinsic value, have particularly pertinent application in the area of cellular injury by ionizing radiation (*4*).

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3 December 1956

Influence of Gibberellins on Stem Elongation and Flowering of Endive

The gibberellins, which are metabolites of the fungus *Gibberella fujikuroi* (Saw.) Wollenweber (*1*), produce rapid stem elongation in numerous plants even when they are applied in quantities as small as 5 μg per plant (*2*). Lang (*3*) induced flower formation in biennial *Hyoscyamus niger* L. by applying a total of 60 μg of gibberellin under conditions of warm temperature and short day. *Hyoscyamus* normally requires a cold period followed by long days for flower induction. Flowering in endive, *Cichorum endiva* L., is hastened by a period of growth under long days, in bright light, or in the cold either as seed vernalization or during early development (*4*).

This report describes the interaction of vernalization and gibberellin on growth and flowering in endive. The gibberellins used were a mixture of gibberellin A and gibberellic acid (*5*) hereinafter referred to as "gibberellins." Seed of variety Fullheart No. 5 from Nunhem, Haelen, Holland, was used. For the vernalized seed, sufficient water was added to raise the moisture content to 40 percent on a dry-weight basis. The

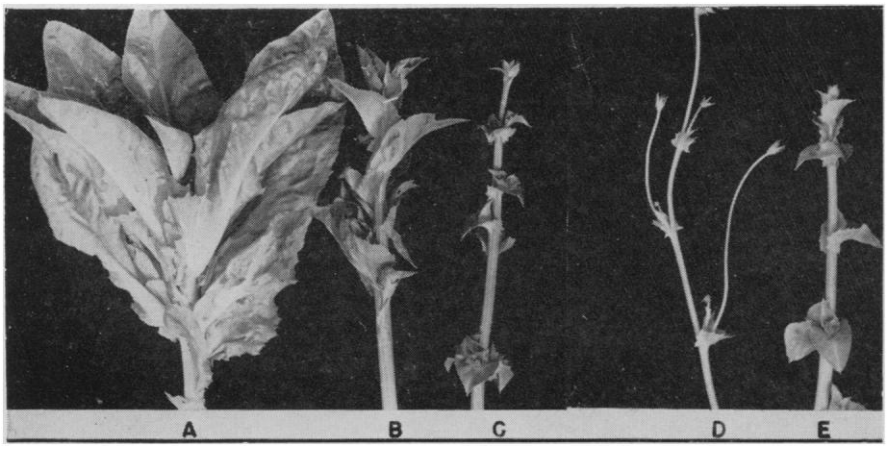

Fig. 1. Apical portions of vernalized and nonvernalized endive as affected by single and repeated applications of gibberellins. (*A*) Not vernalized, 50 μg; (*B*) not vernalized, 450 μg; (*C*) vernalized, no treatment; (*D*) vernalized, 450 μg; (*E*) vernalized, 50 μg. Photographed 27 Sept. 1956.

moist seed was held for 6 hours at 25°C and then placed in a saturated atmosphere at 2.5°C for 4 weeks beginning 29 June 1956. On 27 July, the vernalized and unvernalized seeds were planted in flats in a greenhouse that was kept at a night temperature of 18°C and a day temperature of 22°C or higher.

After 1 Oct., the plants received a 16-hour day, supplementary incandescent light being used to obtain it. Treatments with the mixture of gibberellin A and gibberellic acid were begun on 3 Aug. 1956, when the seedlings emerged. An application of 0.05 ml of an aqueous solution of the mixture of gibberellins (1000 μg/ml) was applied to the stem apex of each seedling. There were six treatments, as is shown in Table 1. The total amount of gibberellins applied to the plants that received only one application, on 3 Aug. was 50 μg; the plants that were treated weekly from 3 Aug. until first anthesis on 4 Oct. received a total of 450 μg.

Ten days after the first application of the gibberellins, stem elongation was visible on all treated plants, irrespective of vernalization. Thirty-one days after treatments began, stem elongation of untreated vernalized plants became evident. By comparison, untreated, unvernalized plants at this date continued to exhibit typical rosette growth with 20 to 30 leaves.

Table 1 shows the marked elongation that occurred in plants treated with gibberellins. Repeated applications resulted in the greatest stem elongation. Especially noteworthy is the increase of stem height exhibited by the unvernalized plants (167.5 cm), as compared with vernalized plants (135.2 cm) following repeated applications of the mixture. This is probably explained by earlier flower induction in the vernalized plants (Table 1).

By 9 Oct., repeated applications of gibberellins to the vernalized plants had induced a greater percentage of incipient inflorescences as well as earlier flowering than vernalization alone (treatments 4 and 6, Table 1). However, development through flowering of the treated plants was distinctly different from that of untreated, vernalized plants. In the plants that were not treated with gibberellins, flower primordia were observed under the binocular microscope almost as soon as elongation was visible. The flowers were sessile and normal in size, color, pollen content, and seed set. On the other hand, the vernalized plants that received repeated applications had stems 75 to 100 cm high before flower primordia could be observed under the binocular microscope. The first flower heads occurred on very long peduncles (Fig. 1); the flowers were small and pale blue and had brownish stamens with very little pollen. The pollen appeared to be viable, for it stained pink with acetocarmine, although no seed developed from them. Following cessation of treatment, blossoms were normal in all respects.

Flowering occurred on vernalized plants of all treatments, but, significantly, weekly applications of gibberellins also induced flowering in nonvernalized plants. In the latter plants, the first flowers produced were similar to the abnormal ones on vernalized plants that received repeated applications of gibberellins (treatment 6). When the mixture of gibberellins was applied once to vernalized plants, elongation occurred to about 30 cm, after which (9 Oct.) vegetative rosettes formed at the stem apices (Fig. 1). Eventually, 78 percent of these plants produced flower parts (Table 1).

The production of normal blossoms following termination of repeated treatments is an indication that gibberellins have limited residual properties. However, the early induction of flowering by a single application to the first leaves of endive (treatment 2) further suggests (*6*) a possible basic role for gibberellins in the flowering mechanism.

Table 1. Effects of a mixture of gibberellin A and gibberellic acid on stem height and flowering of endive.

Expt. No.	Gibberellin treatment	No. of plants	Avg. stem length (cm)	9 Oct. 1956		26 Oct. 1956		3 Dec. 1956	
				Incipient inflorescences (%)	Open flowers (%)	Incipient inflorescences (%)	Open flowers (%)	Incipient inflorescences (%)	Open flowers (%)
Unvernalized									
1	Untreated	39	< 1.0	0	0	0	0	22	0
2	50 μg once	36	31.7	0	0	28	0	78	65
3	50 μg/wk	10	167.5	30	0	100	30	100	100
Vernalized									
4	Untreated	27	14.6	59	0	85	7	100	51
5	50 μg once	29	80.2	90	0	90	41	94	89
6	50 μg/wk	34	135.2	100	21	100	86	100	100

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3 January 1957