7. KORNBERG, H. L. and BEEVERS, H. The glyoxylate cycle as a stage in the conversion of fat to carbohydrate in castor bean. Biochem. Biophys. Acta 26: 531–537. 1957.
8. KORNBERG, H. L. and KREBS, H. A. Synthesis of cell constituents from C_2-units by a modified tricarboxylic acid cycle. Nature 179: 988–991. 1957.
9. KORNBERG, H. L. and MADSEN, N. B. The metabolism of C_2 compounds in microorganisms. II. Synthesis of malate from acetate via the glyoxylate cycle. Biochem. Jour. 68: 549–557. 1958.
10. LAYNE, E. In: Methods in Enzymology, S. P. Colowick and N. O. Kaplan, eds. Vol. III. Pp. 447–454. Academic Press, N. Y. 1957.
11. OLSON, J. A. The D-isocitric lyase system: The formation of glyoxylic and succinic acids from D-isocitric acid. Nature 174: 695–696. 1954.
12. SAZ, H. J. and HILARY, E. P. The formation of glyoxylate and succinate from tricarboxylic acids by *Pseudomonas aeruginosa*. Biochem. Jour. 63: 563–569. 1956.
13. SMITH, R. A. and GUNSALUS, I. C. Distribution and formation of isocitritase. Nature. 175: 774–775. 1955.
14. SMITH, R. A. and GUNSALUS, I. C. Isocitritase: Enzyme properties and reaction equilibrium. Jour. Biol. Chem. 229: 305–319. 1957.
15. ZELLER, A. In: Encyclopedia of Plant Physiology, W. Ruhland, ed. Vol. III. Pp. 323–353. Springer, Berlin 1957.

CHANGES IN ENDOGENOUS GROWTH SUBSTANCES DURING FLOWER DEVELOPMENT [1]

H. HARADA [2] AND J. P. NITSCH [2]
DEPARTMENT OF FLORICULTURE AND ORNAMENTAL HORTICULTURE,
CORNELL UNIVERSITY, ITHACA, NEW YORK

The theories advanced to explain the flowering phenomenon generally involve the assumption that specific substances are manufactured by plants at the time of flower initiation. Such are, for example, the views of Cajlachjan (1) who suggested the existence of a "florigen," of Melchers (15) who thought that a different substance, "vernalin," was involved in the flowering of biennials, of Hamner and Bonner (8), of Resende (25) who speculated on the possible role of native auxins and antiauxins, of Lang et al (14) who actually caused flowering in Hyoscyamus and Samolus with extracts of Echinocystis endosperm, etc.

Nevertheless, investigations on the natural regulators actually present in plants during the onset of flowering are rare and, in general deceiving. Thus, Cooke (3) found that the auxin level in Xanthium was related to day-length rather than to flowering, flowering plants placed under long days having higher auxin contents than similar plants placed under short days. On the contrary, Vlitos and Meudt (26) detected more auxin under short than under long days in 2 other short-day plants, soybean and the Maryland Mammoth tobacco. In long-day plants, Konishi (13) did not find drastic differences between the auxin levels of rosetted and bolting plants, despite the dramatic morphological difference between these 2 states. Finally, Gilson (6) concluded from his auxin determinations in induced and non-induced Hyoscyamus that the observed auxin fluctuations were a consequence rather than a cause of the flowering process.

The reason why the results of the cited experiments are often unconvincing is probably 2-fold: 1) the techniques used were crude and unspecific, and 2) no convenient and quantitative test for flower-forming substances has yet been devised.

In the light of this situation, it was attempted to take advantage of the more accurate methods known today for the determination of auxins and gibberellins in order to get a clearer picture of the fluctuations of these substances during flower development, even though these substances may have only an accessory role in the mechanism of flowering. Such a study is reported below. It has been presented as a Master's thesis (9) and short summaries of it have been published (11, 18).

MATERIALS AND METHODS

PLANT MATERIAL: Three different physiological types of plants were used in the present study, namely: a long-day plant, *Rudbeckia speciosa* Wenderoth; a cold-requiring plant, *Chrysanthemum morifolium* Ram. variety Shuokan (cuttings were kindly supplied by Dr. H. M. Cathey, U.S.D.A., Beltsville, Maryland); and a short-day plant, *Chrysanthemum morifolium* variety Shasta. Rudbeckia appeared to be a favorable material because of its clear-cut response to photoperiodic treatments and also because its photoperiodic behavior has been studied in detail (5, 7, 13, 16). The Shuokan chrysanthemum is a Japanese variety (large pompon type, yellow) which bolts and flowers rapidly after a low-temperature treatment (23, 24). Unlike other chrysanthemums, it initiates and develops flowers regardless of the day-length. If the plant has not been exposed to cold, it remains vegetative and in a rosetted state for a long time[3]. The chrysanthemum Shasta is a short-day, commercial variety (anemone type, white) which usually requires 10 weeks to full bloom from the beginning of the short-day treatment.

[1] Received for publication October 24, 1958.

[2] Present address: Laboratoire du Phytotron, Gif-sur-Yvette (S. & O.), France.

[3] At a minimum temperature of 16° C, bolting and flowering finally occur without cold treatment, but only after some 30 weeks, as was observed during the course of these experiments.

GROWING CONDITIONS: All plants were grown in a greenhouse which was maintained at a temperature of at least 20° C during the day and at 16° C during the night. Short-day treatments were given by means of covering the plants with black satin cloth from 5 P.M. to 8 A.M. every day. Long days were produced by lengthening the natural day to 18 hours with 60-watt incandescent light bulbs installed 5 feet apart and 2 to 3 feet above the plants.

The Rudbeckia seedlings were grown under short days until they reached a suitable size. The long-day treatments were started on Dec. 16, 1957. From that date on, 1 lot of 10 plants was moved to the long-day condition every week until the 1st lot had received 10 weeks of long days, at which time all the plants were harvested.

Cuttings of Shuokan chrysanthemums were taken from devernalized stock plants. Fifty uniform plants were selected, and were divided into 5 treatments (10 plants per treatment) as follows: 1 week, 2 weeks, 3 weeks, and 4 weeks of exposure to 1° C during the night, plus a control receiving no cold treatment at all. The cold treatments were given by moving the plants into a cold storage room (1° C) from 5 P.M. to 8 A.M. every day during the required period. During the day, the plants were kept in a greenhouse at about 20° C. The cold treatments for the 4 lots were started at the same time. Samples were taken immediately after the cold treatments.

The cuttings of Shasta chrysanthemums were placed under long days. A few days after potting, the terminal tips were removed, and 3 or 4 strong lateral shoots per plant were allowed to develop. The short-day treatments were started on Dec. 16; from this date on, 1 lot of 14 plants was brought into the short-day condition every week. After 10 weeks, samples were taken from all lots at the same time. The control lot consisted of plants which had been kept under long days during the entire period.

EXTRACTION OF THE ACTIVE SUBSTANCES: At the end of the various treatments the tips of the plants were harvested. Only the actual shoot apices with the young leaves enclosing them were used as material for extraction. The samples were brought immediately into a deep-freeze unit and stored at −23° C until lyophilized. Prior to lyophilization the frozen samples were crushed into small pieces in the deep-freeze with chilled mortar and pestle. The completely dried material was ground to a fine powder, and stored dry in a dessicator at 1 to 5° C.

Throughout this series of experiments, a uniform method of extraction was employed in order to obtain comparable pictures of the active substances occurring in the plant tissues. To identify the actual growth substances, or to purify the crude extracts, several other methods were tried. Cold methanol was used as the main extracting solvent as recommended by Nitsch (17). Unless mentioned otherwise, 50 mg (dry weight) of plant material were used for each extraction in the case of Rudbeckia and Shasta, and 25 mg in the case of the Shuokan chrysanthemum. The extraction was repeated 3 times using 20 ml of methanol each time. During the extracting process, the flasks containing the samples plus the extracting solvents were kept in an ice-bath in the refrigerator. The extracting solvent was changed every 20 minutes, so that the total extracting time for each sample was 1 hour. The methanol extract was evaporated to dryness under reduced pressure, the flasks being placed in lukewarm water. In general, the crude methanol extracts were used directly for chromatography.

CHROMATOGRAPHY AND BIOLOGICAL ASSAYS: Paper chromatography was used to separate the various active substances present in the extracts. The procedures and the techniques of ascending paper chromatography were based on the method described by Nitsch and Nitsch (20), using paper strips of Whatman 3 MM, 2 to 3 cm wide. Generally, isopropanol : water (80 : 20, v/v) was the developing solvent, though various other solvents were employed occasionally. The solvent was allowed to ascend 20 cm beyond the original spot in the dark.

The Avena 1st-internode (mesocotyl) test (21) was used in the present study to detect both auxins and gibberellins. The chromatograms were cut transversally into twenty 1-cm pieces, each of which was placed in a small tube with 10 1st-internode sections and 1 ml of the testing solution (citrate-phosphate buffer pH 5.0, about 10^{-2} M; Tween 80, 0.1 %; sucrose, 2 g/100 ml). The tubes were rotated in the dark for 20 hours at 25° C on a roller-tube apparatus (1 rpm). After this time, the length of the mesocotyl was measured to the nearest 0.1 mm using a binocular with an ocular micrometer.

Since the Avena 1st-internode test reacts to both auxins and gibberellins (21, 22), an "Avena leaf test" was used to distinguish between these 2 types of substances. This leaf test was performed as follows: Brighton oat seeds were soaked in water for 2 hours, then laid down on several layers of wet tissue paper disposed at the bottom of a transparent plastic box. The box with the seeds was exposed to fluorescent red light (filtered through red cellophane) for 3 hours on the 1st day and for 1 hour each on the 2nd and 3rd days, in order to prevent the growth of the 1st-internodes. Altogether, the seedlings were grown for 72 hours in a darkroom maintained at 25° C with about 85 % relative humidity. The part of the seedling used for this test was a 4-mm section cut 4-mm above the 1st node. This section contained a segment of both the coleoptile and the enclosed 1st leaf. Measurements of the elongation of the 1st leaf were made after 48 hours. All other procedures and techniques employed in this test were the same as the ones used in the 1st-internode test, except that no presoaking was required and that distilled water was used instead of the buffer-sucrose mixture. The elongation of the leaf sections was found to be promoted by gibberellin A_1 and gibberellic acid (down to a concentration of 10^{-9} M), but not by indole-3-acetic acid. In solutions containing 1, 10 and 100 μg/l of indole-3-

acetic acid (IAA) and 0.1, 1, 10 and 100 μg/l of gibberellic acid (GA) were prepared as controls in the 1st-internode and the 1st-leaf tests, respectively.

Results

A Long-day Plant, *Rudbeckia speciosa*: *Active substances*: Figure 1 gives the histograms obtained with cold methanol extracts of Rudbeckia grown under short days and under 1 to 5 weeks of long days. In these histograms any elongation which is equal or superior to that given by 1 μg/l of IAA is statistically highly significant.

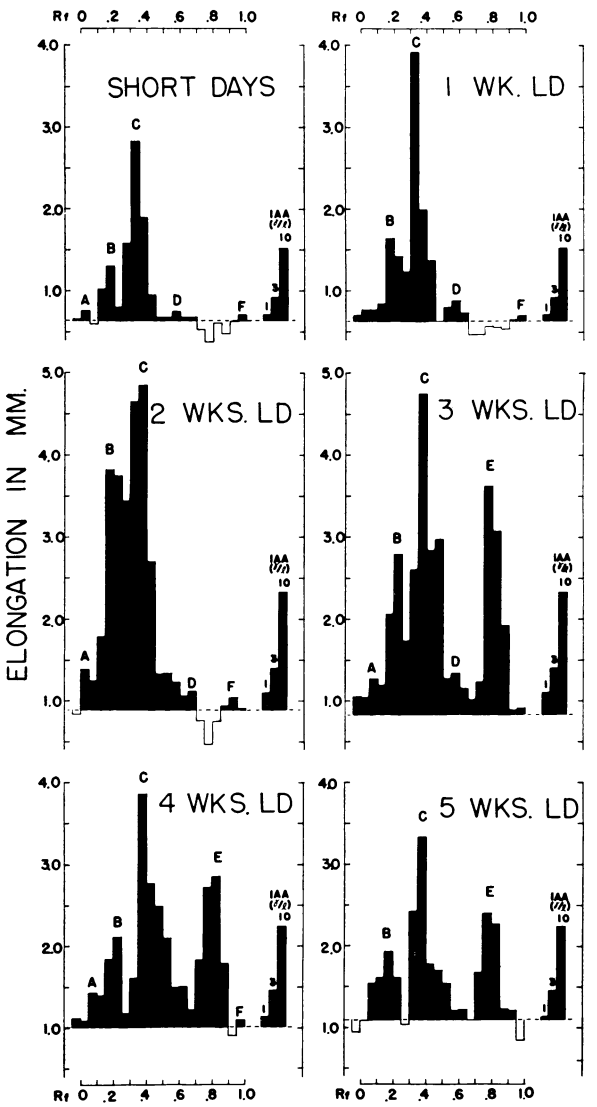

Fig. 1. *Rudbeckia speciosa*. Histograms representing the growth-promoting effect of 1-cm sections cut from paper strips on which the cold methanol extracts of 50 mg (dry weight) of stem tips have been chromatographed in 80 % isopropanol. Ordinates; average elongation of 10 oat 1st-internodes in 24 hours above the initial 4-mm length. Abscissa; R_f. The elongation produced by 1 ml of standard solutions containing 1, 3, 10 μg l of IAA is indicated on the right of each histogram.

As can be seen on the histograms, at least 6 substances promoting the growth of Avena 1st-internodes over the controls in buffer plus sucrose can be distinguished in the crude, cold methanol extracts of the Rudbeckia tips. These substances have been called provisionally A_1, B_1, etc, and have the following R_f values in 80 % isopropanol: A_1 (0.0 to 0.1), B_1 (0.1 to 0.25), C_1 (0.25 to 0.45), D_1 (0.5 to 0.65), E_1 (0.65 to 0.85), and F_1 (0.9 to 1.0). Substances A_1, D_1 and F_1 appeared either to have a low growth-promoting activity or to be present in very small amounts. On the other hand, substances B_1, C_1 and E_1 were major growth substances which promoted a remarkable elongation of the 1st internodes. Substance E_1, for example, extracted from only 50 mg of dried material caused in 20 hours a 3-fold increase in length of the 1st-internode sections.

Preliminary investigations on the nature and properties of substances B_1, C_1 and E_1 have yielded the following results:

Substance B_1 remains in the aqueous phase when shaken with either ether or chloroform at pH 2.8 to 3.0. It is not tryptophane (which has an R_f of 0.3 in 70 % isopropanol, whereas the R_f of B_1 is 0.1 to 0.2 in that solvent). It is active on both the 1st-internode test and the Avena leaf test.

Substance C_1 moves to the IAA position in 80 % isopropanol, but is not IAA because it also remains in the aqueous phase when partitioned between water and chloroform or ether at pH 2.8 to 3.0.

Substance E_1 is neither the nitrile nor the ethyl ester of IAA for its R_f is 0.0 in petroleum ether (boiling point 30 to 60° C) : chloroform : H_2O (75 : 15 : 10 v/v), whereas that of IAN is 0.40 and that of IAE is 0.80 in the same solvent. It is not gibberellin A_1 nor gibberellic acid either (which have an R_f of 0.45 in 80 % isopropanol), although it is active on both the 1st-internode and the Avena leaf tests.

Changes occurring during flowering: When grown under short days, *Rudbeckia speciosa* remains a rosette of leaves without bolting or flowering. At that time, substances B_1 and C_1 are the main growth substances which can be seen on the chromatograms (fig 1).

If the plants are moved to long days of 18 hours, then a physiological process is triggered off which results in bolting and flowering. After 1 week of long days, however, no change can be noticed in the growth rate of the stem of the plant, although the color of the leaves has become a lighter green and their position has changed from horizontal to erect. Yet, the chromatograms of the growth substances extracted from the stem tips already show a 10-fold increase in the level of substance C_1 (fig 2). There is still little change in the growth rate after the 2nd week of long days, but at that time the concentration of substance B_1 also increases to about 10 times the concentration present in the controls remaining under short days (fig 2). The 1st signs of bolting

can be seen around the 3rd week of long-day treatment. At this very moment, a new substance appears on the chromatograms, substance E_1 (fig 1 and 2). From that time on, until anthesis, this remarkable substance has been found in all the extracts prepared from the stem tips. As shown in figure 2, there are 2 periods during which stem elongation is rapid, 1 corresponds to bolting, the other to the growth of the pedicel of the inflorescence. Both periods are preceded by an increase in the levels of substances B_1 and E_1.

A COLD-REQUIRING PLANT, THE SHUOKAN CHRYSANTHEMUM: *Active substances*: In the methanol extracts of the stem tips of Shuokan chrysanthemums, several substances promoting the elongation of oat 1st-internodes could be separated and were tentatively named A_2, B_2, etc. Among these substances, B_2, C_2 and E_2 were most prominent (fig 3). It should be noted that the R_f values of these substances in 80 % isopropanol (B_2 (0.1 to 0.25), C_2 (0.25 to 0.4), E_2 (0.65 to 0.75)) were practically identical with the R_f values of the corresponding substances extracted from Rudbeckia. In addition to this similarity, a partitioning experiment indicated that substance B_2 remained in the aqueous phase when partitioned between water and ether at pH 2.8, as did substance B_1 of Rudbeckia.

Changes occurring during the cold treatment:

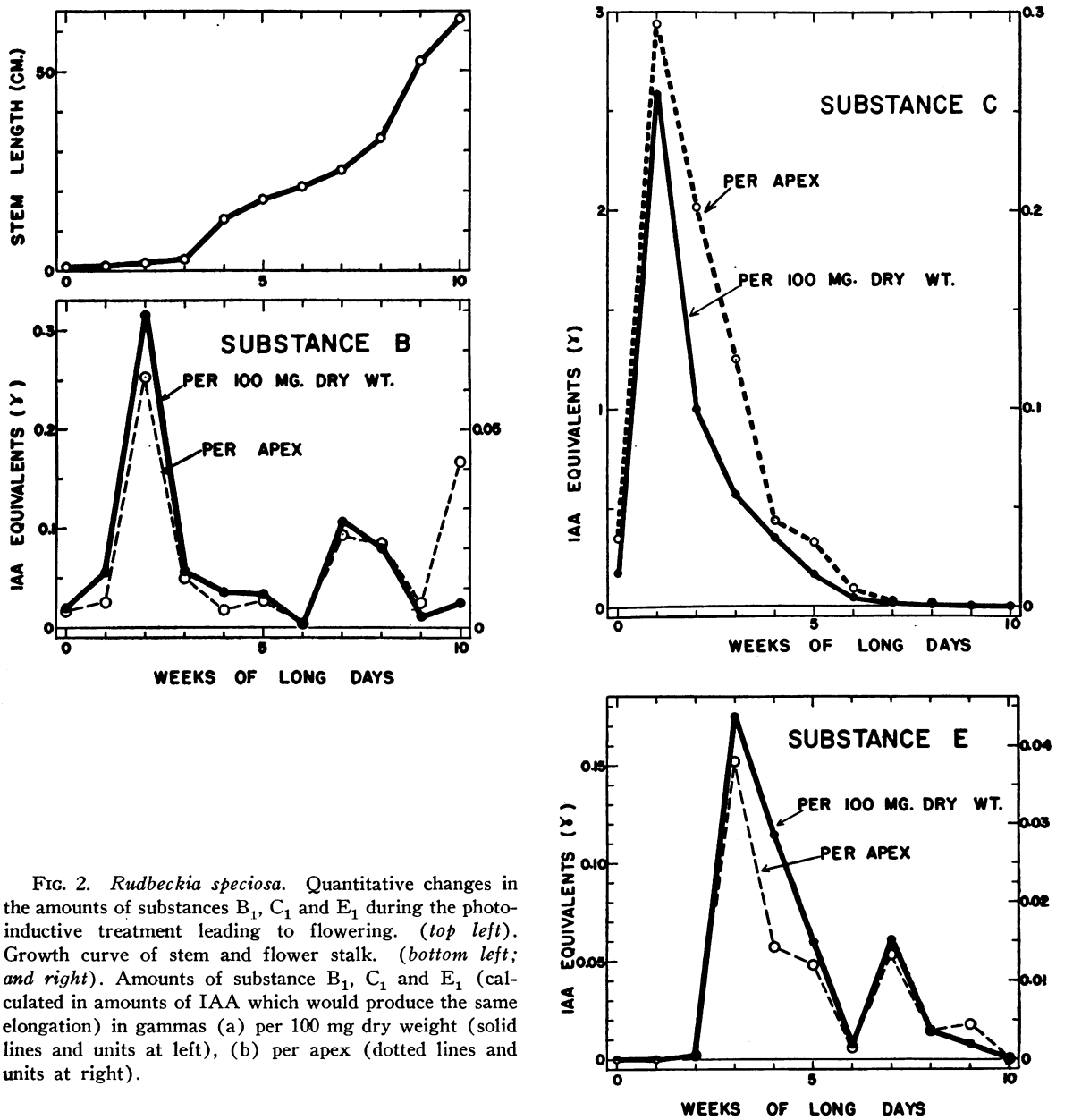

FIG. 2. *Rudbeckia speciosa*. Quantitative changes in the amounts of substances B_1, C_1 and E_1 during the photoinductive treatment leading to flowering. (*top left*). Growth curve of stem and flower stalk. (*bottom left; and right*). Amounts of substance B_1, C_1 and E_1 (calculated in amounts of IAA which would produce the same elongation) in gammas (a) per 100 mg dry weight (solid lines and units at left), (b) per apex (dotted lines and units at right).

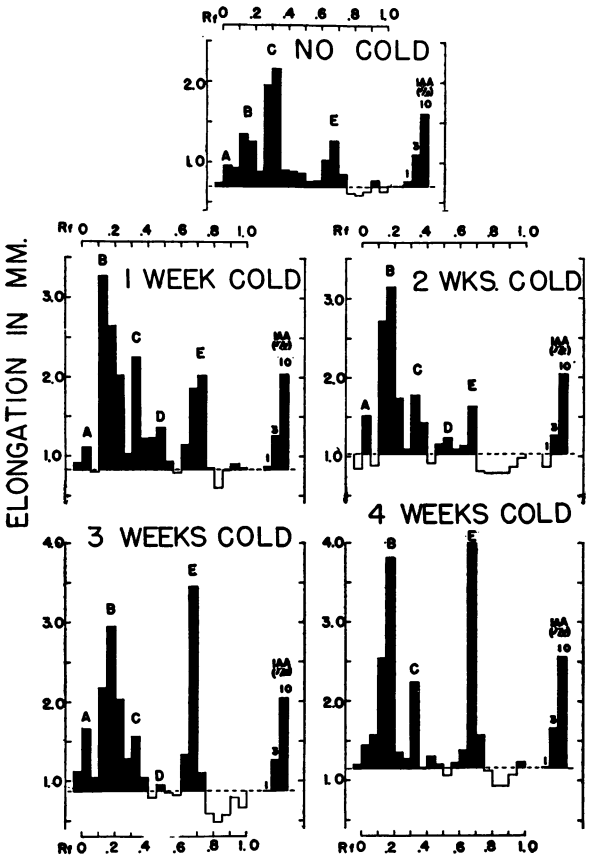

FIG. 3. Chrysanthemum variety Shuokan. Histograms corresponding to the cold methanol extracts of 25 mg (dry weight) of stem tips chromatographed in 80 % isopropanol. Ordinates and abscissa as in figure 1.

The Shuokan chrysanthemums were grown above a 16° C minimum temperature. Under these conditions they remained vegetative and rosetted for a very long time. When extracted in this state, the stem tips were found to contain relatively small amounts of growth substances, the main substance being substance C_2. Low amounts of substances B_2 and E_2 were also present (fig 3). During the vernalization process, the level of substance C_2 decreased, but that of B_2 increased, the concentration of B_2 rising to 10-fold its original level after 1 week of cold treatment (fig 4). On the 3rd week of cold treatment, substance E_2 suddenly rose to 10 times its concentration in the unvernalized controls. The timing of this change in the concentration of substance E_2 is worth noting, for it coincides with the minimum length of time which is necessary to vernalize the Shuokan chrysanthemum.

A SHORT-DAY PLANT, THE SHASTA CHRYSANTHEMUM: *Active substances*: As shown in figure 5, several growth substances were detected in the extracts of the tips of Shasta chrysanthemums. They had the following R_f values: A_3 (0.0 to 0.1), B_3 (0.1 to 0.3), C_3 (0.3 to 0.4), D_3 (0.45 to 0.65) and, perhaps, F_3 (0.9 to 1.0). A toxic substance (which practically killed the 1st-internode sections) moved to the region between R_f 0.6 and 0.9 (in 80 % isopropanol) and prevented the detection of growth-promoting substances in that area. Several techniques were tried in order to remove this inhibitor from the other substances, partitioning between water and ether at various pH values, selective adsorbtion on alumina columns and stepwise extraction of the lyophilized samples. It was found that the toxic substance was a neutral compound which could be extracted with ether from an aqueous solution at pH 8.0. None of the techniques tried, however, gave very satisfactory results, for the pattern of the growth substances changed after purification of the methanolic extract, indicating possible decomposition of the extracted substances.

In view of these results, the various samples of Shasta chrysanthemum were analyzed using the crude methanol extracts, as had been done with Rudbeckia and the Shuokan chrysanthemum.

Changes occurring during flowering: As shown in figure 5, the inhibitor was present at all stages of development, masking the possible presence of substances of the E type which would chromatograph at the same R_f. However, the changes in 2 prominent growth substances which were clearly separated from the inhibitor, namely B_3 and C_3 could be followed. Until the 5th week of short days, the amount of substance C_3 was greater than that of B_3. From the 6th week on, the reverse was true. This change coincided with the rapid development of the flower bud.

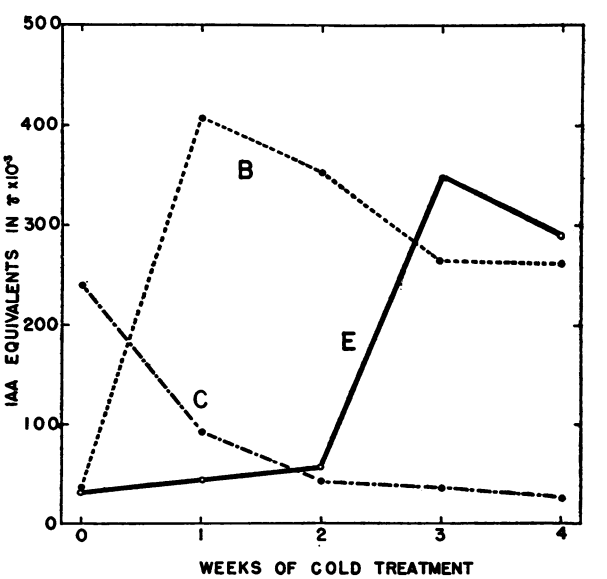

FIG. 4. Chrysanthemum variety Shuokan. Quantitative changes in substances B_2, C_2 and E_2 in stem tips during vernalization. Results expressed in amounts of IAA producing equal elongation of 1st internodes. Values given either per 100 mg dry weight or per apex (each apex happening to weigh about 100 mg dry weight).

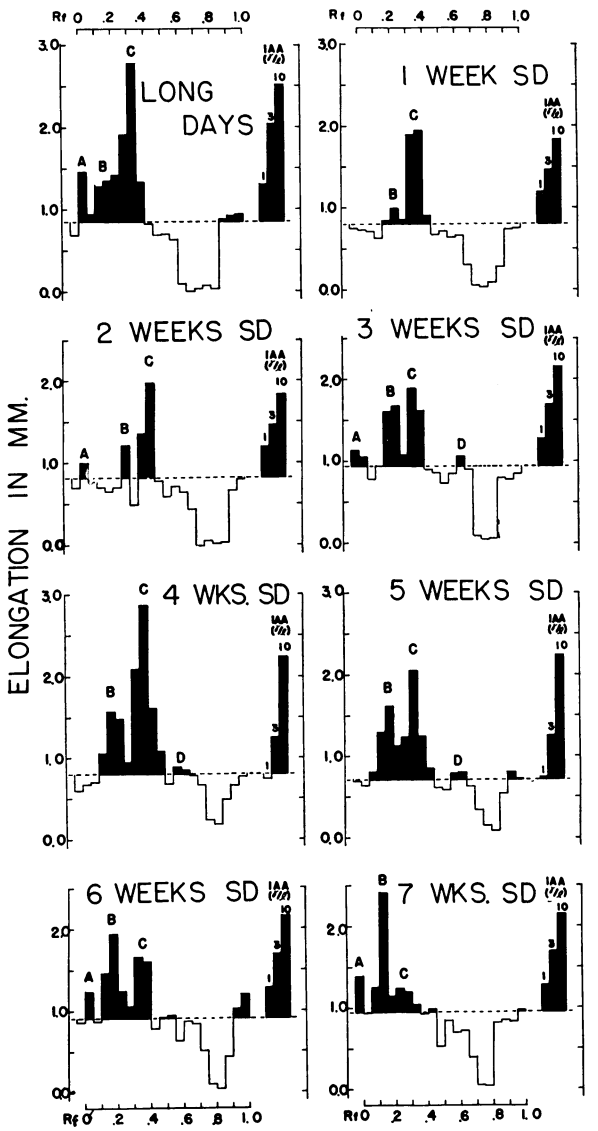

FIG. 5. Shasta chrysanthemum. Histograms corresponding to the cold methanol extracts of 50 mg (dry weight) of stem tips chromatographed in 80% isopropanol. Ordinates and abscissa as in figure 1.

DISCUSSION

The results which have been presented above contribute experimental evidence to the idea that profound hormonal changes occur during flower development. These changes are both of quantitative and qualitative nature. As soon as the inductive treatment is started, the relative importance of the existing growth substances changes. When a plant becomes ready to bolt and flower, at least in the long-day and cold-requiring species studied, a new substance appears in the methanolic extract of the apices. It is to be remarked that the sudden increase in substances E_1 and E_2 precedes the actual bolting phenomenon by about 1 week in Rudbeckia and 2 weeks in the Shuokan chrysanthemum. It is more likely, therefore, that substance E is a cause rather than a consequence of the bolting phenomenon. Another observation seems to confirm the correlation between the presence of substance E and bolting; unvernalized Shuokan chrysanthemums eventually will bolt and flower after a very long time when grown above 16° C; this is a species in which low amounts of substance E exist even before vernalization.

Is substance E a florigen? This intriguing question must await further experimentation to be answered. The crucial point would be to know if substance E occurs in short-day plants once they are induced to flower. Unfortunately, the presence of a substance E in the Shasta chrysanthemum was completely masked by the toxic substance present in the methanolic extracts. At the present time it seems more likely that substance E may be a vernalin for it resembles the substance moving at R_f 0.70 to 0.85 in isopropanol : ammonia : water (80 : 10 : 10, v/v) detected by Fukui et al (4) in vernalized lettuce seed.

In the preceding sentences, "substance E" has been spoken of as if substances E_1 and E_2 were identical. This point has not been proven as yet. However, there seems to be a resemblance between the pattern found in Rudbeckia and that found in the Shuokan chrysanthemum. In both cases, the main growth substances move to similar positions on the chromatograms and their quantitative changes follow the same pattern. The substances C (after an initial peak produced by long days in Rudbeckia) decrease steadily during flower development, the substances B increase then decrease, and substances E appear just before bolting. These resemblances between successive waves of endogenous growth regulators in a long-day and in a cold-requiring plant are reminescent of other resemblances between the flowering process in these 2 types of plants. Among these similarities can be mentioned the induction of flowering by applied GA which produces bolting and flowering in the Shuokan chrysanthemum (10) as well as in *Rudbeckia speciosa* (19), whereas it does not cause flowering in short-day chrysanthemums (2, 12).

SUMMARY

1. Changes in endogenous growth substances occurring during flower induction and development were investigated in 3 types of plants: a long-day plant, *Rudbeckia speciosa*, a cold-requiring plant, the Japanese chrysanthemum variety Shuokan, and a short-day plant, the Shasta chrysanthemum, using improved methods of extraction, chromatography and bioassay.

2. In stem tips of *Rudbeckia speciosa*, 3 major (B_1, C_1, E_1) and 3 minor (A_1, D_1, F_1) substances stimulating the elongation of oat 1st-internodes were separated chromatographically. Substance C_1 reached its peak after 1 week, substance B_1 after 2 weeks of long-day treatment, whereas substance E_1 which was thought to be responsible for bolting, appeared after 3 weeks of long days, just preceding bolting.

3. Three major growth substances (B_2, C_2, E_2) were found in the Shuokan Chrysanthemum. Substance B_2 reached its maximum 1 week after the beginning of the cold treatment, while substance C_2 decreased steadily during the vernalization process. Substance E_2 appeared after 3 weeks of cold treatment, which corresponds to the minimum vernalization period capable of inducing rapid bolting and flowering.

4. In methanolic extracts of the Shasta chrysanthemum, a strong inhibitor, present at all stages of flower development, prevented the detection of growth substances in the area between R_f 0.6 and 0.9 in 80 % isopropanol, where substances of the E type are expected to move. Several other growth-promoting substances (A_3, B_3, C_3, D_3, F_3,) were detected on the chromatograms.

5. Preliminary data on the nature and properties of the active substances B, C and E are given.

6. These findings contribute experimental evidence for the existence of both quantitative and qualitative changes in the pattern of endogenous growth substances during flower induction and flower development.

Grateful acknowledgment is made to Mrs. Colette Nitsch for her invaluable help during the course of these experiments.

This work was supported in part by an assistantship given by the Department of Floriculture and Ornamental Horticulture of Cornell University, by the National Science Foundation (Grant G-4046) and by the National Institutes of Health, Bethesda, Maryland (Grant RG-4840).

Literature Cited

1. Cajlachjan, M. H. Concerning the hormonal nature of plant development. Compt. rend. acad. sci., U.R.S.S. 16: 227–230. 1957.
2. Cathey, M. C. and Stuart, N. W. Growth and flowering of *Chrysanthemum morifolium* Ramat. as affected by time of application of gibberellic acid. Proc. Amer. Soc. Hort. Sci. 71: 547–554. 1958.
3. Cooke, A. R. Changes in free auxin content during the photoinduction of short-day plants. Plant Physiol. 29: 440–444. 1954.
4. Fukui, H. H., Weller, L. E., Wittwer, S. H. and Sell, H. M. Natural growth substances in vernalized and nonvernalized lettuce seedlings. Amer. Jour. Bot. 45: 73–74. 1958.
5. Garner. W. W. and Allard, H. A. Further studies in photoperiodism: The response of the plant to relative length of day and night. Jour. Agr. Res. 23: 871–920. 1923.
6. Gilson, M. Evolution quantitative de l'acide β-indol-acétique chez *Hyosciamus niger*, en rapport avec la sexualisation et la floraison. Acad. roy. Belg., Bull. classe sci., Serie 5, 43: 778–798. 1957.
7. Greulach, V. A. Photoperiodic after-effect in six composites. Bot. Gaz. 103: 698–709. 1942.
8. Hamner, K. C. and Bonner, J. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. 100: 388–431. 1938.
9. Harada, H. Changes in endogenous growth substances during flower development and effects of synthetic substances on flowering. M. S. Thesis, Dept. of Flor. and Ornam. Hort., Cornell Univ., Ithaca 1958.
10. Harada, H. and Nitsch, J. P. Flower induction in Japanese chrysanthemums with gibberellic acid. Science 129: 777–778. 1959.
11. Harada, H. and Nitsch, J. P. Changes in endogenous auxins during flower development. Plant Physiol. 33 Suppl.: iv. 1958.
12. Kohl, H. C. and Kofranek, A. M. Gibberellin on flower crops. Calif. Agric. 11(5): 9. 1957.
13. Konishi, M. Studies on development of flowering stalks in long-day plants in relation to auxin metabolism. Mem. Coll. Agr., Kyoto Univ. 75: 1–70. 1956.
14. Lang, A., Sandoval, J. A. and Bedri, A. Induction of bolting and flowering in Hyoscyamus and Samolus by gibberellin-like material from a seed-plant. Proc. Natl. Acad. Sci., U.S. 43: 960–964. 1957.
15. Melchers, G. Die Blünhormone. Ber. deut. bot. Ges. 57: 29–48. 1939.
16. Murneek, A. E. Length of day and temperature effects in Rudbeckia. Bot. Gaz. 102: 269–279. 1940.
17. Nitsch, J. P. Methods for the investigation of natural auxins and growth inhibitors. In: The Chemistry and Mode of Action of Plant Growth Substances, R. L. Wain and F. Wightman ed. Pp. 3–31. Butterworth Scientific Publications, London 1955.
18. Nitsch, J. P. Changes in endogenous growth—regulating substances during flower initiation. Proc. IVth. Int. Congr. of Biochem., Symp. VI., Vienna 1958.
19. Nitsch, J. P. and Harada, H. Production de fleurs en jours courts par l'alcool furfurylique chez *Rudbeckia speciosa*. Bull. soc. botan. France 105: 319–322. 1958.
20. Nitsch, J. P. and Nitsch, C. The separation of natural plant growth substances by paper chromatography. Beitr. Biol. Pflanz. 31: 387–408. 1955.
21. Nitsch, J. P. and Nitsch, C. Studies on the growth of coleoptile and first internode sections. A new, sensitive, straight-growth test for auxins. Plant Physiol. 31: 94–111. 1956.
22. Nitsch, J. P. and Nitsch, C. Activités comparées de plusieurs gibbérellines sur les tests "coléoptile" et "mésocotyle". Bull. soc. botan. France (In press.)
23. Okada, M. The forcing culture of chrysanthemum. Agr. and Hort. 30: 1597–1601. 1955 (In Japanese.)
24. Okada, M. Classification of chrysanthemum varieties in view of their environmental responses to flowering. Jour. Hort. Assoc. Japan. 26: 59–72. 1957. (In Japanese with English summary.)
25. Resende, F. Auxin and antiauxin, the hormones responsible for the change of the vegetative into floral phenotypes. Bol. soc. Portug. C. Naturais, Series 2a, 2: 174. 1949.
26. Vlitos, A. J. and Meudt, W. The role of auxin in plant flowering. III. Free indole acids in short-day plants grown under photoinductive and non-photoinductive daylengths. Contr. Boyce Thompson Inst. 17: 413–417. 1954.