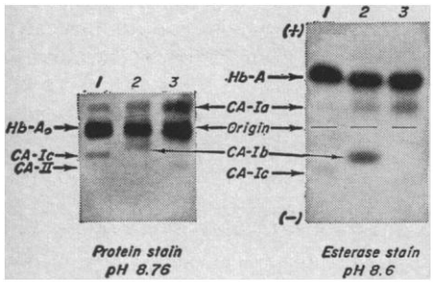

Fig. 1. Starch gel electrophoresis patterns of protein stain and esterase activity stain of normal and variant forms of erythrocyte carbonic anhydrase: *1*, CA-Ic variant; *2*, CA-Ib variant, and *3*, normal pattern. Esterase activity of CA-II not evident in photograph. Electrophoresis for esterase pattern carried out in borate buffer (*p*H 8.6, 0.02*M*). Enzyme activity visualized with Blue RR salt as dye-coupler and β-naphthyl acetate as substrate. In protein stain (nigrosin), electrophoresis carried out at *p*H 8.76 to prevent overlapping of variant forms by Hb-A_2.

ferred to in a later report (*2*) as CA-I and CA-II. Thus, the normal carbonic anhydrase form which migrates anodally at *p*H 8.6 in our starch gel electrophoresis system (*2*) is now designated as CA-Ia (previously Da_1), and the normal cathodally migrating form as CA-II (previously *Db*). A variant of one of these forms, apparently under the control of a single autosomal gene, has been described from a Caucasian family residing in Michigan (*3*) and is now referred to as CA-Ib (previously Da_2). We now describe another variant of CA-Ia, CA-Ic.

The new variant (CA-Ic) was discovered in four "Chamorros" (*5*) during a survey of hemolysate esterase patterns from 490 adult, native inhabitants of Guam, Saipan, and Tinian in the Mariana Islands of western Micronesia. Two of the propositi (one male and one female) were residents of Guam and the other two (one male and one female) were from the island of Saipan. The new variant can be readily distinguished from the CA-Ib variant by both its increased cathodal migration and differential esterase activity (Fig. 1). In hemolysates from individuals with the CA-Ic variant, the esterase activity of the CA-Ic band is moderately increased over that of the normal CA-Ia form; whereas in hemolysates with the CA-Ib variant, the esterase activity of the CA-Ib band is markedly increased over the normal form. The characteristic decrease in protein stain intensity and esterase activity of the CA-Ia band in hemolysates containing the variant forms suggests that CA-Ia, CA-Ib, and CA-Ic are allelic products. As can be seen in the protein stain pattern in Fig. 1, the quantity of CA-II is not noticeably altered in the variant hemolysates.

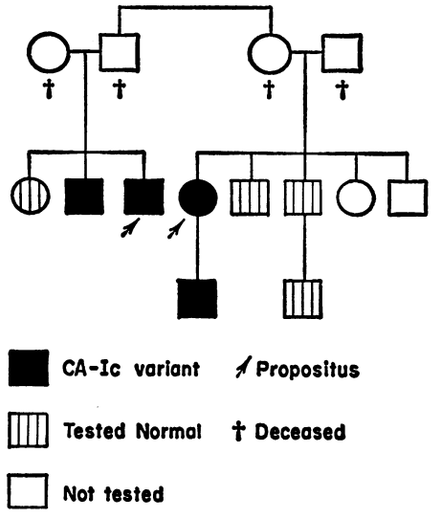

Fig. 2. Pedigree showing segregation of CA-Ic variant.

Preliminary studies of four pedigrees with the CA-Ic trait revealed the following: the first pedigree showed a father to daughter inheritance of the variant (his brother was normal); in the second pedigree three of four female sibs and their paternal uncle possessed the variant (the mother was normal and the father was dead); the third pedigree showed only one female with the trait (her two sons and two maternal half brothers were normal); and in the fourth pedigree, which is shown in Fig. 2, the male and female propositi were found to be first cousins and it appears that the variant was inherited through their respective father and mother. After correcting for ascertainment, the ratio of variant to normal in all segregating sibships tested was 7 : 7. These findings are consistent with single autosomal gene control of the CA-Ic variant.

The starch gel electrophoresis and dye-coupling procedures used in this study have been described elsewhere (*2*). Carbonic anhydrase activity of the electrophoretically separated forms on starch gel was demonstrated by the use of a modification of the Kurata histochemical technique (*6*). Both the dehydrase activity (Kurata stain) and the esterase activity (coupling azo dye stain) of the carbonic anhydrase forms were selectively inhibited when acetazolamide ($10^{-5}M$) was present in the incubation mixtures. In addition, colorimetric assays for hydrase activity (*7*) and esterase activity, and their inhibition by acetazolamide, have been demonstrated on human erythrocyte carbonic anhydrase-Ia after purification on diethylaminoethyl cellulose (*4; 8*).

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References and Notes

1. P. O. Nyman, *Biochim. Biophys. Acta* **52**, 1 (1961); E. E. Rickli and J. T. Edsall, *J. Biol. Chem.* **237**, PC258 (1962).
2. R. E. Tashian and M. W. Shaw, *Am. J. Human Genet.* **14**, 295 (1962).
3. C. R. Shaw, F. N. Syner, R. E. Tashian, *Science* **138**, 31 (1962).
4. R. E. Tashian and D. D. Douglas, in preparation.
5. The "Chamorros" and "Carolinians" are the principal Micronesian people populating the Mariana Islands. The survey included 305 "Chamorros" and 147 "Carolinians" as well as 38 individuals of "Chamorro-Carolinian" admixture. Propositi were classified as "Chamorro" or "Carolinian" if they indicated that all four grandparents belonged to the same ethnic group.
6. Y. Kurata, *Stain Technol.* **28**, 231 (1953).
7. F. J. W. Roughton and V. H. Booth, *Biochem. J.* **40**, 309 (1946).
8. Supported in part by research grant 9252 from the U.S. Public Health Service. We thank Drs. David Dunn, Jose Torres, and Manuel Cruz for collecting the blood specimens from Saipan, and Drs. Charles R. Shaw and Zwi Hart for the blood sample of the CA-Ib variant.

14 November 1962

Floral Induction and the Stimulation of Cell Division in Xanthium

Abstract. *The mitotic index in the apical region of the stem of* Xanthium *seedlings doubled within the 24 hours immediately following a single inductive dark period and an increase occurred within 16 hours in one case. The significance of this finding in relation to the onset of reproductive development is discussed.*

Vegetative plants of the short-day species *Xanthium pennsylvanicum* can be induced to flower by exposure of their leaves to a single long dark period (*1*). The flowering stimulus so formed begins to move into the stem

from the leaves about 20 to 22 hours after the start of the inductive dark period (*2*), and a further two to three days are reported to elapse before the appearance of the morphological signs of the transition of the stem apex from a vegetative to a reproductive state (*3–5*). However, one of the earliest indications of the transition to the reproductive state is the spread of mitotic activity throughout the stem apex (*4*), and this stage presumably precedes the appearance of the first morphological changes at the apex. An investigation was therefore undertaken to determine how soon after the start of inductive treatment a change in the mitotic index could be detected.

Xanthium seedlings were grown for approximately 14 days in continuous light (natural daylight supplemented at night with artificial light at an intensity of about 500 ft-ca from 200-watt incandescent lamps) in a greenhouse maintained at 20° to 26°C. They were then transferred to growth cabinets in which they received continuous light at an intensity of about 3500 ft-ca from a combination of 250-watt incandescent lamps and 80-watt "daylight" type fluorescent tubes. The plants remained within these cabinets at a temperature of 21° ± 1°C throughout the experimental treatments. After growing for 15 to 20 days under these nearly constant conditions, by the end of which time any rhythms of cell division induced by the more variable greenhouse environment would probably have been eliminated, plants were divided into three groups to receive the following treatments: (i) continuous light, (ii) one 16-hour dark period, and (iii) one 16-hour dark period interrupted at its midpoint by a 75-minute high-intensity light break. Plants in groups (ii) and (iii) were returned to continuous light after receiving their dark treatment. As expected, the plants in group (ii) flowered, whereas those in groups (i) and (iii) remained vegetative.

The effect of induction to flower on the number of dividing cells in the apical region of the stem was assessed by examining apexes at various times in relation to the start of the dark period. Apexes from six plants were harvested from each group at each sampling time. Apical segments consisting of the meristematic dome together with the youngest detectable leaf primordium were excised under a dissecting microscope, macerated in 0.1*N* hydrochloric acid for 3 minutes, and squashed in acetic orcein on a microscope slide. The segments were then examined at a magnification of 400× and the mitotic state of each of their 2000 to 5000 cells recorded. The data obtained for all stages of the mitotic cycle showed similar results, but, as metaphase was detected most readily, only the data for this stage are presented in the figures.

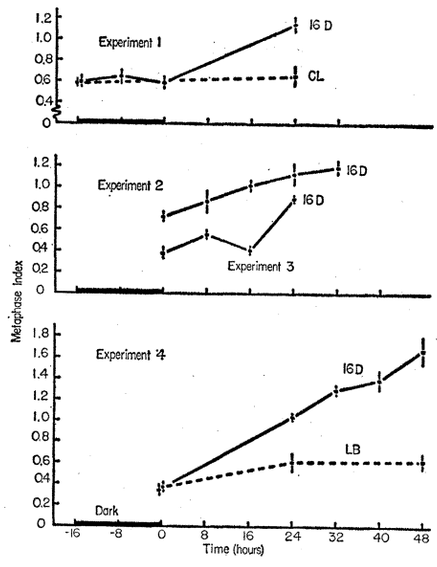

Fig. 1. Effect of darkness on the metaphase index at the stem apex. Plants were exposed to one 16-hour dark period (16D), one 16-hour dark period interrupted by a light break (LB), or to continuous light (CL). Darkness was given from time −16 to 0 hours. Vertical bars indicate ± standard error.

The results of the four experiments presented lead to the following conclusions.

1) No change in the metaphase index (100 × No. of cells at metaphase / total No. of cells) was detectable during the 16-hour dark period (experiment 1).

2) A highly significant increase in the metaphase index occurred 24 hours after the end of the 16-hour dark period in all experiments, and in experiment 2 there was a significant increase after only 16 hours. On the average, the metaphase index increased from 0.505 at the end of darkness to 1.05 24 hours later and 1.68 48 hours after the end of the dark period.

3) The effect of a 16-hour dark period on the metaphase index was prevented by the interruption of the dark period by a light break (experiment 4). The increase in metaphase index thus seems not to be dependent on the total number of hours of darkness received per se, but it appears instead to be closely correlated with the induction of flowering.

4) Assuming, as previously reported (*2*), that the floral stimulus did not start to move out of the leaves to the apex until at least 20 hours after the dark treatment began, an increase in metaphase index was detected as early as 12 hours later.

The increase in the metaphase index reported here is the earliest recorded sign of the transition of the apical region from a vegetative to a reproductive condition. Although this increase does not necessarily imply an increase in the rate of cell division (*6*), it is known that increased meristematic activity in the apical region of the stem accompanies the transition to flowering in many plants (*7*). It thus seems reasonable to interpret the present results as indicating a very early stimulation of cell division at the apex in response to floral induction in *Xanthium*. Such a relationship between floral induction and the early stimulation of cell division is possibly widespread. In both the short-day plants *Chenopodium album* (*8*) and *Pharbitis nil* (*9*) available evidence indicates that the number of cells undergoing mitosis at the stem apex certainly increases 2 days after the start of inductive treatment. In addition, the observations that the initiation of axillary-bud primordia is markedly stimulated at induction in several other short- and long-day plants (*7*, *10*) suggest that an increased cell division rate in apical and/or axillary meristems might occur quite commonly at a very early stage in the transition to the reproductive phase.

These results can now be considered in relation to the role of nucleic acids in the transition to the reproductive phase. In the plants studied so far, it is apparent that the cells of the apical meristem cannot perceive and/or fix the floral stimulus unless they are actively synthesizing DNA (*11*) or RNA (*9*). Furthermore, the stimulus having been fixed, it now appears that one of its primary actions results in the acceleration of mitosis in the apex, which in itself involves an increase in the rate of DNA synthesis. This situation could be explained by supposing that the rate of DNA synthesis is controlled in the vegetative apex by a regulatory antagonist or inhibitor (*7*), and that the rate

of cell division is limited by the rate of DNA synthesis. The action of the floral stimulus might then be to promote DNA synthesis without concomitantly promoting the synthesis of antagonist. Such an upset in the normal balance of DNA:regulator could then lead to the continued more rapid DNA synthesis and cell division which is characteristic of the early stages of reproductive development.

The experiments reported here represent part of a more comprehensive study, now in progress, to elucidate the effects of light and dark on cell division in the stem apex of *Xanthium* and other plants in immediate response to photoperiodic induction (*12*).

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References and Notes

1. K. C. Hamner and J. Bonner, *Botan. Gaz.* **100**, 388 (1938).
2. J. Skok and N. J. Scully, *ibid.* **116**, 142 (1954).
3. F. L. Naylor, *ibid.* **103**, 146 (1942).
4. A. Lance, *Ann. Sci. Nat. Botan., II sér.* (1957), p. 91.
5. R. H. Wetmore, E. M. Gifford, M. C. Green, in *Photoperiodism and Related Phenomena in Plants and Animals* (AAAS, Washington, D.C., 1959), p. 255.
6. R. Brown, *J. Exptl. Botany* **2**, 96 (1952).
7. R. G. Thomas, *Ann. Botany London* **25**, 138 (1961).
8. E. M. Gifford and H. B. Tepper, *Am. J. Botany* **48**, 657 (1961).
9. J. A. D. Zeevaart, *Plant Physiol.* **37**, 296 (1962).
10. R. G. Thomas, *Ann. Botany* **26**, 329 (1962).
11. J. Bonner and J. A. D. Zeevaart, *Plant Physiol.* **37**, 43 (1962).
12. I thank Dr. J. Bonner, California Institute of Technology, Pasadena, for providing the *Xanthium* seeds from which the experimental plants originated, and Miss E. Hunt for laboratory assistance.

4 February 1963

Retention in Immediate Memory Estimated without Retrieval

Abstract. *Report of the missing member of a set depends upon retention of the other members presented. Such a missing scan reveals greater retention than does a digit span and, unlike the digit span, better retention of later than earlier presentations.*

Studies of short-term memory have classically depended upon retrieval by recall or recognition methods (*1, 2*). Recently developed methods requiring only partial retrieval from visual short-term memory have shown greater retention than indicated by methods which require complete retrieval (*3*). Investigation of both retention and retrieval would be further advanced by methods permitting study of retention unconstrained by the effects of retrieval. The "missing scan" described in this report provides such a method, affording a greater estimate of retention without retrieval than may be obtained by retrieval.

Retention in immediate memory may be estimated independently of retrieval by requiring subjects to report which member of a set was *not* presented. This does not necessitate either recall or recognition but does presuppose that subjects know the members of the class. The use of overlearned sequences (*4*) provides an apt illustration. If a subject is told that he will be presented with nine randomly ordered numbers of the set 1 to 10 and asked to report the missing number, he must retain the numbers presented to decide correctly which number of the set was missing. Such a "missing scan" may be contrasted with a retrieval method such as the digit span, which requires that the subject recall those numbers which were presented. Both the missing scan and the modified digit span, as tested in this study, involve transmission of the same amount of information, since both specify the same state of the same class of alternatives, although by messages of different lengths.

The subjects of this experiment were ten males without clinically demonstrable mental deficit who were patients on the Neurology Service of the Palo Alto Veterans Hospital. Their ages ranged from 33 to 67, with a mean of 44.8 years. All subjects were tested for both modified digit span and missing scan, half of the subjects taking the former first, the rest taking the latter first. Each test session lasted about 50 minutes.

The test items were the numbers from one to sixteen. Series containing 4, 6, 8, 10, 12, or 14 randomly ordered numbers were prepared so that in the 15 series for each number of items the missing number was randomly 1 through 15. This was achieved by using appropriate segments of the sequence one to sixteen as the classes from which one number was missing. The missing number was never a limiting number of the class. Before *both* digit-span and missing-scan tests the subjects were told the class or sequential series of numbers to which the numbers presented belonged.

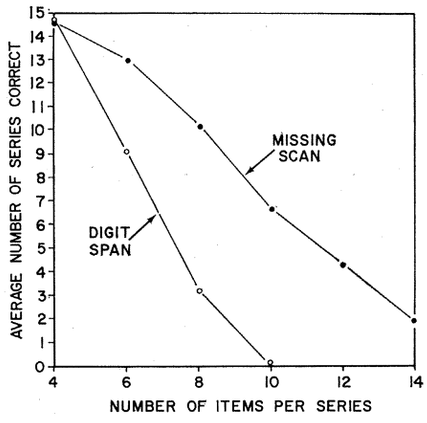

Fig. 1. Average digit span and missing scan of ten subjects.

Subjects were tested individually, the examiner reading the numbers at a rate of one per second. In the missing-scan test subjects were instructed to report the number missing from the series presented. In the modified digit-span test the subjects were instructed to report all of the numbers presented, *without regard to order of presentation.* The usual digit-span test was modified in this manner so that subjects would *not* have to retain the order of presentation as well as the numbers presented. These modifications were designed not only to make the two tests as comparable as possible but also to maximize the estimate of retention obtained with retrieval by the digit span, since one aim of this study was to demonstrate that the estimate of retention obtained without retrieval by the missing scan is greater than the estimate of retention obtained with retrieval by the digit span. Because the same series of numbers were presented in both tests, the only difference between them was in the

Table 1. Comparison of digit span (DS) and missing scan (MS).

Subject	Items retained		Errors in first half of list (%)	
	DS*	MS*	DS	MS
1	7	10	45	80
2	5	8	32	75
3	5	9	10	79
4	4	6	13	73
5	6	7	24	82
6	6	9	28	65
7	6	8	43	87
8	6	9	42	90
9	5	10	34	75
10	5	10	23	84
Mean	5.5	8.6	29	79

*Determined by the method of Woodworth and Schlosberg (*1*).