occurs if this angle is 90°, 60°, or 30° but does not occur if it is 120°, 150°, or 180°. Oscillation does not occur if a single beam is used. It was also discovered that a sporangiophore might be under conditions favorable for oscillation and yet remain in the nonoscillating state; in this case it is only necessary to give a short tropic stimulus in order to initiate the oscillation, which is then self-sustaining.

D. S. DENNISON*
California Institute of Technology, Pasadena

References and Notes

1. When the beams are in the vertical plane the equilibrium is affected by negative geotropism.
2. D. S. Dennison, Ph.D. thesis, California Institute of Technology (1958).
3. R. Cohen and M. Delbrück, *J. Cellular Comp. Physiol.*, in press.
4. O. Hagem, *Bergens Museums Arbok. Naturv. Rekke* No. 3 (1911).

* Present address: Department of Zoology, Dartmouth College, Hanover, N.H.

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Flower Induction in Japanese Chrysanthemums with Gibberellic Acid

Abstract. Gibberellic acid, when applied in lanolin to apices of three Japanese varieties of *Chrysanthemum morifolium*, induced bolting and flowering. These varieties are not sensitive to photoperiod but require a cold treatment in order to flower. On the other hand, the varieties of *Chrysanthemum* which belong to the short-day group are not induced to flower by gibberellic acid.

The discovery that gibberellic acid (GA) induces bolting and flowering in several species (*1, 2*) has stimulated much research on the flower-inducing properties of this compound. Gibberellic acid, however, seems to be unable to induce flowering in the cocklebur (*Xanthium pennsylvanicum*) (*2*) and in short-day varieties of chrysanthemum (*Chrysanthemum morifolium*) (*3*). This situation raises the following point: Is gibberellic acid ineffective, in the cited cases, because the two species, *Xanthium pennsylvanicum* and *Chrysanthemum morifolium*, happen to be nonresponsive for genetic reasons, or is it ineffective because of the physiological short-day character which these species have in common?

In order to elucidate this question, three varieties of Japanese chrysanthemums which can be induced to flower regardless of the photoperiod were selected: Shuokan, Kinkazan, and Shin-misono (*4*). These varieties require a cold treatment near 1°C for 3 to 4 weeks in order to be able to flower, whether under long-day or short-day illumination; without a cold treatment, they may remain in a rosetted condition for almost a year.

The following procedures were carried out, with eight replications per treatment for each of the three varieties: (i) controls were kept in a greenhouse at a temperature above 15°C during the whole growing period; (ii) plants were subjected to temperatures of 1° to 5°C for 4 weeks in an outdoor cold frame, then returned to the greenhouse; (iii) plants were kept in the greenhouse but were treated once, at the growing point, with about 5 mg of a lanolin paste containing 10 μg of gibberellic acid per milligram (*5*). At all times, including the periods of cold treatment, all the plants were given long, 18-hour days by supplementing the hours of natural daylight with periods of incandescent light. From each plant the lateral shoots were removed, only one main stem being left.

The results obtained were as follows. Two weeks after treatment with either gibberellic acid or cold, the stems of the respective plants started to elongate (Fig. 1). Later on, flower buds appeared, and 19 weeks after the beginning of the treatments the plants were in full bloom (Fig. 2). At that time the controls were still in a rosetted state and without any flowers. The controls eventually bolted and finally bloomed also, but much later —some 11 weeks after the treated plants had bloomed. Results were essentially similar in all three varieties, except that in the Shin-misono variety the plants treated with gibberellic acid bloomed 2 weeks later than the cold-treated ones. As is shown in Table 1, the cold-treated plants flowered at a lower node than those treated with gibberellic acid and had longer internodes.

These experiments show that gibberellic acid can induce bolting and flowering in varieties of chrysanthemum which normally require a cold treatment in order to flower. They indicate that it is not the species *Chrysanthemum morifolium*, as such, which is insensitive to the flower-promoting effect of gibberellic acid but rather the short-day characteristic of some of the varieties belonging to this species. This result strengthens the idea that gibberellic acid is not effective in inducing flowering in short-day plants (*6*).

H. HARADA*
J. P. NITSCH*
Department of Floriculture and Ornamental Horticulture, Cornell University, Ithaca, New York

Table 1. Effect of flower-inducing treatments on three varieties of Japanese chrysanthemums.

Variety	Controls	Gibberellic acid	Cold
	Total height (cm)*		
Shuokan	17.8	95.4	85.0
Kinkazan	36.0	106.8	99.4
Shin-misono	25.2	64.0	66.6
	Average length of internodes (cm)*		
Shuokan	0.31	2.27	2.64
Kinkazan	0.49	2.07	2.42
Shin-misono	0.39	1.40	2.08
	*Average nodes to flower**		
Shuokan		42.0	32.2
Kinkazan		51.6	41.0
Shin-misono		45.6	32.0
	Number of weeks to anthesis (from beginning of treatments)		
Shuokan	30	19	19
Kinkazan	29	20	19
Shin-misono	31	22	20

* Nineteen weeks after the beginning of treatments.

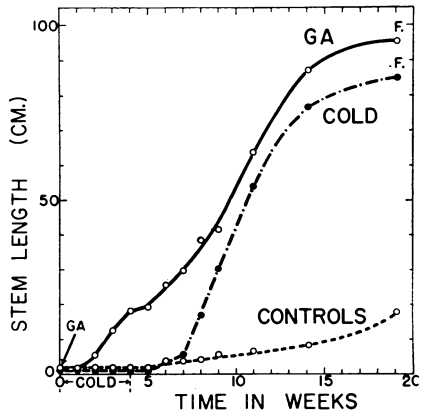

Fig. 1. Growth curves of Japanese chrysanthemums, var. Shuokan, subjected to the following treatments: (open circles and solid line) about 50 μg of gibberellic acid applied in a lanolin paste to the growing points at time 0; (solid circles) 4 weeks of cold treatment; (open circles and dashed line) controls. (Each point represents the average of eight replications.)

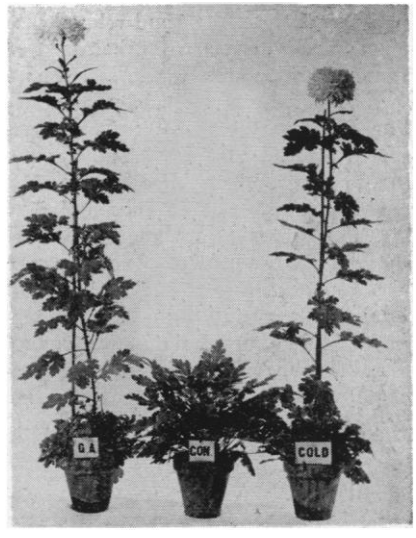

Fig. 2. Induction of bolting and flowering in Japanese chrysanthemums, var. Shuokan, 19 weeks after the application of gibberellic acid (left) or the beginning of a 4-week cold treatment (right). The control (middle) remained rosetted and vegetative.

References and Notes

1. A. Lang, *Naturwissenschaften* 43, 285 (1956).
2. —→, *Proc. Natl. Acad. Sci. U.S.* 43, 709 (1957).
3. H. C. Kohl and A. M. Kofranek, *Calif. Agr.* 11, 9 (1957).
4. The chrysanthemums used in this study were kindly supplied by H. M. Cathey of the U.S. Department of Agriculture, Beltsville, Md.
5. Donated by Eli Lilly and Co., Greenfield, Ind., and by Merck and Co., Inc., Rahway, N.J.
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* Present address: Laboratoire du Phytotron. Gif-sur-Yvette (Seine-et-Oise), France.

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Bromination of Phenol Red by the Dogfish, Squalus acanthias

Abstract. The uterus of the pregnant spiny dogfish, *Squalus acanthias,* can convert phenol red into a new purplish-blue dye. Evidence shows that the new dye is bromophenol blue. This is the first example of biological bromination that has been observed in a vertebrate.

Apart from thyroxin and related substances, naturally occurring halogenated organic compounds are rare in animals. Certain species of shellfish, particularly *Murex brandaris* and *M. trunculus,* secrete a colorless liquid which, on exposure to light and air, gives the ancient dye, Tyrian purple, 6,6′-dibromoindigo (*1*). The occurrence of another bromocompound, 3,5-dibromotyrosine, has also been reported (*2*) in the skeletons of certain anthozoans. However, there appears to be no recorded instance of bromine being incorporated into an experimentally introduced exogenous material. The data presented in this report (*3*) indicate that the uterus of the pregnant spiny dogfish, *Squalus acanthias,* an elasmobranch, can convert the dye phenol red (phenolsulfonphthalein) into bromophenol blue (3,3′,5,5′-tetrabromophenolsulfonphthalein) (*4*).

The spiny dogfish has a gestation period of almost 2 years. In the first summer of pregnancy, the eggs in each uterus are enclosed in a common capsule, with the uterine wall in close apposition to the capsule. The capsule is broken, and in the second summer the embryos—small fish with yolk sacs, now called pups—lie free in the uterine cavity. The uterine fluid from the capsule stage (usually a few milliliters) has an electrolyte and urea content resembling that of elasmobranch blood. The uterine fluid of the pup stage, normally 50 to 100 ml, has no urea and has the same electrolyte composition as sea water, but with a *p*H of 5 to 6 (occasionally somewhat higher). The uterocloacal pore becomes flaccid, so that one can easily insert one's finger, and some uterine fluid is frequently lost when the fish is handled. Spontaneous intermittent emptying and filling of the uterus overnight was observed experimentally; this suggested that the sea water may gain direct entrance into the uterus. Whether the bromine used in the conversion of the phenol red comes from this uterine water or comes directly from the dogfish awaits further investigation.

In connection with a study on uterine function in the dogfish, phenol red, shown by paper chromatography to be free from any contaminating dyes, was dissolved in sea water, and 50- to 100-ml portions were introduced by funnel into the uteri of dogfish in the second summer of pregnancy. Saturated solutions as well as various dilutions of the dye were used. After 24 to 48 hours, all or most of the phenol red was replaced by a purplish blue dye, later identified as bromophenol blue. The conversion occurred without exception in each of the eight fish tested. Throughout the experiment, the dogfish were kept alive in sea water at 14° to 15°C.

Although the new dye (*X*) obtained from the uterine fluid (*p*H about 5 to 6) after the phenol red treatment is purplish blue in color, it soon turned yellow upon acidification. Further, the color change covered a *p*H range from 3.5 to 4.5, in agreement with that reported for bromophenol blue (*5*). The structural relationship between the two dyes suggests that this new dye may in fact be bromophenol blue or may be closely related to it. Chromatographic and light-absorption evidence supports the first possibility.

The uterine fluid containing the blue dye was acidified to about *p*H 1 with dilute HCl and extracted three times with benzene; each time about one half the volume of the aqueous phase was used. The benzene extract was washed with an equal volume of water and was then allowed to evaporate almost to dryness. The orange residue was finally recrystallized from methanol. Unfortunately the minute quantity of pure material did not permit a satisfactory ultimate analysis (*6*). Microfusion with sodium followed by micro- qualitative analysis did, however, result in a positive test for bromine and a negative test for iodine (*7*).

Chromatographic behavior in four solvent systems failed to show any significant difference between the blue dye *X* and bromophenol blue (Table 1). Additional faint spots of fluorescence were seen in two of the systems employed, as is indicated in the same table.

The light-absorption characteristics of the two dyes, in acidic and alkaline media, respectively, are listed in Table 2. The spectra of *X* and bromophenol blue were exactly superposable in the visible region, from 400 to 600 mμ. Between 220 and 360 mμ, the dye *X* demonstrated slightly stronger light absorption than bromophenol blue, but the maximal absorption of both occurred at exactly the same wavelength. In any event, the evidence at hand strongly suggests that the dye *X* and bromophenol blue are really one and the same.

No perceptible bromination of phenol red was observed under the following conditions: incubation of living or dead embryos with phenol red in sea water for 30 hours and incubation of phenol red with uterine fluid, blood plasma, and urine, respectively, for 72 hours at room temperature and also in the refrigerator at about 4°C. Uteri with embryos killed by asphyxia resulting from withdrawal of

Table 1. Comparison of the chromatographic behavior of the dye *X* and of authentic bromophenol blue (BPB). (Whatman No. 1 paper, ascending flow.) The spots indicated in the table appeared red under ultraviolet light in the 366-mμ region.

Compound	R_f			
	3% NaCl	BuOH-HOAc-H_2O (4:1:1)	BuOH-EtOH-H_2O (4:1:5)	BuOH saturated with 1.5*N* NH_4OH
X	0.65	0.76*	0.41†	0.41
BPB	0.61	0.76	0.44	0.42

* An additional faint spot of yellow fluorescence with R_f value 0.64 was also visible.
† An additional faint spot of yellow fluorescence with R_f value 0.61 was also visible.

Table 2. Optical densities (OD) of the dye *X* and bromophenol blue at their respective wavelengths of maximal absorption. The spectra were all determined in methanol. The concentration of bromophenol blue (BPB) was 10 μg/ml.

Compound	*p*H	λ_{max_1} (mμ)	OD	λ_{max_2} (mμ)	OD	λ_{max_3} (mμ)	OD
X	1.6	278	0.26	421	0.33		
BPB	1.6	278	0.17	421	0.33		
X	10.7	314	0.28	380	0.10	594	1.16
BPB	10.7	314	0.24	380	0.10	594	1.16