Ribonucleic Acid Synthesis in the Bud an Essential Component of Floral Induction in Xanthium [1, 2]

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It has been shown in earlier papers (8, 9) that photoperiodic induction of the cocklebur is inhibited by application to the plant of the pyrimidine, 5-fluorouracil (5-FU). It was further found that the inhibitor is most effective in this function if applied directly to the bud at the beginning of the otherwise inductive dark period. It has been further concluded that the inhibitor functions by adversely affecting processes which take place in the bud during the inductive dark period and which are essential to photoperiodic induction.

In this paper we shall show that the process in the bud which is inhibited by 5-FU and which is related to inhibition of induction is the synthesis of ribonucleic acid (RNA).

Methods

The cocklebur (*Xanthium pensylvanicum* Wall, 9) plants used were of our standard inbred strain. They were grown in a controlled environment greenhouse (Campbell Plant Research Lab.) at a day (9 hr) temperature of 23 C and a night (15 hr) temperature of 17 C. The natural day length was lengthened to 18 hours (9 hr at 23 C & 9 hr at 17 C) with low (ca. 50 ft-c) intensity supplementary light to maintain the plants in the vegetative condition. The plants were used for experimentation after the appearance of the sixth leaf by which time they had become fully photoperiodically sensitive. Under the present environmental conditions, the plants were ready for use approximately 30 days after planting the seed.

In preparation for each experiment the plants (200–250 in number) were first defoliated, leaving only a single leaf and this the most rapidly growing one (approximately 7 cm long) which is most sensitive to induction. Such defoliation included removal of young leaves down to approximately seven millimeters in length. The plants were then randomly distributed into groups of 15 to 20. In general a single such group served for a single treatment in the experiment, although, as noted below, duplicate or even quadruplicate groups were used in particular experimental designs. In addition, each experiment reported below has been repeated at least twice.

In experiments or treatments dealing with the influence of added metabolites on the course of induction the plants were treated with the metabolite, induced by exposure to a single 16-hour dark period and returned to a long (22 hr) day in the Dolk evaporatively cooled greenhouse for 9 days at which time the apical buds were dissected and classified according to the floral stage system of Salisbury (7). Metabolites such as 5-FU were applied in these experiments in aqueous solution containing a small amount (ca. 0.1 %) of Tween 20. Treatment consisted of immersing the apical bud, or bud and remaining leaf briefly in the treatment solution.

In experiments concerned with chemical activities of the apical bud, a further procedure for standardization of initial bud size was introduced. Each plant after defoliation was subjected to measurement of the length of its apical bud with a vernier micrometer. The bud was measured on the ventral side of its largest leaf primordium and from tip to base. By this measurement the plants were classified into three groups possessing apical buds, respectively, 8, 7, or 6 mm long. All plants with buds not in these three categories were discarded. The treatment groups for the experiment were then made up so that all contained the same number of 8 mm buds, etc. In all experiments concerned with chemical matters, the 15 to 20 plants of a single treatment group were harvested, pooled, and treated as a single sample. In such experiments, therefore, duplicate, triplicate, or quadruplicate groups were used for each treatment.

In certain of the experiments reported below, C^{14}-labeled metabolites were applied to bud or to leaf. The labeled metabolite, made up in water with Tween 20, was applied in measured volume with a micro syringe, 0.01 ml per bud or 0.1 ml per leaf.

Separation of and determination of RNA and DNA was carried out according to the procedure of Schmidt and Thannhauser (10): The freshly harvested sample was weighed (ca. 150 mg fresh weight in the case of 15 buds) and immediately extracted with 80 % ethanol at 100 C. It was then ground in a glass homogenizer and re-extracted with 80 % ethanol to yield a pigment-free powder. This was subjected to three successive extractions with 5 % TCA at ice bath temperature. The TCA was then removed by ethanol and ethanol-ether (2:1 by volume). RNA was next

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hydrolyzed by incubation with 0.3 N KOH for 16 hours at 37 C. The resultant slurry was now cooled and separated into supernatant and residue by centrifugation and twice repeated re-extraction of the residue with distilled water. The supernatant was next acidified to about pH 3 with perchloric acid, and the resultant precipitate which contains the DNA as well as potassium perchlorate centrifuged off. Ribonucleotide content of the sample was determined on the supernatant spectrophotometrically with a Carey Model 11 spectrophotometer [O.D. at max. (258–260 mμ) — O.D. at 340 mμ △ O.D. × 0.031 = mg RNA]. Radioactivity of the RNA was determined by counting aliquots of this same fraction with a model D 47 Nuclear Chicago gas flow counting system equipped with a micromil window.

The DNA-containing potassium perchlorate precipitate was washed twice with 5% TCA at 0 C (to remove residual RNA-tides), the TCA removed with ethanol and ethanol ether (2:1), and the DNA then hydrolized by incubation for 10 minutes at 90 C in 0.5 N perchloric acid. The hydrolysate was next centrifuged, the supernatant neutralized with KOH, the resultant potassium perchlorate centrifuged off, and the DNA-tide-containing supernatant subjected to spectrophotometry [O.D. at max. (268 mμ) — O.D. at 340 mμ △ O.D. × 0.031 = DNA] or counting as above.

Ribonucleotides were separated in ascending paper chromatography on Whatman paper No. 1 with a mixture of *iso*-propanol-concentrated HCl and water as the solvent (11). Nucleotides were located under an ultraviolet lamp, eluted in 0.1 N HCl and identified by means of spectrophotometry. Distribution of radioactivity in chromatograms was determined in a strip counter.

Results

► Transport of 5-FU. It has been previously shown that although 5-FU is most effective in inhibiting induction when the compound is applied to the bud, it is also effective when applied to the leaf (9). In order to find out whether 5-FU applied to the bud inhibits induction directly or whether 5-FU applied to the bud is translocated to the leaf, there to influence some aspect of the inductive process, experiments with labeled 5-FU have been carried out. One such experiment is summarized in the data of table I. For this experiment 2-C^{14}-labeled 5-FU (California Corp. for Biochemical Research, Spec. act. 5mc/mmole) was applied either to the leaves or to the buds of cocklebur plants. Application was made at the beginning of a 16 hour inductive dark period. At the end of the 16 hour inductive dark period the buds and leaves were harvested, extracted as described above, and distribution of label determined. The data of table I show that when labeled 5-FU is applied to the bud it is recoverable in large amounts in the bud and in the RNA of the bud. No detectable activity was, however, transported to the leaf during the 16 hour dark period. Labeled 5-FU applied to the leaf, on the contrary, is not only recoverable in the leaf, but also in the bud. The amount of labeled 5-FU recovered in the bud as the result of leaf application is somewhat smaller than that found in the bud after bud application even though the extent of inhibition of induction is approximately the same in the two cases. Nonetheless, the data serve to demonstrate quantitatively that 5-FU can and does exert its inhibitory effect upon photoperiodic induction by acting directly on the bud. This is true even though the bud itself is not the photoreceptor; it does not perceive and sense the length of the dark period, a function which is, rather, the property of the leaf. The fact that leaf application of 5-FU is also effective is apparently due to the fact that the material is readily transported to the bud even during a single 16 hour dark period.

Results similar to those above have been obtained with C-14-labeled orotic acid which like labeled 5-FU is readily transported from leaf to bud during a 16 hour dark period but is not transported from bud to leaf during the same period.

The fact that 5-FU exerts its inhibition of photoperiodic induction in the bud, and this during a 16 hour inductive dark treatment of the leaf, indicates that processes essential to induction take place in the bud during this period. This is true even though it is known from defoliation experiments that the transport from the leaf of a material or materials required for induction in the bud commences during the light period subsequent to the inductive dark period (7). The 5-FU-inhibitable processes of the bud must therefore be ones which are essential to the subsequent successful receipt of and action upon the leaf-produced floral stimulus. In any case, the present experiments indicate that the study of the mechanism by which 5-FU inhibits photoperiodic induction should concern itself with the tissues of the bud alone. All of the subsequent material in this paper is, therefore, concerned with the metabolism of the bud.

► Kinetics of 5-FU inhibition. It has been shown in an earlier paper (9) that 5-FU is effective in inhibiting photoperiodic induction only if applied early in or at the beginning of the inductive dark period and

Table I

Transport of C^{14}-labeled, 5-fluorouracil (5-FU) From Leaf to Apical Bud of Xanthium & Absence of Transport From Bud to Leaf During Inductive Dark Period*

C^{14}-5-FU Applied to	cpm Applied × 10^{-3}	Radioactivity detected, cpm × 10^{-3} per 15 buds			
		In bud extract	In bud RNA	In leaf extract	In leaf RNA
Bud	840	69	9.1	0	0
Leaf	8,400	26	2.2	1,500	26

* 0.02 μmole 5-FU per bud, 0.2 μmole per leaf. Applications made at beginning of 16-hour dark period and harvest at the end of same dark period.

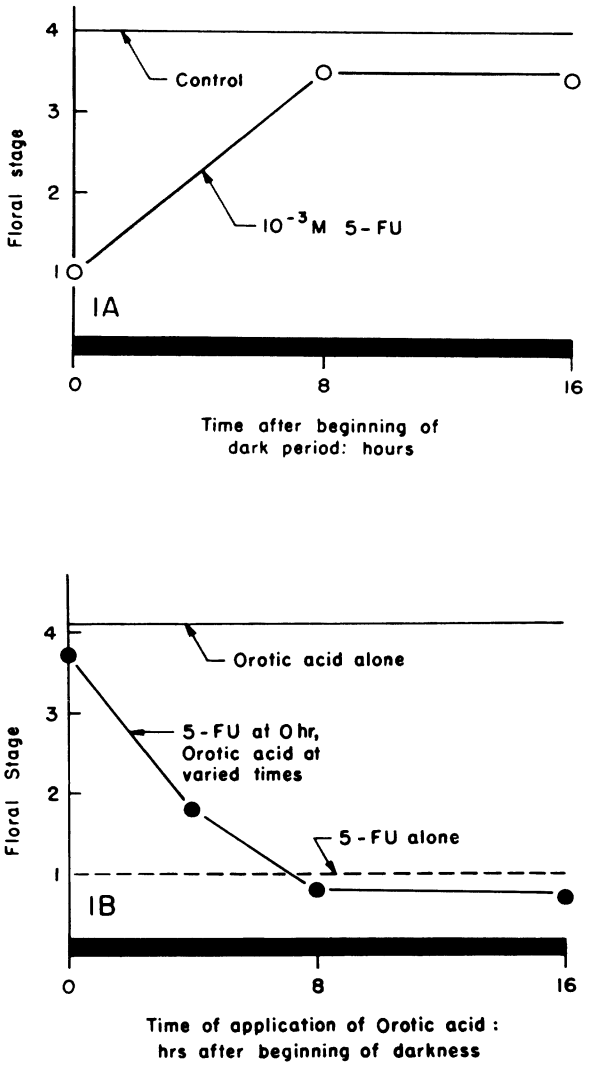

Fig. 1. Inhibition of photoperiodic induction of Xanthium as a function of time of application of 5-fluorouracil (A) and of time of antidoting the 5-fluorouracil inhibition by orotic acid (B). 5-Fluorouracil: 10^{-3} M, orotic acid: 8×10^{-3} M. Both substances applied to leaf and bud.

is ineffective if applied at the end of this period. It has, in addition, been shown that the inhibitory effect of 5-FU is alleviated and can even be totally suppressed by the simultaneous application of orotic acid, an intermediate in the biogenesis of pyrimidines. The fact that orotic acid possesses the ability to antidote the inhibition caused by 5-FU gives us a tool to determine more precisely the interval during which 5-FU exerts its inhibitory effect. This matter is considered in the experiments of figure 1. In the experiment of figure 1A 5-FU was applied in appropriate concentration (10^{-3} M) at the beginning, middle, or end of the inductive dark period. In confirmation of earlier results (9) it is clear that 5-FU is almost ineffective if applied more than 8 hours after the beginning of the dark period. In an experiment in which labeled 5-FU was applied to buds at 0, 8, or 16 hours after the beginning of a 16 hour dark period, the amounts of label incorporated into RNA during the following 8 hours were essentially identical. The results of figure 1A are, therefore, not due to differences in RNA-synthesizing activity of the bud, but rather to differences in the kinds of RNA synthesized during the different portions of the dark period.

In the experiment of figure 1B 5-FU was applied in all treatments at the beginning of the inductive dark period and orotic acid in appropriate concentration applied to antidote the 5-FU at various times after the beginning of the inductive dark period. The data of figure 1B show that orotic acid applied at the beginning of the dark period completely antidotes the inhibitory effect of 5-FU simultaneously applied. If orotic acid is applied at the end of the inductive dark period it possesses no power to antidote the inhibitory effects of the 5-FU. All the inhibition of induction exerted by 5-FU has, therefore, been exercised during the 16 hour inductive dark period. Furthermore, orotic acid is almost ineffective in reversal of 5-FU inhibition even if applied in the middle of the inductive dark period. The inhibitory effects of 5-FU have, therefore, been principally exerted during the first 8 hours of the inductive dark period. It would appear, therefore, that during the first 8 hours of the inductive dark period something is made in the bud which is required for the subsequent response of the bud to the photoperiodic signal produced by the leaf. This something, whose production is inhibited by 5-FU, appears to be made during the dark period before the bud has determined whether the dark period to which the leaf is

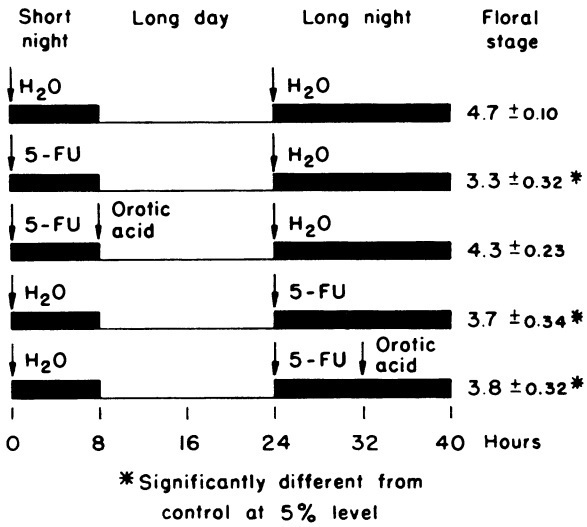

Fig. 2. 5-Fluorouracil applied either at the beginning of a short or long night and antidoted 8 hours later with orotic acid. Both leaf and tip treated. 5-Fluorouracil: 10^{-3} M, orotic acid 8×10^{-3} M.

Table II

Influence of 5-FU on Growth of & Nucleic Acid Synthesis in Xanthium Buds*

Treatment	Bud fr wt mg/15 buds	Total RNA mg/15 buds	Total DNA mg/15 buds	cpm × 10^{-3} C^{14}-orotic acid incorporated into			
				Total RNA	cpm/mg RNA	Total DNA	cpm/mg DNA
Initial harvest	143	0.66	0.26	...	...	...	...
Final harvest, no 5-FU	177	0.73	0.27	34.2	46.5	6.5	24.0
5-FU + orotic acid	168	0.62	0.26	14.1	22.9	1.0	3.9

* During a 16-hour dark period.
10 μl drops containing C^{14}-orotic acid (2×10^{-3} M) without or with 5-FU (5×10^{-3} M) applied to buds at beginning of 16-hour dark period. Harvested at end of same dark period. Each number average of four replicates, each of 13 to 15 plants (calculated per 15 buds).

exposed is going to be a long one or not. It appears reasonable to suppose, therefore, that this 5-FU inhibitable process must go on even in short nights. It is, therefore, of interest to determine if 5-FU given to a cocklebur plant during a short night preceding the photoperiodic inductive dark period similarly exerts an inhibitory effect on flowering. This type of experiment is considered in figure 2. For this experiment plants were supplied with 5-FU either at the beginning of a 16-hour inductive dark period or at the beginning of the 8-hour dark period, one 24-hour cycle before the beginning of the inductive dark period. In each case, the 5-FU was allowed to exert its effect upon the bud for 8 hours and was then antidoted by treatment with orotic acid. It is clear from the data of figure 2 that again the presence of 5-FU in the bud during the first 8 hours of an inductive dark period is inhibitory to subsequent flowering. The presence of 5-FU in the bud during the 8 hours of a non-inductive dark period preceding the inductive one is similarly inhibitory to flowering, but this inhibition can still be reversed after 8 hours. It may be concluded, therefore, that the processes which are inhibited by 5-FU and which are required for a photoperiodic induction start anew each night. These processes are, however, ones which fail safe, are negated in the event that the dark period turns out to be shorter than the critical night length.

► Influence of 5-FU on Nucleic Acid Synthesis in the Bud. Application of 5-FU to the apical bud of Xanthium and in concentrations which inhibit photoperiodic induction cause slight but measurable inhibition in growth of the bud during a 16-hour dark period. This is shown by the fresh weight data of table II. In this and similar experiments 5-FU was applied to the buds of Xanthium plants at the beginning of a 16-hour inductive dark period. The apical buds were harvested as described above at the end of the 16-hour period. In general, and as shown in table II, buds of untreated plants increased in both fresh weight and RNA content by 10 to 20 % during the 16 hours under consideration. Applying 5-FU in an amount which causes approximately 50 % inhibition of induction caused essentially complete inhibition of net synthesis of RNA and substantially depressed increase in bud fresh weight.

A more sensitive measure of RNA synthesis consists in measurement of the incorporation of a C^{14}-labeled precursor of nucleic acid into bud RNA. For this purpose 2-C^{14}-labeled orotic acid (Spec. act. 2.3 mc/mmole) was used. This material was applied to the buds at the beginning of an otherwise inductive dark period in low concentration (ca. 2×10^{-3} M), a concentration insufficient to influence the course of 5-FU inhibition (5×10^{-3} M). It is clear from the data of table II that the incorporation of orotic acid into RNA in the bud is inhibited by the presence of applied 5-FU. This inhibition is found also for the incorporation of label of orotic acid into DNA, which is in fact even more sensitive to 5-FU inhibition than is incorporation into RNA. Therefore, 5-FU appears to be an inhibitor of the synthesis in the bud of both DNA and RNA.

That 5-FU not only inhibits the synthesis of RNA and DNA, but is also itself incorporated into RNA has already been shown by the data of table I. Further data bearing on this matter are presented in table III. In this and similar experiments labeled 5-FU was applied to the bud of cocklebur plants at the beginning of the 16-hour dark period and the buds harvested at the expiration of this time. It is clear again that 5-FU is incorporated into RNA. No ap-

Table III

Incorporation of C^{14}-Labeled 5-FU Into RNA of Xanthium Buds & Inhibition of This Incorporation by Orotic Acid*

Conc applied		Specific activity of RNA cpm/mg RNA	% of label incorporated into RNA
C^{14}-5-FU	Orotic acid		
2×10^{-3} M	...	16,200	0.95
2×10^{-3} M	6×10^{-3} M	10,450	0.61
...	2×10^{-3} M**	63,000	8.58

* 0.01 ml per bud applied at beginning of 16-hour dark period. Buds harvested at end of this dark period.
** C^{14}-labeled orotic acid.

Table IV

Effect of 5-FU & of 5-FDU on Synthesis by Xanthium Buds of RNA & DNA Compared With Effect of These Substances on Rate of Development of Inflorescence Primordia (Induction).*

Inhibitor	Conc	Inhibition of incorporation of C^{14}-orotic acid into		Inhibition of floral development
		RNA	DNA	
5-FU	1.5×10^{-3} M	42 %	64 %	60 %
5-FU	4×10^{-3} M	57 %	85 %	> 75 %
5-FU	5×10^{-3} M	51 %	84 %	> 75 %
5-FDU	2×10^{-4} M	0 %	94 %	50 %
5-FDU	5×10^{-4} M	21 %	96 %	80 %

* In all cases the inhibitor was applied to the bud at the beginning of a single 16-hour dark period. All figures from duplicate lots of 15 plants each.

preciable incorporation of the label of 5-FU into DNA can, however, be detected. Just as incorporation of the C^{14} label of orotic acid into RNA is inhibited by the presence of unlabeled 5-FU, so the incorporation of the label of 5-FU into RNA is inhibited by the presence of unlabeled orotic acid. This behavior parallels the effect of orotic acid on inhibition of photoperiodic induction by 5-FU which is likewise reversed by simultaneous application of orotic acid.

From paper chromatography of RNA-derived nucleotides after incorporation of labeled orotic acid it was revealed that radioactivity is restricted to two spots with Rf values of 0.52 and 0.70, corresponding to those of cytidylic and uridylic acid, respectively. Elution and ultraviolet spectrophotometry confirmed the identity of the two. Chromatography of hydrolysates of bud RNA labeled with 5-FU regularly yielded a radioactive spot (Rf value 0.78) beyond the uridylic acid and 5-FU spot (Rf values for both: 0.70), but elution yielded insufficient amount of material for rigorous identification of the substance as 5-fluorouridylic acid.

Although 5-FU is, itself, incorporated into RNA, this incorporation is much less efficient than incorporation of orotic acid. The data of table III show that 8.58 % of the orotic acid applied to a bud was incorporated into RNA during a 16-hour inductive dark period. This is to be contrasted with the 0.95 % of 5-FU given in identical concentration which was incorporated into bud RNA during the same period of time. This fact doubtless underlies the inhibition by 5-FU of RNA synthesis.

It may in summary be concluded, then, that 5-FU acts as an efficient inhibitor of the synthesis of both RNA and DNA. 5-FU is, however, itself incorporated with low efficiency into the RNA that is made in the presence of the inhibitor, the DNA remaining unlabeled.

► Is inhibition of photoperiodic induction by 5-FU due to inhibition of RNA synthesis or inhibition of DNA synthesis? It is now of interest to determine whether inhibition of photoperiodic induction by applied 5-FU is due to the effect of this material on incorporation into and overall suppression of RNA synthesis, or due to the inhibitory effect of 5-FU on DNA replication. This question has been approached by the use of a further inhibitor, 5-fluorodeoxyuridine (5-FDU). This material is much more specific than 5-FU in the sense that it inhibits primarily synthesis of DNA by inhibiting the methylation of deoxyuridylic acid to thymidylic acid (1, 3) and exerts but little effect upon synthesis of RNA in the cocklebur bud. That this is so is clear from the data of table IV. Thus, for example, 5-FU in a concentration which causes 50 to 75 % inhibition of induction causes approximately 40 % inhibition of rate of RNA synthesis and 80 % inhibition of rate of DNA replication. In a concentration which causes similar inhibition of induction 5-FDU causes 96 % inhibition of DNA replication and only approximately 20 % inhibition of rate of RNA synthesis. Inhibition of induction by 5-FDU is, however, unlike the inhibition exerted by 5-FU in several respects. In the first place, 5-FDU in a concentration which causes substantial inhibition of induction, also greatly inhibits vegetative growth of the plant, that is, its effect is a lasting one, unlike that of 5-FU. In addition, as is shown in the data of table V, inhibition of induction by 5-FDU is reversed by simultaneous application of thymidine, a precursor of and a specific participant in DNA synthesis. It may be concluded, therefore, that 5-FDU exerts its effect on inhibition of induction principally, if not exclu-

Table V

Comparison of Kinetics of Effects of 5-FU & of 5-FDU in Inhibition of Induction & of Effects of Orotic Acid & of Thymidine in Reversal of These Inhibitions.*

Inhibitor applied at 0 hr	Antidote applied	Floral stage after 9 days			
		Antidote applied			Control (No treatment)
		None	at 0 hr	at 16 hr	
5-FU, 10^{-3} M	Orotic acid, 8×10^{-3} M	2.2	4.1	1.5	4.4
5-FU, 10^{-3} M	Orotic acid, 8×10^{-3} M	1.0	3.7	0.7	4.0
5-FDU, 5×10^{-4} M	Thymidine, 4×10^{-3} M	1.7	3.6	3.9	4.5
5-FDU, 2×10^{-4} M	Thymidine, 4×10^{-3} M	2.4	4.6	4.2	4.8

* Beginning of 16-hour dark period designated as 0 hour. Control: no inhibitor applied.

sively, through its effect upon inhibition of DNA synthesis. Finally, the data of table V also show that application of 5-FDU at the beginning of a 16-hour inductive dark period followed by application of thymidine at the end of the same dark period results in full induction. We may conclude, therefore, that although 5-FDU essentially completely suspends DNA replication during a 16-hour dark period, this is not of itself inhibitory to induction. The situation with 5-FDU is to be contrasted to that obtaining for 5-FU as is summarized in table V. In the case of 5-FU applied at the beginning of a dark period, simultaneous application of orotic acid results in antidoting of the inhibition. Application of orotic acid at the end of the dark period does not reverse the bad effect of 5-FU in inhibition of induction. As already concluded above, the inhibitory effects of 5-FU are exerted wholly during the inductive dark period and, in fact, principally during the first 8 hours of this dark period. On the contrary, 5-FDU, although it inhibits DNA replication during the inductive dark period, is without effect upon photoperiodic induction provided that DNA replication is permitted to resume at the end of the inductive dark period by appropriate application of thymidine.

Discussion

Since labeled 5-FU is readily translocated from leaf to bud, but not in the reverse direction it is evident that the inhibitory effect of 5-FU cannot be ascribed to interference with the inductive processes in the leaf which result in the production of floral stimulus. The pyrimidine analog, 2-thiouracil, which is active in inhibition of induction in Xanthium, although less so than is 5-FU (9) inhibits flowering in *Streptocarpus wendlandii* (5) and in *Cannabis sativa* (4). Hess has assumed that such inhibition is exerted in the cotyledon in the case of Streptocarpus. No rigorous demonstration that this is so has, however, been provided (6) and it may equally well act upon the bud itself. Heslop-Harrison (4) has concluded from his work with Cannabis that 2-thiouracil inhibits flowering in this species by causing the apices to become unresponsive to the floral stimulus which emanates from the leaf. The same is true for *Pharbitis nil* in the case of the inhibitors, 5-FU and 5-FDU (Zeevaart, unpublished).

The present results have shown that 5-FU inhibits both the synthesis of RNA and the multiplication of DNA in the bud of the Xanthium plant during an otherwise inductive dark period. It has further been shown, however, that inhibition of DNA multiplication by the specific inhibitor 5-FDU is not inhibitory to induction, provided only that the 5-FDU is ultimately antidoted by an appropriate material such as thymidine. It is clear, therefore, that the process which is inhibited in the bud by 5-FU, and which is related to inhibition of photoperiodic induction, is the process of RNA synthesis. That the two phenomena parallel one another closely has been shown in a variety of ways. Thus, the time courses of the effects of added orotic acid upon the relief of 5-FU inhibition of induction and upon the relief of the incorporation of labeled 5-FU into RNA parallel one another closely.

It has been noted earlier (9) that 5-hydroxyuridine does not inhibit induction in Xanthium as does 5-FU. It is of interest, therefore, to note that even though 5-hydroxyuridine is an inhibitor of RNA synthesis in other organisms, it is without such activity in Xanthium. Thus the presence of 5-hydroxyuridine does not inhibit incorporation of the activity of C^{14}-orotic acid into RNA by Xanthium buds, although it does somewhat depress incorporation into DNA.

The metabolism of 5-FU in Xanthium resembles that of 5-FU in tumor-bearing mice (3). In both cases 5-FU, incorporated into RNA, inhibits the synthesis of both RNA and DNA. These relations obtain, also, for *E. coli* (2), in which case it is additionally clear that the 5-FU-containing RNA which is made by 5-FU-treated *E. coli* is aberrant, and causes synthesis by the cell of aberrant and inactive or little active enzyme molecules. It may be supposed, although we have no direct knowledge of this, that the inhibition of induction by 5-FU in the case of cocklebur is due not only to the partial suppression of RNA synthesis, but also to production by the 5-FU treated cells of aberrant 5-FU-containing RNA.

Summary

► This paper is concerned with the inhibition of photoperiodic induction by 5-fluorouracil. Labeled 5-fluorouracil applied to a leaf of *Xanthium pensylvanicum* at the beginning of an inductive dark period is readily translocated to the apical bud during a 16-hour dark period. Translocation does not take place in detectable amounts in the reverse direction. The inhibition of photoperiodic induction by 5-fluorouracil is exerted, therefore, in the apical bud itself.

► C^{14}-labeled 5-fluorouracil is incorporated into bud RNA, thus forming fraudulent RNA. Simultaneous application of orotic acid decreases such incorporation.

► The application of 5-fluorouracil inhibits synthesis of both RNA and DNA in the apical bud as measured by incorporation of C^{14}-labeled orotic acid into these materials.

► That 5-fluorouracil is most active in inhibition of photoperiodic induction if applied at the beginning of an inductive dark period has been confirmed. Reversal of such inhibition by orotic acid is possible only if the latter is applied simultaneously with the 5-fluorouracil. If orotic acid is applied as little as 8 hours after the application of 5-fluorouracil, reversal of the 5-fluorouracil induced inhibition is incomplete, or nil.

► Application of 5-fluorouracil inhibits photoperiodic induction even if the material is applied at the beginning of the 8-hour short night, preceded by 16 hours of light to the inductive dark period. In this case, however, the inhibition is reversed by applica-

tion of orotic acid at the end of the short-night period. Apparently, therefore, the inductive processes in the bud which are inhibited by 5-fluorouracil are ones which start anew at the beginning of each dark period.

► 5-Fluorodeoxyuridine, a specific inhibitor of DNA multiplication, also inhibits the development of floral primordia, but in contrast to the effects of 5-fluorouracil, this inhibition is fully reversible by thymidine, even if the thymidine is applied at the end of the inductive dark period. These results demonstrate that DNA multiplication in the bud during the inductive dark period is not essential to the act of induction.

► It is concluded that RNA synthesis is the process essential to photoperiodic induction which is inhibited by the presence of 5-fluorouracil in the bud of Xanthium during an otherwise inductive dark period.

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