# EFFECTS OF GIBBERELLIN, KINETIN, THIOUREA, AND PHOTOMORPHOGENIC RADIATION ON MITOTIC ACTIVITY IN DORMANT LETTUCE SEED[1]

ALAN H. HABER AND HELEN J. LUIPPOLD

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY[2], OAK RIDGE, TENNESSEE

Lettuce seed germination can be stimulated, probably by distinct mechanisms, by any of the three widely studied morphogenic agents: gibberellin, kinetin, or red light (11). It has been shown that cellular expansion is both necessary and sufficient and that cell division is neither necessary nor sufficient for the initiation of lettuce seed germination (8). Thus it is now possible to study the effects of germination-stimulating agents on mitotic activity in embryos uncomplicated by germination and growth from expansion. In this paper, mitotic activity as it is affected by gibberellic acid (GA), kinetin, and light, as well as by thiourea, which also stimulates germination of lettuce seed (28), is studied.

## MATERIALS AND METHODS

Seeds (achenes) of lettuce (*Lactuca sativa* L., var. New York) were sown on filter papers moistened with appropriate solutions at pH 5.7 as previously described (8). Unless otherwise indicated, the Petri dishes were placed in closed copper sterilization cans that were submerged in a constant-temperature water bath. The seeds were thus in continuous darkness. Seeds given light treatments were similarly sown in dishes set upon a Cold-Cel truck plate with refrigeration coils through which water was circulated from a 50 gal, constant-temperature water bath. The temperature within the Petri dishes was thus maintained at approximately 31.5° C under the light filters hereafter described. The dishes were enclosed in wooden frames with windows consisting of light filters through which light shone from above. The light source was a multiple water-cooled refluxing lamp unit (33). Seven 150-watt incandescent reflector flood bulbs shone down through 6 cm of water. The red-light filter system was a Corning No. 2403 polished filter and a Plexiglas filter containing a 2 cm thick layer of $CuSO_4 \cdot 5H_2O$ (50 g per liter) plus 0.5 % $H_2SO_4$. The far-red filter system was a Corning No. 7–69 molded filter and a similar Plexiglas filter containing a layer of water 2 cm thick. The red filter system transmits light from 625 to 680 mμ, with a peak near 650 mμ. The far-red filter system (which has a total of 8 cm of water, including the water in the light source) transmits radiation from 710 to 1,150 mμ, with a peak near 820 mμ. Transmission at 740 mμ is 58 % that of the peak transmission. Transmission spectra of the individual components can be found in the following sources: Filters Nos. 2403 and 7–69, color filters catalog available from Corning Glass Works, Optical Sales Department, Corning, N. Y.; copper sulfate solution from Withrow and Price (34), and water from Curcio and Petty (3). The seeds received 1 and 57 microwatts/mm² of radiant energy under the red and the far-red filter systems, respectively. These light treatments were given continuously from the beginning of imbibition.

The criterion of germination was visual detection of radicle protrusion. Radicles (1–2 mm) from nongerminated seeds were excised and prepared as described previously (8). The apical 0.5 mm was examined for mitotic activity. An entire dish of seeds was sampled at one time for germination percentages and mitotic activity in nongerminated seeds. Once sampled, the dish was removed from the experiment.

Respiratory rates were determined by placing 50 seeds in 1.2 ml of solution in the main compartment of Warburg flasks. We placed 0.2 ml of 20 % KOH and a fluted piece of filter paper in the center well to measure oxygen consumption by the direct method (32).

The term "germination" is used here in the physiologic sense of Toole et al (30): ". . . the start of germination depends upon coupling of respiration to growth." Accordingly, seeds that undergo mitotic activity without expansion of the embryo are not designated as germinated. When an embryo undergoes visible expansion (apart from water imbibition) the seeds would be designated as germinated, even if mitosis had not yet begun.

## RESULTS

EFFECTS OF GA, KINETIN, AND THIOUREA ON MITOSIS AT 37° C: To be meaningful, studies on germination and on mitosis in nongerminated seeds should be conducted under identical experimental conditions. Also, there should be no bias from sampling dissimilar populations of nongerminated seeds. We have found these conditions can be satisfied for studies with kinetin and thiourea by incubating the seeds at 37° C in darkness. Both kinetin and thiourea stimulate germination under these conditions (fig 1). The inability of GA to stimulate germination confirms an earlier observation that GA is ineffective in breaking thermodormancy of lettuce seed (11). After 1 day, however, none of the seeds had germinated in any

[1] Received November 5, 1959.

[2] Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

TABLE I

EFFECTS OF GERMINATION-STIMULATING CHEMICALS ON MITOTIC ACTIVITY IN NONGERMINATED SEEDS AFTER 1 DAY AT 37° C IN DARKNESS

| No. of mitotic figs | No. of radicles | | | |
|---|---|---|---|---|
| | Control | GA* | Kinetin* | Thiourea* |
| 0 | 63 | 64 | 45 | 64 |
| 1 | 1 | ... | 9 | 1 |
| 2 | 1 | ... | 5 | ... |
| 3 | ... | ... | 1 | ... |
| 4 | ... | 1 | 1 | ... |
| 5 | ... | ... | 1 | ... |
| 6 | ... | ... | 1 | ... |
| 7 | ... | ... | 1 | ... |
| 8 | ... | ... | ... | ... |
| 9 | ... | ... | ... | ... |
| 10 | ... | ... | 1 | ... |
| >10 | ... | ... | ... | ... |
| Total No. radicles examined | 65 | 65 | 65 | 65 |
| Total No. mitotic figures observed | 3 | 4 | 54 | 1 |
| Radicles with one or more mitotic figures (%) | 3.1** | 1.5 | 30.8** | 1.5 |

* Concentrations given in figure 1.
** Difference significant at level of $P = 10^{-4}$.

of the treatments. Consequently, seeds could be sampled at this time from all treatments for examination of mitotic activity uncomplicated by growth from expansion. After 2 days, none of the seeds had germinated in water or GA, and the germination percentages in kinetin and thiourea were only 0.8 and 5 %, respectively. Thus, after 2 days, these nongerminated seeds could be sampled from the different chemical treatments with very little sampling bias resulting from the germination. After incubation for either 1 or 2 days, 65 radicles were excised from the nongerminated seeds in each treatment for examination of mitotic activity (tables I, II). For each chemical treatment, the distribution of mitotic figures among the radicles deviated from randomness, with a very great discrepancy between the observed number of radicles having no mitotic figures and the number expected from the Poisson distribution having the same mean number of mitotic figures per radicle. These comparisons, which are given only for the water controls in table II, confirm previous results discussed elsewhere (8). For our present purpose, this particular aspect of the deviation from randomness permits the application of contingency tests of the significance of differences (26) in mitotic activity between treatments by comparing the numbers of radicles having one or more mitotic figures detected.

It is apparent that mitotic activity is greater in kinetin than in water in the nongerminated seeds after either 1 or 2 days at 37° C in darkness. The kinetin treatment resulted not only in increased mitosis but also in increased cell division inasmuch as no binucleate cells were observed.

GA had no effect on mitotic activity after either 1 or 2 days. After 2 days mitotic activity was inhibited by thiourea. Since thiourea inhibits mitotic activity in this experiment even though it stimulates germination, we conclude that thiourea is an inhibitor of mitosis in these seeds where mitosis is uncomplicated by growth by expansion. Before concluding definitely that kinetin is a true cell-division factor and that GA can stimulate germination without directly stimulating mitosis, it is necessary to rule out the alternative explanations considered in each of the following three subsections.

STUDIES OF ACTION OF KINETIN AT HIGH TEMPERATURE: Sufficiently high temperatures can inhibit germination (2) or mitosis in nongerminated seeds (8). Accordingly, the actions of kinetin on mitosis (tables I and II) and germination (fig 1) in seeds at superoptimal temperatures could conceivably result from a general protection against high-temperature effects; if so, then the kinetin effects would not necessarily result from more-direct actions upon either germination or mitosis. This possibility has been tested in two types of experiments. First, we gave seeds various high-temperature treatments designed to prevent subsequent germination under continuous white light at 26° C. The results of one ex-

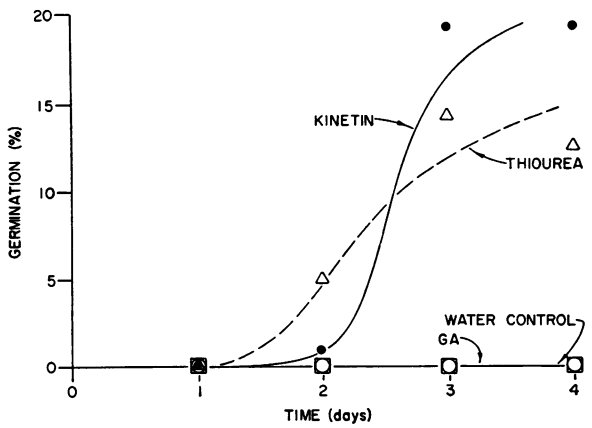
## TABLE II

EFFECTS OF GERMINATION-STIMULATING CHEMICALS ON MITOTIC ACTIVITY IN NONGERMINATED SEEDS AFTER 2 DAYS AT 37° C IN DARKNESS

| No. of mitotic figures | No. of radicles | | | | Poisson distribution (refers to control) |
|---|---|---|---|---|---|
| | Control | GA* | Kinetin* | Thiourea* | |
| 0 | 38 | 38 | 9 | 61 | 0.5 |
| 1 | 4 | 3 | 3 | 2 | 2.4 |
| 2 | 2 | 2 | 4 | ... | 5.9 |
| 3 | ... | ... | 5 | 1 | 9.6 |
| 4 | 1 | 4 | 4 | ... | 11.7 |
| 5 | 4 | 1 | 4 | ... | 11.4 |
| 6 | ... | 1 | 1 | ... | 9.3 |
| 7 | 1 | ... | 2 | ... | 6.4 |
| 8 | 1 | 3 | 2 | 1 | 3.9 |
| 9 | ... | ... | 2 | ... | 2.1 |
| 10 | 2 | 2 | 3 | ... | 1.0 |
| 11 | ... | 1 | 2 | ... | 0.5 |
| 12 | 1 | 2 | 1 | ... | 0.2 |
| 13 | 1 | ... | 2 | ... | 0.1 |
| 14 | 1 | 1 | ... | ... | |
| 15 | ... | ... | ... | ... | |
| 16 | 1 | ... | 3 | ... | |
| 17 | 1 | ... | ... | ... | |
| 18 | 1 | ... | 1 | ... | |
| 19 | 1 | 1 | 1 | ... | |
| 20 | ... | ... | 1 | ... | |
| 21–22 | 1 | ... | 1 | ... | |
| 23–24 | 1 | 1 | 1 | ... | |
| 25–26 | ... | 1 | 2 | ... | |
| 27–28 | 1 | ... | 1 | ... | |
| 29–30 | 1 | 1 | 1 | ... | |
| 31–35 | ... | 1 | 1 | ... | |
| 36–40 | ... | 1 | 2 | ... | |
| 41–50 | 1 | ... | ... | ... | |
| 51–60 | ... | 1 | 2 | ... | |
| 61–70 | ... | ... | ... | ... | |
| 71–80 | ... | ... | 1 | ... | |
| 81–90 | ... | ... | 2 | ... | |
| 91–100 | ... | ... | 1 | ... | |
| >100 | ... | ... | ... | ... | |
| Total No. of radicles examined | 65 | 65 | 65 | 65 | 65 |
| Total No. of mitotic figures observed | 317 | 351 | 1,028 | 13 | 317 |
| Radicles with 1 or more mitotic figures (%) | 41.5[A] | 41.5[B] | 86.2[B] | 6.2[A] | |

* Concentrations given in figure 1.

[A, B] Pairs of figures with the same symbols significantly different from each other, $P = 10^{-4}$. The kinetin data are compared with the GA data rather than with the control data to avoid using the same observations for two different tests of significance. See table I for comparison between kinetin and control treatments.



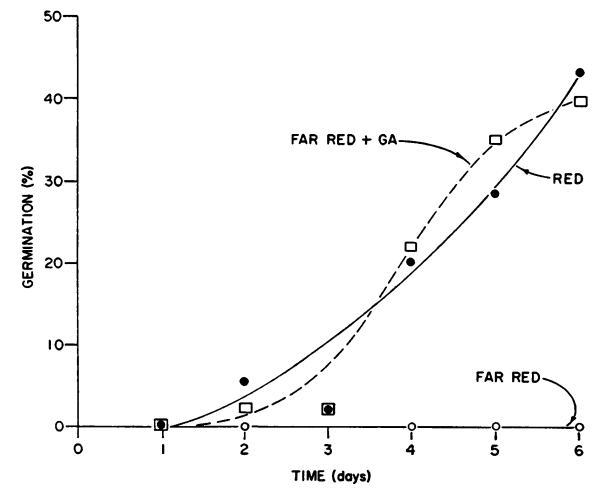




FIG. 1 (*Top*). Effects of GA, kinetin, and thiourea on germination at 37° C in darkness. ○, Water controls; □, $3 \times 10^{-4}$ M GA; ●, $10^{-5}$ M kinetin; and △, $3 \times 10^{-2}$ M thiourea. Each point represents the germination percentage of an independent sample of 120 seeds.

FIG. 2 (*Bottom*). Effects of GA and light on germination at 31.5° C. ○, Far-red; ●, red; and □, far-red + $3 \times 10^{-4}$ M GA. Each point represents the germination percentage of an independent sample of 88 to 94 seeds.

periment are given in table III. Seeds germinated directly gave 95 % germination, whereas seeds first kept 6 hours at 45° C gave only 21 % germination under the same conditions of continuous lighting at 26° C. Incubation in various concentrations of kinetin during the 6 hours at 45° C, however, did not significantly protect against the deleterious effect of the high-temperature treatment. These data suggest that kinetin does not generally increase tolerance of the seeds to high-temperature stress. Consequently, its effects on increasing mitotic activity in nongerminated seeds at 37° C should be regarded as a true acceleration of mitosis. Similarly, the effect of kinetin on germination at superoptimal temperatures should be regarded as a true breaking of thermodormancy rather than a protection of the germination mechanism against heat damage. A similar conclusion is also suggested by studies of the respiratory rates of seeds at 37° C in water or in kinetin. As shown in table IV, the respiratory rate per seed, which is a general indication of over-all metabolic activity, is unaffected by kinetin treatments which stimulate germination. Although all experiments were performed with the same batch of seeds, those cited in tables III and IV were done about 9 and 11 months, respectively, before the other experiments cited in this paper. The somewhat better germination rate of the kinetin-treated seeds (table IV compared with that shown in fig 1) may possibly be related to this and to other differences of experimental method necessitated by the manometric techniques.

SYNERGISTIC ACTION OF GA WITH EITHER KINETIN OR THIOUREA AT HIGH TEMPERATURE: The results given in tables I and II might suggest that GA generally has no effect on mitosis in seeds when such mitosis occurs without growth by expansion. Since treatment with GA alone also fails to allow any germination at this temperature (fig 1), the possibility remains that GA loses all its activity at the high temperature. Accordingly, the data in tables I and II in themselves do not permit the conclusion that GA has no direct effect on mitotic activity under those conditions for which it might still be active with respect to germination. The experiment summarized in table V confirms the ineffectiveness of GA alone to stimulate germination at high temperature. However, GA does retain the capacity to stimulate germination in the presence of either kinetin or thiourea.

TABLE III

EFFECT OF KINETIN ON HIGH-TEMPERATURE-INACTIVATION OF LETTUCE SEED GERMINATION

| HOURS AT 45° C* | KINETIN MOLARITY DURING HIGH-TEMP TREATMENT* | NO. SEEDS | SUBSEQUENT GERMINATION AT 26° C** |
|---|---|---|---|
| | | | (%) |
| 0 | ... | 240 | 95.4 |
| 6 | 0 | 579 | 21.2 |
| 6 | $3 \times 10^{-6}$ | 264 | 26.1 |
| 6 | $10^{-5}$ | 272 | 26.5 |
| 6 | $3 \times 10^{-5}$ | 134 | 13.4 |
| 6 | $10^{-4}$ | 285 | 21.4 |

* Batches of approximately 130 to 140 seeds were sown on one piece of filter paper moistened with 5 ml of appropriate solution in the bottom of 250 ml Erlenmeyer flasks. The opening of the flask was covered with Parafilm, and the main body of the flask was submerged in a 45° C water bath.

** After exactly 6 hours, seeds were washed out of each flask into a cheesecloth bag and repeatedly washed with distilled water. The seeds were then transferred to 9 cm Petri dishes containing one piece of filter paper moistened with 3.5 ml of water. Germination percentages were determined after 3 days at 26° C under continuous white light of 15 ft-c.

TABLE IV

EFFECT OF KINETIN ON RESPIRATION AND GERMINATION AT 37° C

| TIME* (HOURS) | $Q_{O_2}$ (MICROLITERS/50 SEEDS**) | |
|---|---|---|
| | WATER CONTROLS | 5 × $10^{-5}$ M KINETIN |
| 3–4 | 36.4 | 38.8 |
| | 39.7 | 37.5 |
| | 32.4 | 36.0 |
| | 35.2 | 39.8 |
| | average 35.9 | average 38.0 |
| 4–5 | 36.4 | 42.2 |
| | 33.1 | 35.9 |
| | 38.9 | 37.6 |
| | 46.4 | 35.2 |
| | average 38.7 | average 37.7 |
| 5–6 | 36.4 | 38.8 |
| | 34.7 | 39.1 |
| | 38.9 | 40.9 |
| | 32.0 | 35.2 |
| | average 35.5 | average 38.5 |
| 6–7 | 38.0 | 43.9 |
| | 41.3 | 45.6 |
| | 42.1 | 45.8 |
| | 49.6 | 41.3 |
| | average 42.8 | average 44.2 |
| Germination (%)*** | 0.0 | 32.5 |

* Time from beginning of imbibition in Warburg flasks at 37° C.

** Each entry represents a single flask containing 50 seeds.

*** Percentages refer to germination after 2 days for the 200 seeds in each treatment. Germination in kinetin had begun by 24 hours in this experiment.

At the very high temperature GA is effective under conditions (i.e., in the presence of either kinetin or thiourea) where some germination is possible in its absence. This is in contrast to the data of table II which show that GA did not affect the mitotic activity in the nongerminating seeds. These considerations therefore favor the conclusion that the failure of GA to affect mitotic activity at 37° C (tables I and II) cannot be related to its incapacity to stimulate germination at 37° C. If so, we should expect GA to have no effect on mitosis in nongerminated seeds under conditions for which treatment with GA alone does significantly stimulate germination. The results of the next subsection will indicate that this is correct.

EFFECTS OF GA AND PHOTOMORPHOGENIC RADIATION ON MITOSIS AT 31.5 ° C: A temperature of 31.5° C permits experiments with GA and light analogous to those with kinetin and thiourea described in the first subsection and summarized in figure 1 and tables I and II. As can be seen from figure 2, germination in water is negligible at 31.5° C under continuous far-red light. However, treatment with GA stimulates germination under the same conditions of temperature and far-red lighting. Similarly, continuous exposure to red light permits germination of seeds in water. We examined 50 radicles from nongerminated seeds after 1, 2, or 3 days for each of the three treatments: red, far-red, or far-red plus GA (see table VI). In each of the three treatments, contingency tests showed there was no significant difference in the occurrence of radicles having one or more mitotic figures detected after 1, 2, or 3 days. Consequently, the data for each of the three time periods are combined within any one treatment at the bottom of table VI. There are no significant differences in mitotic activity that can be attributed to treatment with GA or to the different light conditions. These results show that GA has no direct effect on mitotic activity even at a temperature where it stimulates germination. Similarly, red and far-red light treatments, which have such different effects on germination, do not give significantly different results on mitotic activity in nongerminated seeds. After only 1 day under far-red light at 31.5° C, seeds in $10^{-5}$ M kinetin or 3 × $10^{-2}$ M thiourea gave 28 and 12 % germination, respectively.

## DISCUSSION

VALIDITY OF CONCLUSIONS DRAWN FROM OBSERVATION OF MITOTIC FIGURES: In general, conclusions on the effect of any given treatment on mitosis that are drawn from numbers of mitotic figures observed may be open to any of the following criticisms: A. Detection of increased numbers of mitotic figures for a given treatment may not necessarily result from a stimulation of greater numbers of cells to undergo mitosis but rather from an effect on the time course

TABLE V

SYNERGISTIC ACTION OF GA WITH EITHER KINETIN OR THIOUREA ON GERMINATION AT 37.5° C

| SUPPLEMENT* | NO. SEEDS | GERMINATION** |
|---|---|---|
| | | (%) |
| None | 195*** | 0.0 |
| GA | 183*** | 0.0 |
| Kinetin | 453† | 8.2[A] |
| Kinetin + GA | 464† | 19.4[A] |
| Thiourea | 465† | 2.8[B] |
| Thiourea + GA | 462† | 13.6[B] |

* Concentrations as in figure 1.

** After 3 days at 37.5° C in darkness.

*** From two dishes of approximately 90 to 100 seeds each.

† From five dishes of approximately 90 to 100 seeds each.

[A, B] Pairs of figures with the same symbols significantly different from each other, P = $10^{-4}$.

of the mitotic cycle. B. Increased numbers of mitotic figures may not result from a given treatment, but may rather result from biological variability in which some organisms sampled have either a vigorous extent of cell division or are undergoing a wave of cell division at the time of fixation. C. Treatments with growth-regulating agents that seem to affect the mitotic ratio may do so by altering the growth pattern of the tissue so that cells examined after different treatments may not have come from equivalent or comparable morphological regions. The conclusions presented here are not invalidated by the first two criticisms since the pattern of deviation from randomness in the detection of mitotic figures permits the application of contingency tests on the basis of radicles having some mitosis vs. no detected mitosis. Moreover, the large numbers of individual radicles examined tend to minimize the second criticism if the total numbers of mitotic figures observed among all radicles examined in one given treatment are considered. The third criticism does not apply because all radicles examined were excised from nongerminated seeds; consequently, no total enlargement had occurred. Moreover, the data are not expressed as mitotic ratios but as numbers of mitotic figures detected per radicle.

TABLE VI

EFFECTS OF LIGHT AND GA ON MITOTIC ACTIVITY IN NONGERMINATED SEEDS AT 31.5° C

| No. of mitotic figures | Treatment → | Numbers of radicles | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Red | | | Far-red | | | Far-red + $3 \times 10^{-4}$ M GA | | |
| | Days → | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0 | | 21 | 35 | 34 | 29 | 34 | 32 | 36 | 34 | 35 |
| 1 | | 7 | 6 | 5 | 10 | 6 | 2 | 4 | 2 | 7 |
| 2 | | 5 | 1 | ... | 3 | 4 | 2 | 1 | 1 | 2 |
| 3 | | 6 | 2 | 2 | 4 | ... | 2 | 3 | 3 | 1 |
| 4 | | 2 | 2 | ... | 1 | 1 | 3 | 2 | 2 | ... |
| 5 | | ... | ... | 1 | 1 | ... | ... | ... | 2 | ... |
| 6 | | 1 | 2 | ... | ... | ... | ... | 2 | ... | 1 |
| 7 | | ... | 1 | 2 | 1 | ... | 3 | ... | 1 | ... |
| 8 | | 2 | ... | 1 | ... | ... | ... | ... | 1 | 1 |
| 9 | | ... | ... | 1 | ... | 1 | 4 | ... | 1 | ... |
| 10 | | ... | ... | 2 | ... | ... | ... | ... | ... | ... |
| 11 | | 1 | ... | ... | ... | ... | 1 | ... | ... | 1 |
| 12 | | 1 | ... | ... | ... | 1 | 1 | ... | ... | ... |
| 13 | | ... | 1 | 1 | ... | ... | ... | ... | ... | ... |
| 14 | | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 15 | | 1 | ... | ... | ... | 2 | ... | ... | ... | ... |
| 16 | | 2 | ... | ... | ... | ... | ... | ... | ... | ... |
| 17 | | 1 | ... | ... | ... | ... | ... | ... | 1 | ... |
| 18 | | ... | ... | ... | 1 | ... | ... | ... | ... | ... |
| 19 | | ... | ... | ... | ... | ... | ... | 1 | ... | ... |
| 20 | | ... | ... | ... | ... | 1 | ... | ... | ... | 1 |
| 21 | | ... | ... | ... | ... | ... | ... | 1 | ... | ... |
| 22 | | ... | ... | 1 | ... | ... | ... | ... | 1 | 1 |
| 23 | | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 24 | | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 25 | | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 26 | | ... | ... | ... | ... | ... | ... | ... | 1 | ... |
| >26 | | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Total No. of radicles examined | | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Total No. of mitotic figures observed | | | 308 | | | 255 | | | 276 | |
| Radicles with 1 or more mitotic figures (%) | | | 40.0 | | | 36.7 | | | 30.0 | |

FURTHER EVIDENCE THAT CELL DIVISION AND CELL EXPANSION ARE INITIATED BY DIFFERENT MECHANISMS DURING GERMINATION: We previously concluded that cell division and cell expansion were initiated by different mechanisms during germination (8). If this is so, we may predict that there should be no necessary and general relation between the activity of any particular treatment on germination and its activity on mitosis in the absence of germination. Of the four germination-stimulating agents studied in this paper, only kinetin also stimulated mitosis in nongerminated seeds; GA or red light had no effect on such mitosis, and thiourea inhibited it. Thus these results amply confirm that cell division and the initiation of germination by cell expansion are controlled by different mechanisms during germination.

WHAT IS DORMANCY? Table IV shows that the effect of kinetin on breaking thermodormancy was unaccompanied by any readily discernable difference in oxygen consumption. In similar studies on the dormancy resulting from $\gamma$-irradiation of lettuce seed, it was found that GA or kinetin (which produced 15-fold or 20-fold increases in germination percentages, resp., compared to water controls) gave no difference in oxygen consumption per seed until root growth was visible (9). Similarly, although white light gave a 28-fold greater germination percentage of Grand Rapids lettuce seeds than far-red light, the fixation of $C^{14}$ from externally supplied radioactive bicarbonate into the alcohol- and water-soluble fraction was not significantly different under the two light conditions (12). Although other investigators have reported some changes in oxygen consumption per seed resulting from light treatments known to affect dormancy (5, 13, 15), our results suggest that the breaking of dormancy is not generally related to gross metabolic alterations until after root growth has begun. Long before growth begins, lettuce seeds couple energy to endergonic metabolic processes (12). Accordingly, it is not surprising that the metabolism of dormant seeds can be sufficient to sustain mitotic activity and that such activity can be modified by some growth-regulating chemicals (e.g., kinetin or thiourea). It would therefore seem that dormancy in seeds is not generally related either to a significant depression of over-all metabolism or to a block preventing cellular division. Because the term "dormancy" connotes a relatively inactive metabolism and a cessation of all processes concomitant to growth, it may be an inappropriate term for describing the physiologic state of seeds prevented from germinating by withholding morphogenic stimuli (e.g. red light, GA, etc.) under otherwise favorable conditions. These considerations lead to the conclusion that the definitive characteristic of dormancy in seeds is a subtle block that specifically prevents the initiation of cellular expansion. If thiourea generally inhibits mitosis (see p. 493), then the breaking of dormancy in potato tubers by thiourea (4) must similarly be related to an initial stimulation of growth by cellular expansion and not to cell division. Thus the definitive characteristic of dormancy in seeds may possibly be the definitive characteristic of dormancy in general.

GIBBERELLIN AND MITOSIS: Whereas previous studies showed that the action of GA in breaking lettuce seed dormancy must be attributed to an activation of cell expansion (8), our present findings further suggest that GA does not generally influence growth by directly affecting mitotic activity. It should be remembered that the mitotic activity studied here occurred in the radicle, the first organ to grow during lettuce seed germination. Gamma-irradiated wheat can provide a system for growth studies complementary to those studied here, i.e., growth can occur by cellular expansion in the absence of any cell division (10). In such a system, GA was found to give typical effects on seedling growth (10). Considerations of growth as a function of radiation dose further suggested that the increased growth induced by GA in unirradiated young wheat seedlings can be attributed largely or entirely to a direct effect on cell expansion rather than a direct effect on cell division (10). Thus the experiments performed on the two complementary systems (lettuce seeds in which cell division occurs in the absence of growth by cell expansion and irradiated wheat in which seedling growth by cell expansion occurs in the absence of cell division) suggest a single consistent theory. The growth-stimulatory effects of GA are caused solely by a direct effect on cellular expansion; thus the apparent effects on cell division are results, and not causes, of such growth stimulation (see ref. 10 for additional discussion and literature citations).

SYNERGISTIC ACTIONS INVOLVING GA ON SEED GERMINATION: Synergistic actions on lettuce seed germination from combinations of GA and kinetin (or kinetin analogues) have been reported (11, 23, 24). However, no such synergistic effect has been reported for combinations of gibberellin and thiourea, which is demonstrated here (table V). Inasmuch as kinetin and thiourea have such different over-all effects on lettuce seeds (as evidenced by their opposite effects on mitotic activity in nongerminated seeds), the fact that GA can give synergistic effects with either compound tends to weaken the suggestion of Skinner et al that gibberellin and 6-(substituted) purines "are closely related in their biochemical functions" (24). The present considerations agree with studies that showed that the actions of GA and kinetin on lettuce seed germination are separable and distinct (11).

ACTION OF KINETIN: Since the discovery of kinetin and its activity in cell division (18), it has been found to produce diverse effects, other than promotion of cell division, in several systems (see ref. 27 for review). Its effect on leaf disk expansion has been attributed to cell enlargement (6, 16). Its activity in stimulating lettuce seed germination must also be attributed to cell expansion (8). Kinetin can break dormancy resulting from $\gamma$-irradiation under

conditions where the initiation of mitosis lags several days behind the initiation of germination by cell expansion (8, 9). Consequently, this initial stimulation of germination in γ-irradiated seeds cannot possibly be related to cell division since there is no division at the time. Since kinetin has been shown to promote cell expansion in several instances, an increased cell number resulting from kinetin treatment (7, 19) does not in itself imply a primary action on cell division. Since the stimulation of cell division in nongerminated seeds occurs without over-all expansion, our studies show that kinetin can indeed act as an accelerator of cell division. That this stimulation of cell division can not be attributed to the stimulatory effect of kinetin on germination follows from the failure of the other germination-stimulators (GA, thiourea, red light) to similarly stimulate cell division in nongerminated seeds. This demonstration of a true stimulation of cell division by kinetin is in accord with the work of Skoog and his associates. These workers found that, in the presence of indoleacetic acid, kinetin treatment of tobacco pith resulted in clusters of very small cells (25). The action of kinetin in the nongerminated seeds differs from its action in tobacco pith in that no *exogenous* auxin is necessary for activity.

EFFECTS OF LIGHT: In the classic studies by the Beltsville workers (2), red and far-red light were applied to seeds as short pulses instead of the continuous light treatments used in the present studies. Since we found no difference in the mitotic activity resulting from continuous red or far-red light treatments, there should have been no differences in mitotic activity from light treatments of short duration. We chose continuous light treatment to obtain the great differences in germination percentages (fig 2), in contrast to which the lack of effect on mitosis (table VI) is all the more striking.

That the over-all effect of light on seeds differs from the over-all effects of either kinetin or thiourea is indicated by the fact that light apparently has no effect on mitotic activity whereas kinetin or thiourea do have. The effects of red light and kinetin on the initiation of germination proper are mediated by different mechanisms (11, 17). Although apparently neither red light nor GA influence mitosis in nongerminated seeds, we do not wish to imply that the effects of these two agents on germination are therefore closely related. We have previously shown that under certain conditions where lettuce seeds are unable to respond to GA they can respond to red light (11). Conversely, under certain conditions where the response to light was poor, there was a very strong response to GA (9).

From considerations analogous to those presented in the subsection on gibberellin and mitosis, it might be suggested that the effects of light on cell division during seedling growth (29) may result from more direct actions on growth by cell expansion.

EFFECTS OF THIOUREA: Although thiourea must stimulate germination via cellular expansion, it nonetheless inhibits cell division in nongerminated seeds. It also greatly inhibits mitotic activity in protruded roots (2–4 mm long) of lettuce under conditions where both treated and untreated seeds have fully germinated (Haber and Luippold, unpub.) Treatment of lettuce seeds with thiourea after γ-irradiation increased production of chromosomal aberrations in the root tips after germination (9). Thus thiourea has (at least) two different effects on the nuclear level: mitotic inhibition and (potentiation of) chromosome breakage. Inhibition of mitosis may explain the drastic inhibitory and lethal effects of thiourea on seedling growth (9, 21, 28); on inhibiting or greatly retarding cleavage of eggs and arrest of growth at the earliest developmental stages in echinoderms (1, 22); and on inhibition of regeneration in planarians (14). It may also be possible that mitotic inhibition may be related to the activity of thiourea as a goitrogenic drug, especially since goiters are characterized by a greater extent of cellular hypertrophy than cellular proliferation (31).

It is most interesting that thiourea, which inhibits growth [including that of lettuce seedlings (9, 28)] as well as mitosis in nongerminated lettuce seeds, should stimulate the initial cellular expansion in breaking dormancy of lettuce seeds and potato tubers (4). This might be partly explained by postulating that the growth-inhibitory action of thiourea can be attributed solely to inhibition of cell division. If so, then it should be relatively ineffective as a growth inhibitor in systems where mitotic activity does not significantly contribute to over-all growth as, for example, in the initial growth by cell expansion in the breaking of dormancy. In this connection, it is interesting to note that Parry found a synergistic action of thiourea and indoleacetic acid on the growth of *Avena sativa* L. seedlings (20). There was, moreover, a slight positive effect of thiourea in the absence of applied indoleacetic acid (20). This might suggest that thiourea stimulates cellular expansion in certain special cases apart from its action in breaking dormancy.

## SUMMARY

The effects of several germination-stimulating agents on mitotic activity in radicles of nongerminated lettuce seeds have been studied using conditions near the upper limits of temperatures permitting germination. Under appropriate conditions where gibberellin, kinetin, thiourea, and red (as compared with far-red) light could stimulate germination, only kinetin stimulated mitotic activity. Kinetin can be considered a true cell division factor in nongerminated lettuce seeds. Neither gibberellin nor red light had any significant effect on mitotic activity under conditions where they were later active in stimulating germination. The conditions under which thiourea inhibited mitosis were the same as those under which it later stimulated germination. The results are discussed in relation to seed germination, the general problem of dormancy,

and the actions of the germination-stimulating agents on other aspects of growth and development.

## Acknowledgments

The authors wish to express their gratitude to Dr. John Jagger for assistance in calibration of the light sources and to Drs. R. F. Kimball and Drew Schwartz for reviewing the manuscript and for their many helpful suggestions.

## Literature Cited

1. Bevelander, G. 1946. The effect of thiourea on the development of the sea urchin, *Arbacia punctulata*. Proc. Soc. Exp. Biol. Med. 61: 268–270.
2. Borthwick, H. A., S. B. Hendricks, E. H. Toole, and V. K. Toole. 1954. Action of light on lettuce-seed germination. Bot. Gaz. 115: 205–225.
3. Curcio, J. and C. C. Petty. 1951. The near infrared absorption spectrum of liquid water. Jour. Opt. Soc. Amer. 41: 302–304.
4. Denny, F. E. 1926. Hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. 13: 118–125.
5. Evenari, M., G. Neumann, and S. Klein. 1955. The influence of red and infrared light on the respiration of photoblastic seeds. Physiol. Plantarum 8: 33–47.
6. Griffith, M. M. and R. D. Powell. 1959. The effect of kinetin and red light on growth and cell size. ASB Bull. 6: 24–25.
7. Guttman, R., and A. Back. 1958. Effects of kinetin on cell division in *Paramecium caudatum*. Nature 181: 852.
8. Haber, A. H. and H. J. Luippold. 1960. Separation of mechanisms initiating cell division and cell expansion in lettuce seed germination. Plant Physiol. 35: 168–173.
9. Haber, A. H. and H. J. Luippold. 1959. Dormancy resulting from gamma-irradiation of lettuce seed. Int. Jour. Radiation Biol. 1: 317–327.
10. Haber, A. H. and H. J. Luippold. 1960. Effects of gibberellin on gamma-irradiated wheat. Amer. Jour. Bot. 47: 140–144.
11. Haber, A. H. and N. E. Tolbert. 1959. Effects of gibberellic acid, kinetin, and light on the germination of lettuce seed. In: Photoperiodism and Related Phenomena in Plants and Animals, R. B. Withrow, ed. Pp. 197–206. Amer. Assoc. Advance Sci., Washington, D. C.
12. Haber, A. H. and N. E. Tolbert. 1959. Metabolism of $C^{14}$-bicarbonate, $P^{32}$-phosphate, or $S^{35}$-sulfate by lettuce seed during germination. Plant Physiol. 34: 376–380.
13. Hagen, C. E., H. A. Borthwick, and S. B. Hendricks. 1954. Oxygen consumption of lettuce seed in relation to photocontrol of germination. Bot. Gaz. 115: 360–364.
14. Jenkins, M. M. 1959. The effects of thiourea and some related compounds on regeneration in planarians. Biol. Bull. 116: 106–114.
15. Leopold, A. C. and F. S. Guernsey. 1954. Respiratory responses to red and infra-red light. Physiol. Plantarum 7: 30–40.
16. Miller, C. O. 1956. Similarity of some kinetin and red light effects. Plant Physiol. 31: 318–319.
17. Miller, C. O. 1958. The relationship of the kinetin and red-light promotions of lettuce seed germination. Plant Physiol. 33: 115–117.
18. Miller, C., F. Skoog, F. Okumura, M. von Saltza, and F. Strong. 1956. Isolation, structure, and synthesis of kinetin, a substance promoting cell division. Jour. Amer. Chem. Soc. 78: 1375–1380.
19. Orr, M. F. and B. McSwain. 1957. The effects of kinetin upon epithelium and fibroblasts in tissue culture. Cancer 10: 617–624.
20. Parry, D. W. 1952. A growth interaction between $\beta$-indolylacetic acid and thiourea (thiocarbamide). Nature 170: 1074.
21. Parry, D. W. 1956. The effects of 2,4-dichlorophenoxyacetic acid, 2,3,5-tri-iodobenzoic acid, and thiourea on the vegetative and reproductive growth of *Avena sativa*. Jour. Exp. Bot. 7: 381–386.
22. Rulon, O. 1950. The modification of developmental patterns in the sand dollar by thiourea. Physiol. Zoöl. 23: 248–257.
23. Skinner, C. G. and W. Shive. 1958. Synergistic effect of gibberellin and 6-(substituted)-purines on germination of lettuce seed. Arch. Biochem. Biophys. 74: 283–285.
24. Skinner, C. G., F. D. Talbert, and W. Shive. 1958. Effect of 6-(substituted) purines and gibberellin on the rate of seed germination. Plant Physiol. 33: 190–194.
25. Skoog, F. and C. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia Society for Exp. Biol. XI: 118–131.
26. Snedecor, G. W. 1956. Statistical Methods. Iowa State College Press, Ames. (5th ed.).
27. Strong, F. M. 1958. Topics in Microbial Chemistry: Antimycin, Coenzyme A, Kinetin, and Kinins. John Wiley & Sons, Inc., New York.
28. Thompson, R. C. and W. F. Kosar. 1939. Stimulation of germination of dormant lettuce seed by sulphur compounds. Plant Physiol. 14: 567–573.
29. Thomson, B. F. 1954. The effect of light on cell division and cell elongation in seedlings of oats and peas. Amer. Jour. Bot. 41: 326–332.
30. Toole, E. H., S. B. Hendricks, H. A. Borthwick, and V. K. Toole. 1956. Physiology of seed germination. Ann. Rev. Plant Physiol. 7: 299–324.
31. Turner, C. D. 1955. General Endocrinology. (2nd. ed.) W. B. Saunders Co., Philadelphia.
32. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1957. Manometric Techniques. (3rd ed.) Burgess Publishing Co., Minneapolis.
33. Withrow, R. B. and V. Elstad. 1953. Water-cooled lamp systems with refluxing aqueous filters. Plant Physiol. 28: 334–338.
34. Withrow, R. B. and L. Price. 1953. Filters for the isolation of narrow regions in the visible and near-visible spectrum. Plant Physiol. 28: 105–114.