ACTION OF MALEIC HYDRAZIDE ON DORMANCY, CELL DIVISION, AND CELL EXPANSION [1]

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Maleic hydrazide (MH) is of practical and theoretical interest as a growth-regulating chemical that can inhibit plant growth without causing obvious morphological abnormalities (13, 14). After recent reports of growth interactions between MH and gibberellic acid (GA) (2, 10), Brian and Hemming called attention to the many growth effects for which MH has actions opposite to those of GA (1). From studies of interactions of MH and GA on pea stem extension, these authors concluded that "MH prevents the response to GA of GA-sensitive plants" and that it is, therefore, likely that "MH inhibits growth by blocking some essential reaction at a stage preceding that where GA normally exerts its effect" (1). In these experiments, we have studied the effects of MH and compared them with those of GA on three systems: lettuce seed germination, mitosis in the absence of growth by expansion in dormant lettuce seeds (5, 7), and growth by cell expansion in the absence of mitosis in γ-irradiated wheat (6). The results show that there is no specific interaction between MH and GA either on lettuce seed germination or on the growth of wheat seedlings, and MH seems to exert direct effects on cell division but not on cell expansion during seedling growth [which is in contrast to previously studied GA effects on these same systems (6, 7)]. Similarities between effects of MH and of ionizing radiation are noted.

MATERIALS AND METHODS

Experiments were performed with lettuce (*Lactuca sativa* L., var. Big Boston White) and wheat (*Triticum vulgare* Vill., (Triticum aestivum L.), var. Thorne). Big Boston White was used because its germination is more sensitive to MH than any of several other varieties we tested. We sowed 100 mg of lettuce seed on one piece of Whatman No. 1 filter paper moistened with 3.5 ml of solution at pH 5.7 in a covered 9 cm Petri dish. The dishes within closed copper sterilization cans (and thus in continuous darkness) were placed in a constant-temperature water bath. The criterion for germination was visual detection of radicle protrusion after 2 days at 26° C. Radicles from nongerminated seeds at 29° C were excised, fixed, and examined for mitotic figures as previously described (5, 7). Some wheat grains were given 800 kr of γ rays at 49 kr/hr from a Co^{60} source described elsewhere (3). The wheat was from the same batch used in previous studies (6) and was grown under the same controlled conditions for 12 days (with light intensity of approximately 450 ft-c during the 16-hour day). Growth was measured as length of the first leaf 12 days after sowing, at which time growth of the irradiated plants growing without mitosis was complete (6).

RESULTS

If the suggestion of Brian and Hemming (1), that MH prevents response to GA, is generally true for gibberellin responses then we should expect GA to be less effective than other germination stimulators in relieving the dormancy imposed by MH. Accordingly, we studied the effects of germination stimulators (GA, kinetin, thiourea) in combination with germination inhibitors (MH, coumarin, 2,4-dinitrophenol, mannitol, and nicotine) on lettuce seed germination. The results given in table I show that GA and the other two stimulators seem to have the same effects relative to one another in relieving dormancy imposed by MH as in relieving dormancy imposed by the other four inhibitors. From these results it seems unlikely that there is any specific interaction between MH and GA on lettuce seed germination. This argument is valid only if the promoters stimulate germination via different mechanisms. That GA and kinetin stimulate lettuce seed germination via different mechanisms is indicated by the findings that these two compounds have different temperature ranges of activity, and that the interaction between them can be either more or less than additive, depending on experimental conditions (9). That GA, kinetin, and thiourea each have different over-all effects on seeds before germination is indicated by their different effects on mitotic activity in nongerminated seeds: no effect, stimulation, and inhibition, respectively (7).

From the original finding that dormant lettuce seeds may show mitotic activity (5), it became possible to study the effect of growth-regulating agents on such activity in the absence of growth by expansion (7). For the lettuce seeds studied in this paper, a temperature of 29° C in darkness prevented germination without completely preventing mitotic activity in the dormant seeds. Thus any effects of MH on such activity should be attributed to direct actions on cell di-

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TABLE I

EFFECTS OF GERMINATION-INHIBITING AND -STIMULATING CHEMICALS ON LETTUCE SEED GERMINATION

STIMULATOR	MOLARITY OF STIMULATOR	GERMINATION %*				
		INHIBITOR				
		MALEIC HYDRAZIDE 1.5×10^{-2} M	COUMARIN 3×10^{-4} M	2,4-DINITROPHENOL 2×10^{-4} M	MANNITOL 0.25 M	NICOTINE 10^{-3} M
None		5	2	1	0	23
GA	10^{-5}	20	5	6	0	22
	10^{-4}	29	11	7	2	36
	10^{-3}	32	14	7	4.5	46
Kinetin	5×10^{-7}	37	7	5	3.5	31
	5×10^{-6}	48	27	6	12.5	59
	5×10^{-5}	68	54	3	24	64
Thiourea	10^{-4}	7	3	2	0.5	28
	10^{-3}	9	4	3	0.5	25
	10^{-2}	32	13	2	5	41

Water controls gave 72 % germination.
* Refers to 250 to 260 seeds among three replicate dishes.

vision and not to indirect effects resulting from arrested growth. The results (table II) show that the mitotic activity in such nongerminated seeds is greatly inhibited by 3×10^{-3} M MH. This is in contrast to previous results from work with GA, which does not affect such mitotic activity (7).

Studies with the system just described, consisting of some cell division in the absence of growth by cell expansion, can be complemented by studies with a system in which growth by cell expansion occurs without cell division (6). It was previously shown that wheat grown from grain given 612 kr of Co^{60} γ rays can grow into small seedlings without any mitotic activity. (No mitotic figures were detected in root and shoot meristems fixed every day for 12 days; whereas several thousand figures were detected in similarly treated unirradiated controls.) Consequently, wheat grown and treated under exactly the same conditions but given 800 kr can be presumed to have no mitotic activity (6). The effect of MH on growth of wheat from grain given 800 kr, as well as of unirradiated wheat, is given in figure 1. Whereas the highest concentrations produce approximately 85 to 90 % inhibition in the growth of unirradiated wheat, there is no significant inhibition of the irradiated wheat. Thus these MH treatments, which can inhibit growth of the unirradiated wheat undergoing both cell division and cell expansion, cannot inhibit growth of irradiated wheat undergoing cell expansion without cell division. This might suggest that the inhibitory effect of MH on seedling growth can generally be attributed to an inhibition of cell division, a suggestion sustained by the finding that MH can inhibit cell division in the absence of growth by expansion (table II). This suggestion will be considered more critically in the Discussion section. Figure 1 shows that, with increasing MH concentra-

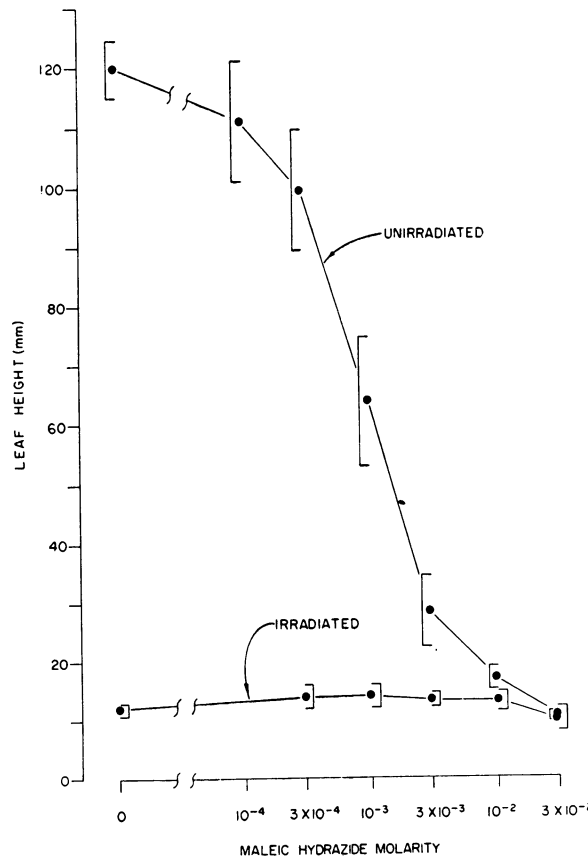

FIG. 1. Height of wheat grown from unirradiated and irradiated (800 kr) grain 12 days after sowing on solutions of MH. Brackets represent 95 % confidence limits.

TABLE II

EFFECT OF MH ON MITOTIC ACTIVITY IN DORMANT LETTUCE SEEDS AFTER 24 HOURS AT 29° C IN DARKNESS

MOLARITY OF MH	GERMINATION*	NO. RADICLES EXAMINED	TOTAL NO. MITOTIC FIGS OBSERVED	NO. RADICLES WITH		
				1 OR MORE FIGS	3 OR MORE FIGS	5 OR MORE FIGS
	(%)					
0	0.9	50	87	13	8	4
3×10^{-3}	0.0	50	1	1	0	0

* Percentages refer to 108 seeds for each treatment.
** Germinated seed excluded.

tion, the growth curve of the unirradiated wheat appears to approach that of the irradiated wheat. If it is assumed that the sole effect of MH on wheat seedlings is to inhibit cell division, these results might suggest that the maximal inhibition by MH would correspond to a concentration that reduces mitotic activity to the extent that cell division does not contribute to growth. If so, then the high concentrations of MH would, like γ rays, restrict growth to cell expansion. When unirradiated wheat grains were sown on solutions of 3×10^{-2} M MH and root and shoot meristems were fixed every day for 12 days, no mitotic figures were found. This is in striking contrast to the thousands of figures observed in untreated wheat from the same batch of grain and growing under the same experimental conditions (6). In contrast to MH, GA does exert characteristic growth effects on this irradiated wheat even at doses as high as 1,225 kr (6).

Interactions of MH and GA on the growth of unirradiated wheat are given in figure 2A. GA and MH each produce their expected effects; moreover, plants treated with MH plus GA show more growth than those treated with MH alone and less growth than those treated with GA alone. The results also confirm the observations of Brian and Hemming that the effects of GA and MH are less than additive (1). It is our contention, however, that the assumption that arithmetic additivity of GA and MH effects is a necessary condition for independent action is incorrect. A more likely requirement for independent action would be that the percentage increase in growth from GA treatment should be the same in the absence of MH as in the presence of MH. The latter condition is fulfilled by the results shown in figure 2A. GA treatment gives approximately a 60 % growth increase over controls. When plants are treated with MH plus GA, the increase is also about 60 % compared to treatment with MH alone. Our conclusion that MH and GA apparently act independently (i.e., exhibit no growth interaction) will be considered more fully in the Discussion section. Independent actions on the growth of wheat seedlings is consistent with the data for lettuce seed germination (table I), which do not indicate any specific interaction of MH and GA. Independent actions would also be expected if MH inhibited seedling growth solely by cell division, since GA apparently exerts its growth-stimulatory effects in this wheat only by cell expansion (6). Therefore, we should expect that the growth response to GA of the irradiated wheat growing without mitosis should be unaffected by MH. This expectation is confirmed by the results given in figure 2B.

DISCUSSION

Many previous investigations have led to a commonly accepted conclusion that MH is an antimitotic agent (see ref 4 and refs cited therein). Our finding that MH inhibits cell division in dormant lettuce seed contributes new information in two respects. First, this inhibition of mitosis shows that the action of MH on cell division is probably direct and cannot be attributed to arrested growth since dormant embryos do not grow. Second, this is an instance whereby MH and GA do not produce opposite effects since GA has no effect on mitosis in nongerminated lettuce seeds, even under conditions where it stimulates germination (7). The experiments with irradiated

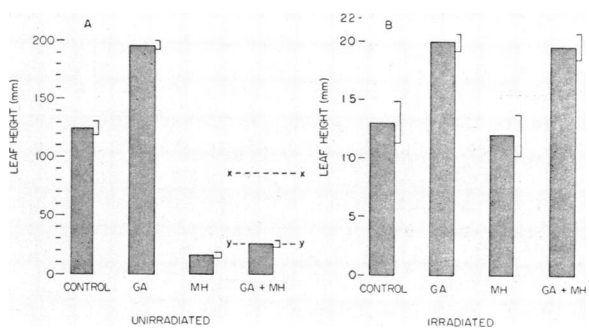

FIG. 2. Height of wheat 12 days after sowing on solutions of GA, MH, or both. Concentrations, GA, 3×10^{-4} M; MH, 10^{-2} M; GA + MH, 3×10^{-4} M GA plus 10^{-2} M MH. Brackets represent 95 % confidence limits. A. Wheat grown from unirradiated grain. x --- x represents height expected if the effects of MH and GA are arithmetically additive (i.e., $h_{GA} - h_{control} = h_{GA + MH} - h_{MH}$). y --- y represents height expected if the GA effect on MH-treated plants is in proportion to the GA effect in the absence of MH (i.e., $h_{GA}/h_{control} = h_{GA + MH}/h_{MH}$). B. Wheat grown from irradiated (800 kr) grain. Note change of scale.

wheat growing without cell division indicate that, in this system at least, MH has no effect on expansion. This growth of the heavily irradiated wheat by cell expansion is true growth sustained by metabolism and is not a physical artifact, since it involves a great increase in dry weight, is prevented by 2,4-dinitrophenol (Haber, unpubl.) and gives typical growth responses to GA [fig 2B; (6)]. These results both with dormant lettuce seed (where cell division occurs without growth by cell expansion) and with γ-irradiated wheat (where growth by cell expansion occurs without cell division) are consistent with the conclusion that MH inhibits cell division but has no effect on expansion (4). However, the results with irradiated wheat may not necessarily parallel the effect of MH on cell expansion in unirradiated wheat; our results do not preclude the possibility that wheat has a radiation-sensitive component of cell expansion that can be inhibited by MH. Moreover, inhibition of lettuce seed germination by MH must be an expression of a prevention of cell expansion and cannot be related to the inhibition of cell division studied here. This is because cell expansion is necessary and sufficient for breaking lettuce seed dormancy, whereas cell division is not (5, 7). Nevertheless, the fact that MH does not affect growth of the irradiated wheat which grows without cell division whereas GA does affect such a system, provides another instance whereby MH and GA do not produce opposite effects.

Since GA apparently affects normal growth of young wheat seedlings solely by stimulating cell expansion (6), the results presented in figure 2A further suggest that MH affects seedling growth solely or primarily by inhibiting cell division. Accordingly, we should expect that for different MH treatments, the GA effect per cell would be the same and would produce the same percentage increase in growth with or without MH, i.e., the effects should appear to be independent. However, failure to find a significant growth interaction in the results of figure 2A does not prove that generally there is no such interaction. Nevertheless, when some of Brian and Hemming's data are reinterpreted, they also seem to show that MH and GA can act independently on the growth rate of Meteor pea seedlings. In these plants pretreated with 0, 0.11, 0.33, or 1.0 g of MH per 100 ml, the ratios of the growth rates for plants given 10 μg GA to those given no GA were 3.3, 3.0, 3.5, and 3.8, respectively (calculated from data of fig 2 of ref 1). Thus, although the effect of MH plus GA was less than would be expected from arithmetic additivity, it was just what would be expected if GA had given the same percentage increase in growth for any MH pretreatment from 0 to 1.0 g per 100 ml. Therefore, according to our interpretation, their data also suggest that MH and GA can act independently. The finding that MH and GA apparently act independently on seedling growth has its parallel in the evidence denying a specific interaction in lettuce seed germination (table I). These considerations might also explain several other relations observed by Brian and Hemming. The greater effect of MH on tall peas as compared to dwarf peas (measured as millimeters growth/day) may have been a consequence of the greater initial height of the tall peas and their nearly fivefold greater growth rate in the absence of any chemical treatments (1). Also, their observation that "MH was slow to produce its maximum retarding effect on growth, whereas GA accelerated growth very rapidly" (1) would be expected, since direct effects on cell division should result in delayed observable effects on over-all growth, whereas direct effects on expansion should result in early observable effects.

The action of MH to induce chromosome breakage (12) might in itself conceivably contribute to growth inhibition. The work of Schwartz and Bay (15) indicates that aneuploidy and cell lethality, resulting from chromosome breakage in dividing cells, may indeed slightly reduce over-all growth. However, our previous data suggest that chromosome breakage in itself could not account for more than a few per cent of the total growth inhibition from γ irradiation (see fig 1 of ref 6). Since γ irradiation can cause reversal in the seedling height-dose curve (6) and MH does not (fig 1), we should expect that the fraction of MH-induced growth inhibition resulting from chromosome breakage would be even less than the corresponding fraction of such γ-irradiation-induced growth inhibition. Any effect of such breakage on over-all growth cannot result in aneuploidy unless the cell divides. Consequently, by using the heavily irradiated wheat, the effect of MH on cell expansion is separated from its action on aneuploidy resulting from chromosome breakage as well as from its action on mitosis.

Our results show several striking similarities between effects of MH and effects of ionizing radiation. It has long been known that chromosome breakage can result from ionizing radiation or from MH (12). Similarly, mitosis in dormant lettuce seeds can be inhibited by MH (table 2) and also, presumably, by ionizing radiation (5, 8). That grain given certain high doses of ionizing radiation can germinate and undergo limited growth without any cell division, was first suggested by Schwartz and Bay (15). This radiation effect seems to be duplicated by MH (see Results section). The growth of this wheat is extraordinarily insensitive to γ radiation in doses greater than those sufficient to prevent mitosis. By this we mean that, from 500 to 1200 kr, there is no significant effect of radiation dose on subsequent growth (6). Also MH treatment of such heavily irradiated wheat does not decrease growth any further (figs 1 and 2B). Consequently, cell expansion in these young wheat seedlings is apparently equally insensitive to γ radiation and to MH, especially since the growth curves for irradiated and unirradiated wheat seem to converge with increasing MH concentration (fig 1). That the inhibitory effects of 800 kr of γ rays and those of MH are not at all additive (figs 1 and 2B) further suggests that these two treatments affect

growth by common mechanisms. Finally, the well-known effects of MH on inducing dormancy [table I; (11)] have their counterpart in the finding that a failure of γ-irradiated lettuce seeds to germinate could be attributed to dormancy resulting from the irradiation (8). In all four of these respects in which MH effects resemble those of ionizing radiation (chromosome breakage, mitotic inhibition, relative ineffectiveness on cell expansion, and dormancy), the only one in which GA exerts effects opposite from MH is on dormancy. We have seen, however, that this cannot be attributed to any specific interaction between MH and GA (table I). Thus, treatment with GA after γ irradiation has no effect on the yield of chromosomal aberrations (8); GA does not seem to influence mitotic activity directly (7); GA, however, does stimulate growth of γ-irradiated wheat growing without mitosis [fig 2B; (6)].

This discussion favors caution in interpreting the nature of growth interactions solely from observations of over-all growth and the criterion of arithmetic additivity. None of the experiments described here bear directly on the reported activity of MH as an inhibitor of auxin action (11). However, since the primary action of MH on seedling growth seems to be inhibition of cell division, it is unlikely that MH and auxin generally and directly influence the same mechanisms that regulate growth. This last suggestion is apparently consistent with the discussion of the relation between MH and auxin presented by Leopold (11).

Summary

MH can inhibit cell division in dormant lettuce seeds in which cell division occurs in the absence of growth by cell expansion. Concentrations of MH that inhibit normal growth of wheat seedlings by 85 to 90 % have no effect on the growth of wheat from irradiated grain, which grows without any cell division. Thus MH affects mitosis in a system where GA does not, and MH has no effect on cell expansion in a system where GA is active. When wheat is treated with a combination of MH and GA, the two chemicals apparently act independently on growth. All these findings suggest that the effect of MH on seedling growth can be largely or entirely attributed to an inhibition of cell division and not to any appreciable effect on cell expansion. MH and GA do not appear to give specific interactions on lettuce seed germination, thereby providing further evidence against the theory that MH and GA affect growth through common mechanisms. A number of similarities between effects of MH and of ionizing radiation are discussed, including the capacity to permit germination and limited seedling growth of wheat without cell division.

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Literature Cited

1. Brian, P. W. and H. G. Hemming. 1957. The effect of maleic hydrazide on the growth response of plants to gibberellic acid. Ann. Appl. Biol. 45: 489–497.
2. Bukovac, M. J. and S. H. Wittwer. 1956. Gibberellic acid and higher plants. I. General growth responses. Quart. Bull. Mich. Agric. Exp. Sta. 39: 307–320.
3. Conger, A. D., M. L. Randolph, C. W. Sheppard, and H. J. Luippold. 1958. Quantitative relation of RBE in Tradescantia and average LET of gamma-rays, X-rays, and 1.3-, 2.5-, and 14.1-Mev fast neutrons. Radiat. Res. 9: 525–547.
4. Greulach, V. A. and J. G. Haesloop. 1954. Some effects of maleic hydrazide on internode elongation, cell enlargement, and stem anatomy. Amer. Jour. Bot. 41: 44–50.
5. 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.
6. Haber, A. H. and H. J. Luippold. 1960. Effects of gibberellin on gamma-irradiated wheat. Amer. Jour. Bot. 47: 140–144.
7. Haber, A. H. and H. J. Luippold. 1960. Effects of gibberellin, kinetin, thiourea, and photomorphogenic radiation on mitotic activity in dormant lettuce seed. Plant Physiol. 35: 486–494.
8. Haber, A. H. and H. J. Luippold. 1959. Dormancy resulting from gamma-irradiation of lettuce seed. Int. Jour. Radiation Biol. 1: 317–327.
9. 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. AAAS, Washington, D. C. Pp. 197–206.
10. Kato, J. 1956. Effect of gibberellin on elongation, water uptake, and respiration of pea-stem sections. Science 123: 1132.
11. Leopold, A. C. 1955. Auxins and Plant Growth. University of California Press, Berkeley.
12. McLeish, J. 1952. The action of maleic hydrazide in Vicia. Heredity 6 (suppl.): 125–147.
13. Naylor, A. W. and E. A. Davis. 1950. Maleic hydrazide as a plant growth inhibitor. Bot. Gaz. 112: 112–126.
14. Schoene, D. L. and O. L. Hoffmann. 1949. Maleic hydrazide, a unique growth regulant. Science 109: 588–590
15. Schwartz, D. and C. E. Bay. 1956. Further studies on the reversal in the seedling height dose curve at very high levels of ionizing radiations. Amer. Naturalist 90: 323–327.