

## References and Notes

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## 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 ovoid 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  $23^{\circ} \pm 1^{\circ}\text{C}$  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  $\mu$ ). Class 5: intermediate-sized, single cells (approx 35 to 70  $\mu$ ). Class 7: short, single, rod-shaped cells approximately 20  $\mu$  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.

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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## 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  $\mu\text{g}$  per plant (2). Lang (3) induced flower formation in biennial *Hyoscyamus niger* L. by applying a total of 60  $\mu\text{g}$  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, *Cichorium 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

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  $1.4 \times 10^6$  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