Effects of 5-Fluorouracil on Photoperiodic Induction and Nucleic Acid Metabolism of Xanthium[1, 2]

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Salisbury and Bonner (13) have shown that the pyrimidine, 5-fluorouracil (5-FU), when applied either to the apical vegetative bud or the leaf just prior to the photoinductive dark period inhibits the development of the floral primordia. The inhibitory effect of 5-FU could be reversed by applying orotic acid at the same time as the application of 5-FU. Later, Bonner and Zeevaart (2) showed that 5-FU inhibited the incorporation of orotic acid into both RNA and DNA. In general, DNA synthesis was inhibited more than RNA synthesis. They concluded that RNA synthesis is the process essential to photoperiodic induction which is inhibited by the presence of 5-FU in the bud of *Xanthium* during an otherwise inductive dark period. However, Zeevaart (14) later showed with *Pharbitis* that 5-FU and 5-fluorodeoxyuridine (5-FDU) inhibited flowering by causing a deficiency of thymidylic acid which resulted in the suppression of DNA multiplication. The inhibition of DNA synthesis caused by 5-FDU also profoundly inhibited cell division as judged by microscopic examination of sections for mitotic figures. Zeevaart finally concluded that the floral stimulus can express itself in the initiation of floral primordia only in an apex with multiplying DNA. In these experiments (2, 13) involving *Xanthium*, the effect of the fluorinated pyrimidines on the development of the floral primordia was determined by classifying the apical buds 9 days after photoinduction into various floral stages according to the method of Salisbury (12). Using this method alone, it is possible that the development of the floral primordia might be suppressed when examined 9 days after photoinduction by a single period of 16 hours of darkness, but if the plants had been left in long days for a much longer time they might overcome the inhibitory action of 5-FU and produce reproductive buds. However, Zeevaart (14) overcame this objection with *Pharbitis* by counting the number of floral buds.

From studies with both *Xanthium* (2, 13) and *Pharbitis* (14), it has been concluded that 5-FU prevents the apex of otherwise adequately photoinduced plants to respond to the floral stimulus. Under these conditions, a minimum of 1 photoperiodic induction period is given to the plants. One period of 16 hours of darkness is sufficient to induce flowering but the floral stimulus from only 1 period is weak, and it requires several days to detect the floral primordia as compared to plants given 2 or more photoperiodic cycles. Thus, if anything is done to the plants at the time of photoinduction which would drastically upset the metabolic balance of the plants, especially inhibiting cell division, a reduction in the rate of development of the floral primordia might be expected.

In this paper, it will be shown that 5-FU retards but does not completely inhibit flowering of *Xanthium*. Inhibition of the development of the floral primordia and subsequent production of *Xanthium* seeds was obtained only when 10^{-2} M 5-FU was applied to the apex of plants at the beginning of a single photoperiodic cycle of 16 hours of darkness. If plants were given 2 or 3 photoperiodic cycles and treated with 5-FU (either 10^{-3} M or 10^{-2} M) at the beginning of each cycle the development of the floral primordia was retarded because of the inhibition of nucleic acid synthesis but the plants eventually produced seeds. It will also be shown that 5-FU profoundly inhibits DNA and ribosomal RNA synthesis, but does not greatly inhibit messenger RNA synthesis.

Materials and Methods

Plant Material. The cocklebur (*Xanthium pensylvanicum Wallr.*) plants used were of a standard inbred strain originally obtained from Dr. Harry Borthwick of Beltsville. The seeds were washed in running tap water for 3 days and then were germinated in moist vermiculite. The seedlings were transplanted and were grown in a controlled environment greenhouse. The day length was maintained at 18 hours with supplementary light and the temperature kept at 23° during the day and 17° at night. The plants were grown in this environment for approximately 6 weeks. At the beginning of each experiment, the plants were defoliated except for a single leaf which was usually the third leaf from the apex (approx. 7 cm long). The plants were randomly selected and placed in either of 2 growth chambers. One growth chamber was programmed for 8 hours of light and 16 hours of darkness (short day) while the second was set for 16 hours of light and 8 hours of darkness (long day). In both chambers, the temperature was maintained at 27° during the light period and 18° during the dark period. These conditions were adequate to control photoperiodic induction.

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In experiments dealing with the influence of 5-FU on the photoinduction process, the chemical (either 10^{-3} M or 10^{-2} M) in 0.1 % Tween-20 was applied by dipping the apex in the solution. The 5-FU was applied immediately at the beginning of each dark period. After the appropriate number of cycles (1, 2 or 3 depending on the experiment), the plants were returned to the greenhouse and kept in an environment of 18-hour days at a temperature of 23° during the day and 17° at night until used. Two weeks after the first induction period the apical buds from 6 plants of each treatment were removed and the floral stage classified according to Salisbury (11). An equal group of the plants was kept under long days for approximately 2 additional months in order to determine the final effect of 5-FU on flowering.

Labeling the Apex with an Isotope. The apex of *Xanthium* plants was labeled isotopically in 2 ways, either intact or excised. In experiments where both 5-FU and an isotope were applied to intact buds, the plants were defoliated and readied for photoinduction as described above. The apical buds were dipped in a solution of 0.1 % Tween-20 containing either 0, 10^{-3} M or 10^{-2} M 5-FU, approximately 1 hour prior to the dark period. After the buds dried, and just prior to the dark period, one drop of an aqueous solution containing 50 μc $Na_2HP^{32}O_4$, pH 7 was placed on the apex. In other experiments designed to determine whether nucleic acid synthesis was different between induced and noninduced plants, the buds were excised immediately after the dark period and labeled with P^{32} in solution. Approximately 3 g of bud tissue was incubated in a solution containing 10^{-4} M citric acid, pH 6.0 with NH_4OH, 1 % sucrose, 10 μg/ml streptomycin and 0.5 mc $Na_2HP^{32}O_4$ for 2 hours at 30°. Still, in other experiments to determine the effect of 5-FU on nucleic acid synthesis, only vegetative buds were used. They were treated with 5-FU, either intact or excised, with P^{32} being applied either to intact buds shortly after 5-FU application or to excised buds in solution. The details are given in table II.

Nucleic Acid Extraction and Fractionation. Nucleic acids were extracted from *Xanthium* buds with a phenol method employing dupanol. The isotopically labeled buds (3 g) were homogenized with a VirTis homogenizer in a solution containing 10 ml 0.01 M Tris-HCl, pH 7.6, 0.06 M KCl and 0.01 M $MgCl_2$; 1 ml bentonite (40 mg); 3.1 ml of 11 % dupanol (sodium lauryl sulfate) and 17 ml of cold phenol. The aqueous solution was removed and treated twice with equal volumes of cold phenol in the presence of bentonite, and nucleic acids were precipitated by the addition of 2 volumes of cold ethanol. The extracted nucleic acids were dialyzed for 2 days against 0.05 M phosphate buffer, pH 6.7. All extraction procedures were carried out at about 2°. This technique was found to be satisfactory for the extraction of nucleic acids from plant tissue (4). Dialyzed samples of nucleic acid labeled with P^{32} were separated on columns of methylated albumin coated on kieselguhr according to the method of Mandell and Hershey (11). Two mg of nucleic acid were added to the column and eluted with a linear gradient of NaCl from 0.4 M to 1.2 M in 0.05 M phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected; the ultraviolet absorbancy and radioactivity were determined on each fraction.

Results

Influence of 5-FU on the Development of Floral Primordia. In fairly close agreement with Salisbury and Bonner (13), data summarized in table I show that application of 5-FU to the apices of *Xanthium* plants prior to photoinduction inhibits the development of the floral primordia. Very strong inhibition is observed if the plants are given only 1 inductive cycle. If, however, 2 or 3 cycles are given with 5-FU applied at the beginning of each cycle the development of the floral primordia is indeed inhibited, but nevertheless the apex has received the floral stimulus. Thus, it is apparent that 5-FU is not a specific inhibitor of floral induction as previously inferred (2). As shown in figure 1, 10^{-3} M 5-FU applied at the beginning of the dark period does not destroy the ability of the plant to develop floral primordia (fig 1 A) and produce seed (fig 1 C). If *Xanthium* plants are given only a single photoinductive dark period, 10^{-2} M 5-FU prevents the development of the floral primordia and production of seed. Apices of these plants are not typical vegetative buds. The growth of the apical bud is diminished and after several weeks after photoinduction it closely resembles floral primordia in an early stage. This strong inhibitory effect, as shown by Zeevaart (14), is the result of the inhibition of DNA multiplication and thus cell division. It is, therefore, concluded from these data (table I and fig 1) and those of

Table I. *Effect of 5-FU on the Development the Floral Primordia*

5-FU was applied to the plants by dipping the buds in a solution containing the 5-FU and 0.1 % Tween-20. The chemical was applied to the bud at the beginning of each dark period. Two weeks after the first induction period the buds were removed and classified according to the development of the floral primordia (12). The values are averages from 6 or more plants.

No. of photoperiodic cycles (16 hr darkness)	Stage of bud development: Conc of 5-FU applied to buds: 0	10^{-3} M	10^{-2} M
0	0	...	...
1	3.3	2.3	0.3
2	7.3	7.0	4.0
3	6.5	7.8	5.0

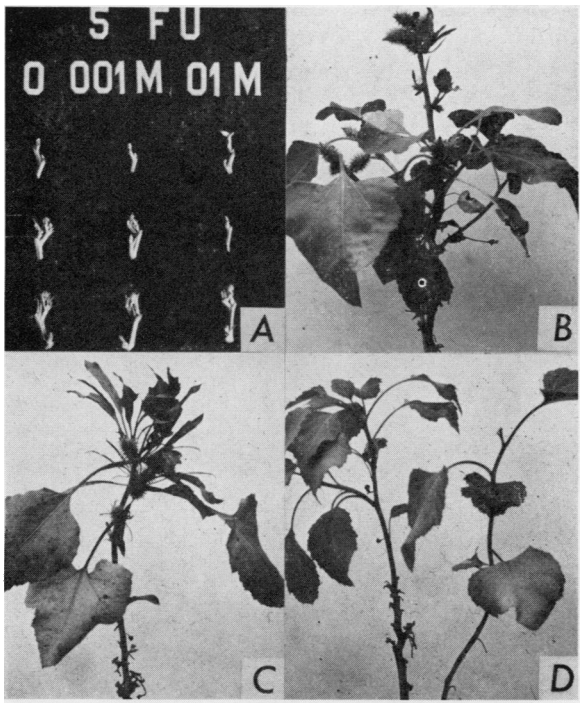

FIG. 1. Effect of 5-FU during the photoinductive dark period on the development of floral primordia of *Xanthium* plants. Figure 1 A shows the development of buds approximately 2 months after photoperiodic induction and treatment with 5-FU. The buds shown in the top row of figure 1 A are from plants receiving 1 dark cycle while the buds in row 2 and 3 were from plants receiving 2 and 3 inductive dark cycles, respectively. In each case, 5-FU was applied at the beginning of each cycle. Figures 1 B, 1 C, and 1 D represent plants treated with 0, 10^{-3} M and 10^{-2} M 5-FU during a single photoperiodic dark period (16 hr) approximately 3 and one-half months prior to the time the photographs were made.

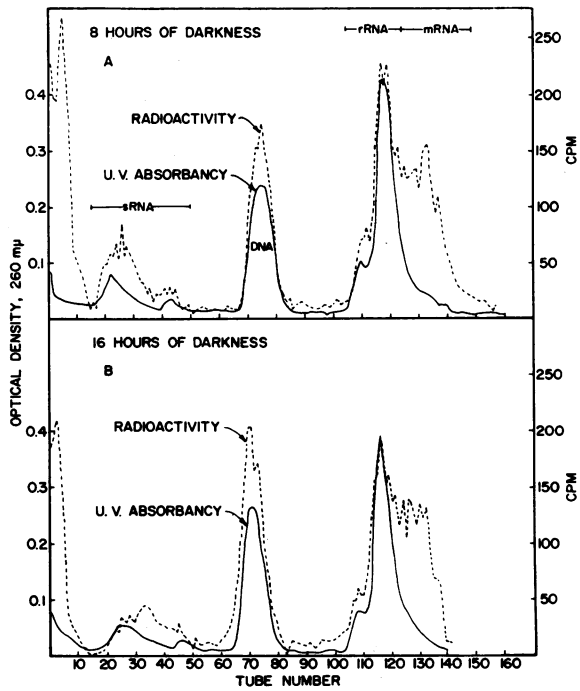

FIG. 2. Nucleic acids obtained from buds of plants not induced to flower (2 A) and from plants induced to flower by two 16-hour dark periods (2 B) by fractionation on MAK columns. After the second dark period the buds were labeled in solution with P^{32} for 3 hours. The nucleic acids were extracted by cold phenol and then dialyzed. The purified RNA was fractionated on MAK columns. Samples containing 5 ml each were collected and the UV absorbancy and radioactivity determined.

other investigators (2, 13, 14) that 5-FU is not a specific inhibitor of floral development in *Xanthium* plants. Rather, it appears that the inhibition of the fluorinated pyrimidine is a function of its action on DNA multiplication which results in a reduction in cell division and subsequent growth.

Comparison of Nucleic Acid Metabolism in Induced and Noninduced Buds. In the initial studies, it seemed desirable to determine whether there is a difference in the nucleic acids produced by noninduced and induced *Xanthium* buds. To approach this question, the nucleic acids were extracted from noninduced and induced (2 photoinductive cycles) excised buds which had been labeled with P^{32} in solution and subsequently fractionated on MAK columns. As can be observed in figure 2, nucleic acids from *Xanthium* are fractionated into 6 fractions by the MAK column similar to that previously shown for other plant tissue (3, 4). Since the radioactivity profiles of the soluble RNAs do not always coincide well with the UV absorbancy peaks, no distinction is made between the 2 general sRNA peaks as has been made previously (4). Likewise, the light ribosomal and heavy ribosomal RNAs are grouped together. The characterization of each of these fractions of nucleic acids has been described previously for peanut cotyledon tissue (4). The RNA fraction eluted near the tail of ribosomal (r)RNA is referred to as mRNA (messenger). Classification of this fraction as mRNA is perhaps debatable. However, since the base composition of the mRNA fraction more nearly resembles DNA (10) and also since our preliminary work [3] shows that this fraction hybridizes with homologous DNA to a much greater extent (at least 2-fold greater) than does soluble or ribosomal RNAs, the authors feel that this fraction contains most of the so-called messenger RNA. While the MAK column technique does not resolve the various fractions of nucleic acids as well as is desired, it appears to be the best method presently available for nucleic acid fractionation. Using this method, we compared

[3] Unpublished data of R. van Huystee and J. H. Cherry.

the nucleic acids extracted from noninduced (fig 2 A) and induced (fig 2 B) *Xanthium* buds which had been excised and labeled in solution with P^{32}. From a comparison of the specific activities and the amount of radioactivity in each fraction, it is not possible to distinguish adequately between the 2 samples of nucleic acid. For example, there is 28 % of the total radioactivity found in mRNA of the noninduced buds while 29 % mRNA is found in induced buds. It will be shown later in this paper that the amount of mRNA can be manipulated, depending on the technique of labeling the tissue with an isotope or by treating with 5-FU. Therefore, it is felt that this technique using P^{32} labeled nucleic acid, is not adequate to quantitatively determine differences in nucleic acid synthesis, especially mRNA in the *Xanthium* buds. To get a qualitative estimate of any difference in mRNA production between noninduced and induced buds, the double labeling technique has been employed.[4]

Effect of 5-FU on Nucleic Acid Metabolism in Xanthium Buds. Since 5-FU impairs the development of the floral primordia in *Xanthium* buds (13), and since 5-FU inhibits both DNA and RNA synthesis, as shown by Bonner and Zeevaart (2), it seemed desirable to determine whether 5-FU affects each of the various fractions of RNAs and DNA in the same way. Initially, in order to estimate the effect of 5-FU on vegetative (noninduced) buds, the apices were dipped in 5-FU and subsequently labeled with P^{32} as either intact or excised buds. As shown in table II, 10^{-3} M 5-FU when applied to intact buds, does not inhibit the incorporation of P^{32} into phenol extractable nucleic acid, regardless of whether the P^{32} was applied to either intact buds or to excised buds. However, 10^{-2} M 5-FU inhibited the incorporation of P^{32} into nucleic acids by 44 %, if the P^{32} was applied to intact buds, but only by 16 % if the 5-FU treated buds were incubated in solution with P^{32}. Surprisingly, if control buds are preincubated with 10^{-2} M 5-FU for 1 hour prior to the 3-hour incubation period with 10^{-2} M 5-FU and P^{32}, the nucleic acids incorporate 49 % more P^{32} than the control tissue.

In order, therefore, to determine the effects of 5-FU on nucleic acid synthesis in vegetative *Xanthium* buds, nucleic acids were extracted from buds labeled in the manner given in table II, and then fractionated on MAK columns. The elution profiles on MAK columns of the nucleic acids extracted from buds in which the 5-FU and P^{32} was applied to intact buds is shown in figure 3. An examination of the elution profiles indicates that application of 10^{-3} M 5-FU to the bud has little effect on nucleic acid metabolism. However, 10^{-2} M 5-FU greatly inhibited DNA and rRNA with little noticeable change in sRNA and mRNA. These observations are illustrated in table III. While 10^{-3} M 5-FU slightly enhanced the incorporation of P^{32} into sRNA and mRNA, 10^{-2} M 5-FU inhibited DNA and rRNA syn-

Table II. *Effect of 5-FU on the Metabolism of Nucleic Acids of* Xanthium *Buds*

Intact buds were labeled with P^{32} for 16 hours while the excised buds were incubated with P^{32} in solution for 3 hours.

Method of application of 5-FU	Method of application of P^{32}	Specific activity of total nucleic acid (cpm/μg)
Control-Intact	Intact	138
10^{-3} M Intact	Intact	140
10^{-2} M Intact	Intact	77
Control-Intact	Excised	55
10^{-3} M Intact	Excised	54
10^{-2} M Intact	Excised	46
Preincubate excised for 1 hr in 10^{-2} M	Excised with 10^{-2} M 5-FU	82

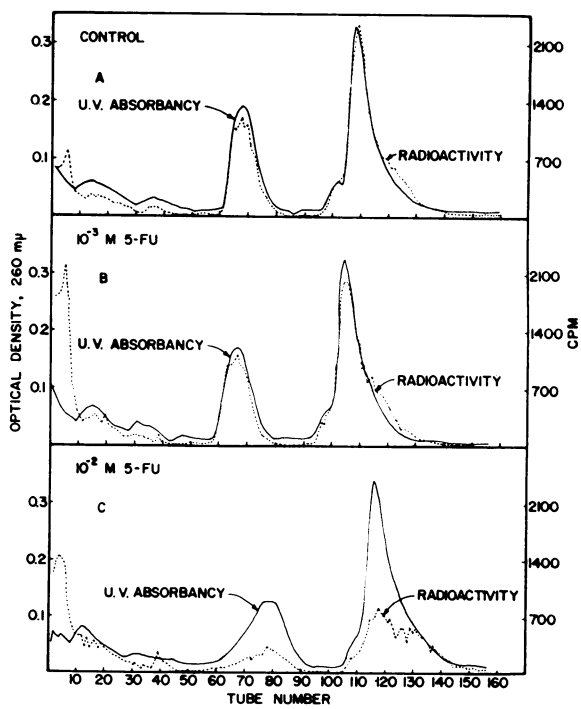

FIG. 3. Effect of 5-FU on nucleic acid metabolism in intact *Xanthium* buds as judged by fractionation on MAK columns. Plants grown under long days were treated with 5-FU about 1 hour prior to application with 50 μc P^{32} to the terminal apex. Sixteen hours later (including 6 hours darkness) the buds were harvested and the nucleic acid extracted, dialyzed and then fractionated on MAK columns.

[4] Unpublished data of J. H. Cherry and R. van Huystee.

thesis by more than 60 %. Messenger RNA synthesis was inhibited by only 33 % and labeling of sRNA was enhanced by 43 %, by the treatment with 10^{-2} M 5-FU. Perhaps, 5-FU promotes the degradation of rRNA to smaller RNA sub-units which are eluted with sRNA.

A comparison of the relative amount of mRNA in intact buds labeled with P^{32} (fig 3) to excised buds labeled in solution (fig 2), indicates that a relatively greater amount of the newly-synthesized nucleic acids is composed of mRNA when the excised tissue is labeled in solution. Therefore, the effects of 5-FU on nucleic acid metabolism in *Xanthium* buds which were treated without or with 5-FU, either on the intact buds or in solution prior to labeling with P^{32}, were compared. Figure 4 presents the elution profiles on MAK columns of the nucleic acids extracted from buds labeled with P^{32} in solution. It is apparent that there is a relatively greater amount of mRNA in control buds when labeled in solution (fig 4 A) as compared to intact control buds labeled with P^{32} (fig 3 A). The application of 10^{-3} M 5-FU to intact buds followed by incubation of the excised buds in P^{32} 16 hours later, indicated little change in the labeling pattern of the nucleic acids. Examination, however, of the elution profile of the nucleic acids extracted from buds treated with 10^{-2} M 5-FU (intact), 16 hours prior to labeling with P^{32} in solution (fig 4 C), show that synthesis of DNA and rRNA is inhibited while mRNA synthesis appears not to be influenced. Most unusual is the fact that, when control buds are preincubated in 10^{-2} M 5-FU for 1 hour prior to the 3-hour incubation period with 10^{-2} M 5-FU and P^{32}, a large apparent stimulation of mRNA synthesis is observed (fig 4 C). Data from the 4 elution profiles are summarized in table IV.

From these data it is concluded that while 10^{-3} M 5-FU, applied to intact buds, has little effect on the incorporation of P^{32} into nucleic acids when labeled in solution, 10^{-2} M 5-FU inhibits DNA and rRNA synthesis by 44 % to 49 %, but does not influence mRNA synthesis. Pretreatment of buds in solution with 10^{-2} M 5-FU inhibits rRNA synthesis (35 %) but greatly enhances mRNA production (7-fold) while sRNA and DNA synthesis are essentially not affected.

It would be desirable to make a direct quantitative comparison between the nucleic acids synthesized in intact (fig 3) and excised (fig 4) buds. However, in the particular experiments described here this is not possible because the method of labeling and the amount of P^{32} used for the intact and excised tissue was different. Thus, the ratio of P^{32} to P^{31} in the phosphorus pool of the tissues would be quite different. Also, the intact buds were labeled for 16 hours, instead of 3 hours for excised buds, in order to get adequate uptake of the isotope. While only a qualitative comparison can be made between the data ob-

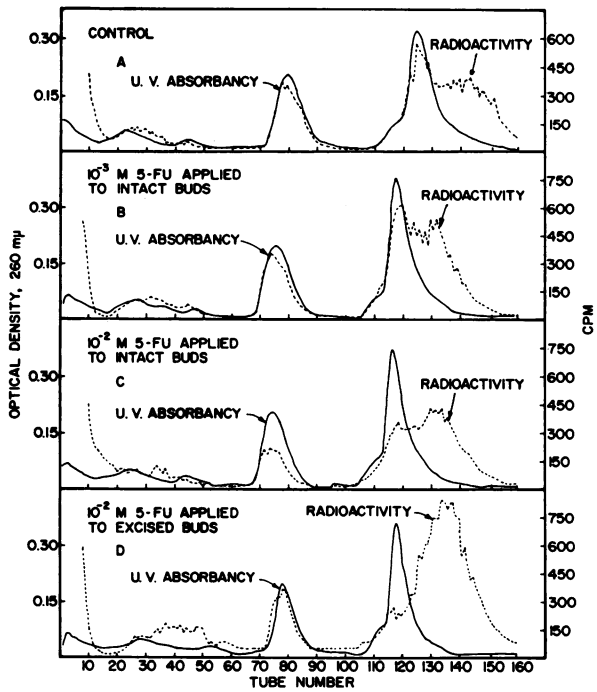

FIG. 4. Effect of 5-FU on nucleic acid metabolism in excised *Xanthium* buds as judged by fractionation on MAK columns. The elution profile presented in figure 4 A represents nucleic acids obtained from non-treated buds labeled in solution with P^{32}. Figures 4 B and 4 C represent nucleic acids from buds treated with 5-FU 16 hours prior to excision and incubation in solution. Figure 4 D represents nucleic acids obtained from buds pretreated with 10^{-2} M 5-FU 1 hour prior to labeling with P^{32} in the presence of 5-FU. In all cases the buds were incubated with P^{32} for the same length of time, 3 hours.

Table III. *Effect of 5-FU on the Metabolism of Nucleic Acids in Intact* Xanthium *Buds*
Both 5-FU and P^{32} were applied to intact buds.

Conc of 5-FU applied to buds	% of total radioactivity incorporated into each of the nucleic acid fractions from MAK columns				Specific activity of each nucleic acid fraction from MAK columns (cpm/μg)			
	sRNA	DNA	rRNA	mRNA	sRNA	DNA	rRNA	mRNA
0	9.2	26.7	44.5	19.7	84	126	143	213
10^{-3} M	12.2	25.9	42.3	19.6	106	127	132	265
10^{-2} M	26.4	15.3	31.1	27.2	120	46	56	143

Table IV. *Effect of 5-FU on the Metabolism of Nucleic Acid Metabolism in Excised* Xanthium *Buds*

5-FU was applied to intact buds. After 16 hours the buds were removed and incubated with P^{32} for 3 hours.

Conc of 5-FU applied to buds	% of total radioactivity incorporated into each of the nucleic acid fractions from MAK columns				Specific activity of each nucleic acid fraction from MAK columns (cpm/μg)			
	sRNA	DNA	rRNA	mRNA	sRNA	DNA	rRNA	mRNA
0, Intact	11.4	19.2	39.5	29.9	49	34	43	144
10^{-3} M, Intact	10.4	18.7	28.3	42.6	43	33	37	147
10^{-2} M, Intact	18.5	12.5	20.0	49.0	50	19	22	161
Excised buds Preincubated in 10^{-2} M 5-FU, 1 hr	11.3	11.6	17.9	59.2	59	34	28	1202

tained from intact and excised buds, it is to be emphasized that a good quantitative comparison can be made between the treatments of either intact or excised buds.

Discussion

In agreement with previous workers (2, 13) we found that 5-FU retards the development of the floral primordia of photoinduced *Xanthium* plants. However, only 10^{-2} M 5-FU when applied at the beginning of a single dark period effectively prevents the vegetative apex from developing into floral primordia and eventually producing seed. When the plants are given 2 or 3 inductive dark periods, and 5-FU is applied at the beginning of each dark period, the initial development of floral primordia is retarded, but the flowering stimulus is not abolished by 5-FU because the plants do develop floral primordia.

It is assumed that the photoperiodic dark cycle triggers the leaf to produce the flowering hormone which is translocated to the plant apex. In the apex, the flowering hormone probably acts to derepress the chromatin DNA. The cells of the apex respond by producing mRNA which codes for enzymes required for the vegetative bud to grow into a floral primordium. If these assumptions are correct, and if 5-FU were to act specifically by blocking the floral stimulus, some part of the transcription or translation of the genetic code would have to be altered. If this were the case, the likely explanation would be the production of a defective mRNA as a result of the fluorine on the 5 position of uracil. The substitution of fluorine for hydrogen on the uracil is thought to render the mRNA containing 5-FU useless. There are, however, at least 2 reasons why 5-FU should not specifically inhibit flowering in *Xanthium*. 1) There are probably hundreds of mRNAs required for normal cell activity, and it is hard to reconcile how only one or a few mRNAs required for floral development would be preferentially made defective while the hundreds of other mRNA are not. 2) Aronson (1) has shown that 5-FU inhibits RNA synthesis of bacteria; yet, the proteins synthesized appear to be normal. Key and Ingle (10) have shown that when 5-FU is applied to soybean hypocotyls to inhibit RNA synthesis by 50 % or more, cell elongation is not affected. They also showed that 5-FU may inhibit RNA synthesis in radish cotyledons by 50 %, while the induction of the enzyme, nitrate reductase was not altered. The total uracil of TMV virus RNA may be replaced by as much as 47 % with fluorinated uracil without losing its ability to produce local lesions on host leaf tissue (7). These results suggest that mRNA containing 5-FU codes for normal protein, and thus 5-FU does not influence cell activity by the production of nonsense mRNA.

On the mechanism of action of 5-FU, it is not clearly understood why certain fractions of nucleic acids are preferentially inhibited much more than others. It is supposed that DNA biosynthesis is impaired because of the inhibition of thymidylate synthetase by the 5-fluorodeoxyuridine converted from 5-FU (9). It has also been thought that 5-FU exerts its effect on RNA biosynthesis by changing the message of informational RNA (mRNA). As explained above, however, this does not seem to be the case. The anomalous result is that rRNA is inhibited approximately twice as much as mRNA (table III). Therefore, 5-FU treated cells, in some way, recognize the presence of 5-FU and preferentially slow down the synthesis of rRNA as compared to the relative amount of mRNA produced. In one instance, when *Xanthium* buds were pretreated with 5-FU, the production of mRNA was actually enhanced 7-fold over the control tissue while rRNA was inhibited. There appears to be some regulator mechanism which acts in the presence of 5-FU to greatly reduce the synthesis of rRNA as compared to mRNA.

These results point up changes in nucleic acid metabolism which come from excising tissue and labeling it in solution with an isotope. This question was investigated previously (5), with peanut cotyledons, and it was found that the pattern of labeling nucleic acids in sliced peanut cotyledons with P^{32} did not differ significantly from cotyledons labeled on intact plants through the root system. Contrary to those results, it is clear with *Xanthium*

buds that the pattern of labeling the nucleic acids with P^{32} is quite different when intact labeled buds (fig 3 A) are compared to excised labeled ones (fig 4 A). The major difference is in the relative amount of mRNA synthesized. Excised labeled tissue contains approximately 50 % or more P^{32}-mRNA than does intact labeled tissue. These data, with *Xanthium* buds, are perhaps comparable to the results of Hayashi and Spiegelman (8) on the step-down culture with bacteria where it was shown that transferring logarithmically growing bacteria to a minimal medium (step-down transition) caused them to preferentially synthesize informational RNAs which possess base sequences complementary to their homologous DNA. No speculation was made concerning the reason for this shift in synthesis of RNA. However, it seems reasonable, since the bulk of the RNA in a cell is ribosomal which possesses a relative long half-life, that when the cells are deprived of nutrients, thereby reducing their growth rate, mostly informational or messenger RNA would be produced. This would be assumed to be the case if there were a regulatory control mechanism which would recognize the deficiencies in nutrients and would react by causing the cell to produce little rRNA, while the synthesis of mRNA might be reduced very little. Similarly, tissue of higher plants would be affected in the same manner. If tissue were removed from the plant, certain nutrients, auxins, etc. might be limiting in the excised tissue after a short time of incubation in solution. This would be equivalent to the step-down culture of bacteria, therefore, causing the tissue to reduce its synthesis of rRNA. Thus, labeling excised tissue with P^{32}, in this instance, would result in the production of RNA containing a relatively larger amount of P^{32}-mRNA than in the intact tissue. This is precisely the result obtained in the present investigation. This phenomenon can be dramatically illustrated by the use of double-labeling techniques.[5]

Summary

The application of 5-fluorouracil (5-FU) to *Xanthium* buds prior to photoperiodic induction reduces the development of the floral primordia. Plants given 2 to 3 photoinductive dark cycles with 5-FU applied at the beginning of each cycle, show less inhibition of the floral development than when only 1 cycle is given. While development of the floral primordia is reduced by 5-FU it appears that only high levels (10^{-2} M) applied to plants given 1 photoinductive dark cycle effectively stop flowering. Therefore, it is concluded that 5-FU is not a specific inhibitor of flowering in *Xanthium* plants.

Estimation of the amounts of various fractions of RNA as revealed by fractionation on MAK columns indicate that nucleic acids from buds of noninduced and induced plants are essentially identical. Using the same technique, it was found that 5-FU inhibits DNA and ribosomal RNA to a much greater extent than messenger RNA. It appears that mRNA is fairly resistant to the inhibitory action of 5-FU. Labeling excised tissue in solution preferentially promotes the synthesis of mRNA as compared to intact labeled tissue. Excising tissue from the plant influences nucleic acid metabolism in a manner similar to depriving bacteria of nutrients required for optimum growth (step-down cultures).

[5] Unpublished data of J. H. Cherry and R. van Huystee.

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