INDUCIBILITY OF THYMIDINE
KINASE BY THYMIDINE AS A
FUNCTION OF INTERPHASE STAGE

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ABSTRACT

Thymidine may act as an inducer of thymidine kinase activity in cells of higher plants. The general response is demonstrable in a randomly developing cell population such as is found in germinating wheat embryos. If a synchronously developing cell population is studied, however, potentially inducible cells are found to be susceptible to the inductive effect of thymidine only during about 10 per cent of the G1 period, and close to the interval when thymidine kinase activity normally appears.

INTRODUCTION

In microspores of lily and trillium, the enzyme thymidine kinase is formed prior to DNA synthesis and disappears within 18 hours (1, 2). The transient presence of this enzyme poses several questions with respect to the mechanisms governing cell development. Of these, the one we wish to consider is concerned with the fact that this enzyme does not appear until about the twentieth day of interphase. The more uniform the conditions under which the anthers housing the microspores are allowed to develop, the more precisely may the time of enzyme appearance be predicted (1). Some regulatory mechanism must therefore exist which leads to the synthesis of enzyme during a narrow interval in the life cycle of the cell. The general significance of thymidine kinase as a regulator of DNA synthesis is open to question, but its particular significance to chromosome replication in the microspores is not. Just prior to DNA synthesis, a pool of deoxyribosides appears, and at approximately the same time, the enzyme is formed (9). The formation is entirely restricted to the microspores; none of the surrounding somatic cells show significant enzyme activity even though deoxyribosides are present in the vicinity of these cells outside the microspores.

In earlier publications we pointed to the likelihood that the immediate cause of thymidine kinase formation was a substrate molecule, most probably thymidine itself. This interpretation was based on the coincidence of deoxyriboside appearance and enzyme formation. In other systems, evidence has also been obtained for the inducibility of this enzyme (4, 5, 6). In microbes, thymidine kinase has been assigned the role of "scavenger" (7, 8), since the main biosynthetic route of DNA precursors is via phosphorylated derivatives. A similar enzymatic route undoubtedly exists in cells of higher plants, although scavenging appears to be a normal and probably essential process for at least some groups of cells within the multicellular community. Whether or not thymidine kinase is a trivial instance of a control mechanism, it is a profound instance of a controlled mechanism. The enzyme may in certain instances make DNA synthesis possible, but it does not make the synthesis mandatory. On the other hand, the fact that synthesis of the enzyme itself is restricted to a brief interval of the cell cycle points to the existence of a fundamental regulatory mechanism. The principal question to which this paper is addressed is whether the appearance of thymidine kinase is controlled en-

tirely by the intracellular level of inducer molecule, or whether other control mechanisms, more closely tied to intracellular development, are superimposed upon the well established relationship between inducer molecule and protein synthesis.

The results reported in this paper fall into two parts. The first of these concerns the demonstration that thymidine does induce thymidine kinase activity in plant tissues which are heterogeneous with respect to stage of cell development; the second part concerns the demonstration that the effectiveness of thymidine as an inducer is limited to a small interval of interphase in a population of cells which is developing synchronously.

METHODS

Induction Studies in Wheat Seedlings

Wheat seeds (var. Pawnee) were germinated by placing 50 seeds in each of several Petri dishes (diameter 3.5 inches) over 3 layers of filter paper wetted with 7 ml of standard Hoagland's medium containing 0.01 per cent streptomycin. After 48 hours, the embryos were excised from the seeds and grouped into lots of 20. Each lot was placed in a 25-ml Erlenmeyer flask containing 2 ml of incubation medium (Hoagland's plus various additions), and the flasks were gently shaken through the incubation period. Triplicate groups were used in all enzyme studies. The small volume of medium and also the small number of embryos per flask were found to be essential to optimal developmental conditions. In early studies the entire experiment was conducted under aseptic conditions. This precaution was found to be unnecessary in later studies, since the time of incubation was 2 to 4 hours.

TISSUE ANALYSIS: At the conclusion of the incubation period, the embryos were washed several times with control medium (0°C). Each lot was homogenized in 1 ml of 0.02 M phosphate buffer (pH 7.2) and the resulting suspension centrifuged for 5 minutes at 3500 g. The sediment was discarded and the supernatant fluid was divided as follows: 0.3 ml for thymidine kinase assay; 0.2 ml for thymidine determination; and 0.3 ml for protein measurement. For those studies involving nucleic acids, a portion of the original suspension was retained for fractionation by the Schmidt-Thannhauser procedure as described in an earlier publication (3).

THYMIDINE KINASE ASSAY: The assay medium consisted of the following: 0.1 ml phosphate buffer (2.0 M, pH 7.2); 0.05 ml MgCl$_2$ (0.1 M); 12.4 mg adenosine triphosphate; 16.8 mg phosphoenol pyruvate; 20.15 μg H^3-thymidine plus 181.65 μg cold thymidine. The volume of the mixture was 10 ml,

and the specific activity of the thymidine was 6(5 mcuries/mmole. For individual assays, 0.1 ml of the medium, supplemented with 0.025 ml of pyruvate kinase (10 mg/ml), was combined with 0.1 ml of tissue extract. The mixture was incubated at 25°C for 20 minutes, and the reaction was stopped by the addition of 0.4 ml of 95 per cent ethanol. The ethanolic suspension was heated for 1 minute in a boiling water bath and then centrifuged to obtain a clear supernatant fluid. The residue was reextracted with 70 per cent (v/v) ethanol. The supernatant fluids were combined, and appropriate aliquots were streaked on filter paper. Chromatograms were developed in either water-saturated n-butanol or isobutyric acid–ammonium hydroxide (13). Radioactivity of the spots containing thymidine, thymidylic acid, and in some cases thymidine di- and triphosphate was determined by means of a scintillation counter after elution of the components with water.

Extracts of those tissues which had been exposed to high concentrations of thymidine had to be dialyzed against 0.02 M phosphate buffer (pH 7.2) for 2 hours at 0°C prior to assay. Without such dialysis, the high endogenous content of thymidine diluted the specific activity of the standard medium and gave spuriously low values. For any given series, all samples were treated in the same way irrespective of the extent of exposure to thymidine.

Several preliminary measurements were made to establish the quantitative validity of the assay procedure. A time course study indicated that the reaction rate was linear for the first 30 minutes; 20 minutes was therefore chosen as the standard incubation time. A plot of activity $vs.$ concentration of protein in reaction mixture indicated a proportionality up to 200 μg of protein. Individual assays were therefore conducted with amounts of protein below this maximum.

Induction Studies with Lily Microspores

Excised anthers of *Lilium longiflorum* (var. Ace) were cultured as previously described (1). Concentrations of thymidine added to the culture media are reported under Results. At the termination of the culture period, the microspores were removed from the anthers and purified in a solution of 0.35 M sucrose containing 0.003 M MgCl$_2$. In previous experiments anthers were removed singly so that for any given bud length a series of six sequential measurements could be made. Generally, this procedure was unsuitable for the present studies because the extracts were dialyzed, and several anthers had to be combined to yield volumes of extract which could be conveniently handled. Such combination partly obscured the sharp peaks in activity obtained by the original procedure, but, as will be seen, it did not obscure the relationship sought. In this series of analyses the assay medium for

thymidine kinase was as follows: 0.35 ml phosphate buffer (2.0 M, pH 7.2); 0.04 ml $MgCl_2$ (1.0 M); 0.01 ml $MnCl_2$ (1.0 M); 4 mg bovine serum albumin; 0.334 ml H^3-thymidine plus 121 μg thymidine. The volume of the mixture was made up to 7.0 ml with water, and the specific activity of the thymidine was 574 mcuries/mmole. Of this solution, 0.1 ml was mixed with an equal volume of cell suspension containing 20 to 200 μg protein in 0.1 M phosphate buffer (pH 7.2). This medium was introduced after the publication of Okazaki and Kornberg (7, 8) became available. However, in these studies, no difference in results was found between the new and the old medium.

For reasons which will be discussed under Results, measurements were made of the amount of thymidine absorbed and retained by the microspores. Groups of excised anthers from 10 buds were cultured in standard medium containing a mixture of radioactive and cold thymidine. At the conclusion of the culture period the microspores were extruded from the anthers and suspended in 20 ml of sucrose medium. The suspension was centrifuged for 2 minutes at 2500 g and the wash was made 70 per cent (v/v) with respect to ethanol. The several washes obtained in this procedure were thus retained as a check on the efficacy of the washing method. The sedimented microspores were resuspended in 40 ml of sucrose medium and again centrifuged. After this washing the microspores were suspended in 5 ml of medium and layered over 0.4 M sucrose. They were then sedimented by centrifugation for 5 minutes at 3500 g, and the step was repeated once more. The washes in these last two steps showed negligible radioactivity, and the microspores were therefore considered to be as free as possible from radioactive contamination by extracellular sources. For the sake of caution, however, the microspores were washed twice more with sucrose medium and then extracted twice with 2-ml portions of 70 per cent ethanol. The residue was then extracted with 10 per cent (w/v) trichloroacetic acid, and the acid-extracted residues were fractionated by the Schmidt-Thannhauser procedure after the lipid components had been extracted with a 1:3 ether:ethanol mixture. The soluble fractions were resolved by paper chromatography to identify the thymidine. Radioactivity was determined for all fractions.

RESULTS

Studies with Wheat Seedlings

The objective of these experiments was to determine whether thymidine could induce thymidine kinase formation in cells of higher plants. To fulfill this objective, without introducing complications which might arise either from a loss of enzyme-forming capacity due to differentiation or from the

shortness of the time during which such a capacity might be expressed, a tissue was chosen in which thymidine kinase was known to be formed and in which the cells were heterogeneous with respect to developmental stage. Although the fact that germinating wheat embryos form thymidine kinase would appear to contradict the purpose of the experiment, this was not actually so. Given the capacity to form thymidine kinase, the question could be asked whether exogenously supplied thymidine had any effect on enzyme formation. The alternative question—whether thymidine could induce enzyme formation in a tissue which normally lacks the enzyme—could not be easily answered because our testing of plant tissues which were suitable for experimental study (such as various regions of the young root) revealed no tissue in which thymidine kinase activity was absent. These experiments were intended to provide background information for microspore studies, but the results are of sufficient interest in regard to the phenomenon of enzyme regulation in general to merit description.

Forty-eight-hour-old wheat seedlings double their thymidine kinase but not total protein content within 24 hours (10). When the embryos are excised from such seedlings and grown in a nutrient medium for 4 to 6 hours, no increase in thymidine kinase activity is observed (Fig. 1). Whatever the cause of this sudden cessation in enzyme synthesis, it is not due to a general debilitation of the protein-forming machinery. The excised seedlings grow normally, though at a reduced rate, and, as may be seen in subsequent figures, synthesis of protein and of nucleic acid continues during the test period. If such excised seedlings are exposed to various concentrations of thymidine, significant increases in enzyme activity are observed (Fig. 1). The activities have been plotted in terms of protein to obviate differences arising due to variations in embryo size. The relationship is essentially the same if plotted on a per embryo basis. The extent of the increase obtained under culture conditions is best compared with that occurring in intact seedlings. As already stated, a doubling of thymidine kinase activity occurs normally during the growth interval from 48 to 72 hours. Assuming uniformity in rate, an increase of about 8 per cent would be expected in 2 hours. This expected rate is to be compared with the observed rate of 86 per cent for the excised embryos exposed to optimal concentrations of thymidine. From these experi-
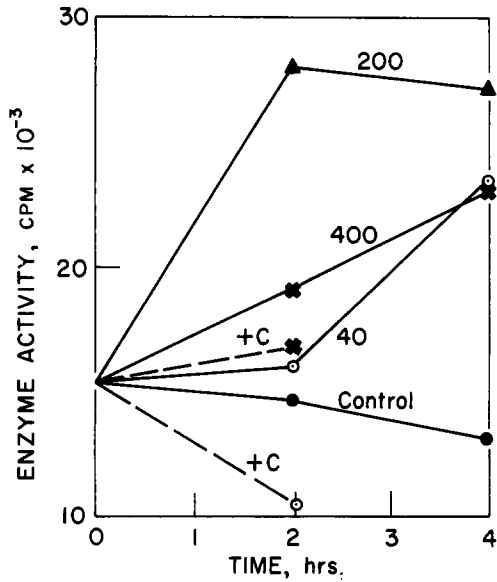
FIGURE 1 Effect of thymidine on thymidine kinase activity in wheat embryos excised from 48-hour-old seedlings. At zero time the embryos were exposed to various concentrations of thymidine and then removed after 2 or 4 hours for enzyme assay. The numbers next to each of the curves represent the concentrations (μg/ml) of thymidine in the incubation media. At two of the concentrations (40, 400), parallel runs were made in the presence of chloramphenicol (50 μg/ml); these are indicated by +C. Activity is expressed as counts per minute in thymidylic acid formed per milligram seedling protein in 20 minutes.
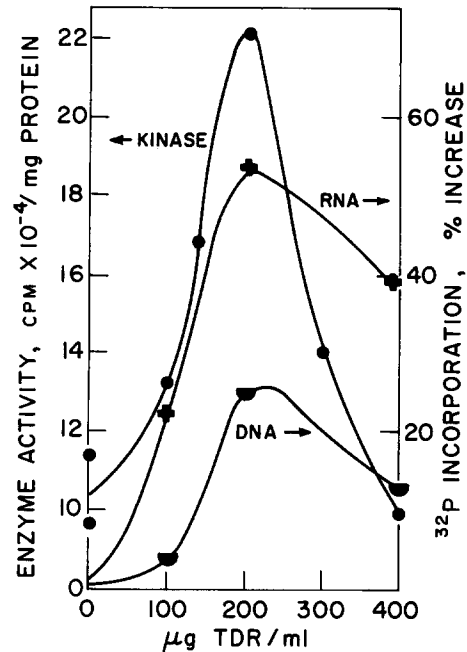
FIGURE 2 Response of excised wheat seedlings to the presence of various concentrations of thymidine (TDR) in growth medium. Conditions were similar to those described in Fig. 1, except for the addition of ^{32}P-phosphate to the medium. RNA and DNA were separated by alkaline hydrolysis as described under Methods. All excised seedlings were incubated for 2 hours. ^{32}P incorporation into nucleic acids is expressed as percentage of values found in control seedlings after the same period in culture.

ments we therefore infer that exogenously supplied thymidine does act as a stimulant to the formation of thymidine kinase. If equivalent concentrations of deoxyuridine are supplied to the excised embryos under the same conditions, no effect is observed. Stimulation of thymidine kinase formation would thus appear to be specifically effected by thymidine.

The patterns of enzyme increase shown in Fig. 1 are not open to a simple interpretation. In the presence of an optimal concentration of thymidine (200 μg/ml), the peak value is reached in 2 hours; no further increase is observed during the latter half of the incubation interval. The arrest is not due to physiological deterioration, since at other concentrations of thymidine the rate of increase in enzyme activity is either the same or greater during the last 2 hours. Other factors clearly enter into the achievement of what appears to be a steady state condition, but these are largely beyond the province of this communication. The curves in

Fig. 2 have been included to show that the rates of nucleic acid synthesis as measured by ^{32}P incorporation roughly parallel the rates of thymidine kinase activity when plotted against the external concentration of thymidine. The increase in thymidine kinase formation is thus associated with increases in other parameters of cell activity. The parallel responses of DNA and RNA synthesis to the level of exogenously supplied thymidine are interesting but unclear. Although a relatively simple feedback mechanism might be advanced to explain the DNA–thymidine kinase relationship, no obvious explanation exists for the relationship between thymidine and RNA. However, whether or not thymidine kinase is the limiting reaction in DNA synthesis in wheat seedlings, the parallel between enzyme activity which is measured in extracts and DNA synthesis which is measured *in vivo* reinforces the conclusion that
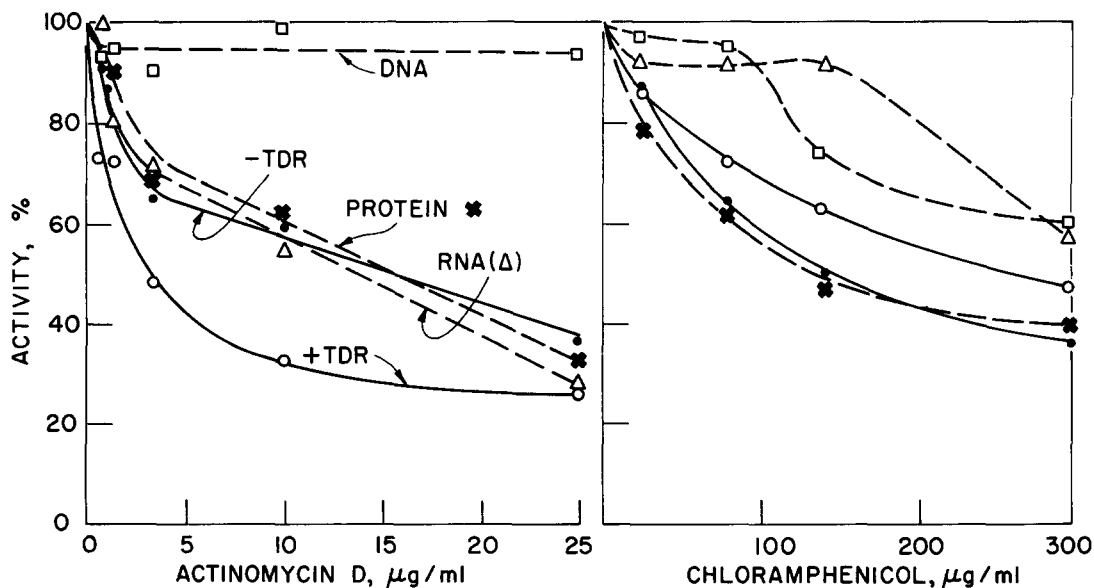
FIGURE 3   Responses of excised wheat seedlings to various concentrations of Actinomycin D and chloramphenicol. Conditions were the same as in preceding experiments except for the addition of antibiotics in the concentrations indicated. The seedlings were incubated for 2 hours at 25°C. Protein synthesis was measured by the incorporation of 14C-leucine; RNA and DNA synthesis by incorporation of 32P-inorganic phosphate. Solid lines represent thymidine kinase activities in the presence (open circles) and absence (closed circles) of thymidine (TDR). Values are plotted as percentages of activities in seedlings incubated without the antibiotics. One interesting feature of the data is the differences in response of +TDR and —TDR seedlings to actinomycin and chloramphenicol. The inhibitory effect of chloramphenicol is counteracted by thymidine, but the patterns of response to increasing concentrations of the drug are similar. At low levels of Actinomycin D, on the other hand, inhibition is much greater in the TDR-exposed seedling, indicating a greater sensitivity of the induced system to the action of the drug.

exogenously supplied thymidine has a profound effect on this particular aspect of cellular activity.

The experiments just described do not distinguish between enzyme activation and enzyme synthesis, although previous studies on germinating embryos point to an actual synthesis (12). Support for the latter conclusion may be drawn from another set of experiments in which chloramphenicol and Actinomycin D were used. In Fig. 3, the inhibitory effect of the drugs on thymidine kinase activity may be seen. Two types of response are evident in the results. The first of these relates to seedlings which were not exposed to thymidine, the "controls." In such controls both reagents cause a loss in activity after two hours' exposure. Thus, either the reagents destroy the enzyme in vivo or the enzyme is continuously turning over and the reagents inhibit an otherwise unobserved synthesis. The first alternative would be inconsistent with the recognized action of these drugs; the second alternative would be compatible

with the behavior of microspores, in which enzyme synthesis and removal have been demonstrated (1, 2). The fact that in vivo amino acid incorporation into protein parallels the course of thymidine kinase activity with respect to reagent concentration supports the second alternative.

A distinctive response may however be noted if the activities of seedlings grown in the presence and absence of thymidine are compared. Although the two groups behave similarly toward chloramphenicol, the thymidine-exposed group is far more sensitive to Actinomycin D than are the "controls." Such differential sensitivity has previously been noted in lily microspores with respect to general protein synthesis and endogenously induced thymidine kinase synthesis (1). If the assumption is made that Actinomycin D acts at the level of gene transcription, one may explain the results by supposing that the sluggish response in the "controls" is due to the presence of message RNA which continues to be translated, whereas
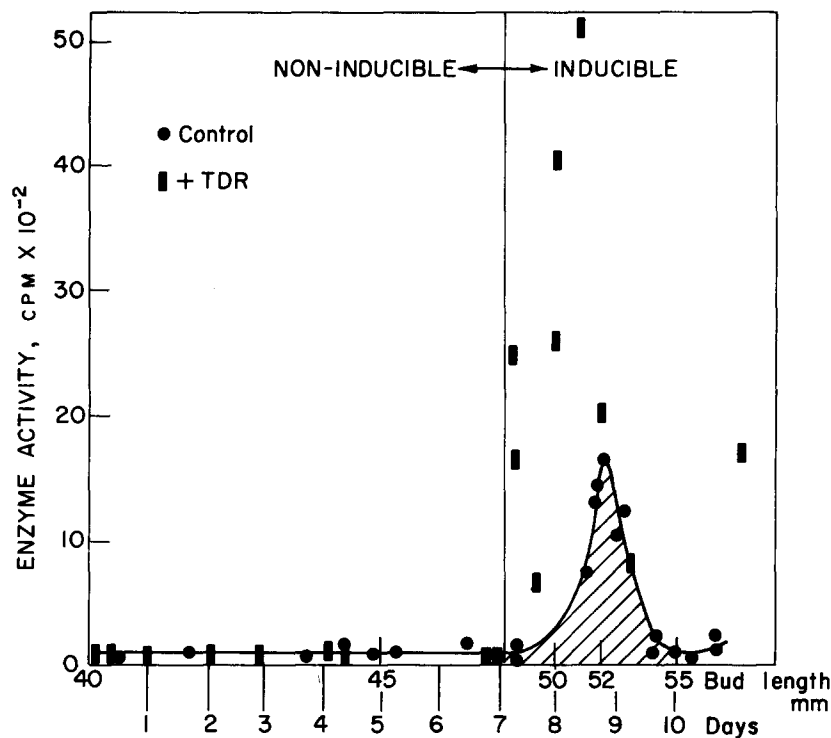
Figure 4   Thymidine kinase activities in microspores of *Lilium longiflorum* (var. Ace) at different developmental stages and under conditions of exposure to different levels of thymidine. The kinase activities are expressed as counts per minute in thymidylic acid formed in 20 minutes by microspores from *one* anther. Solid circles represent control values; their pattern is the same as that previously reported for the Croft variety (1). The bars represent values for microspores which had been removed from anthers cultured in the presence of thymidine (50 μg to 2.5 mg/ml) for 4 to 6 days before removal. The significant feature of the results is that no effect of thymidine is observed until 1 to 2 days before the normal appearance of enzyme. The abscissae are marked in both bud lengths (in millimeters) and days. The bud length serves as a developmental index of microspores at the time when anthers are removed from the plant and put into culture.

the heightened *de novo* synthesis induced by thymidine is readily susceptible to inhibition. The relationship nevertheless appears to be complex, and the single conclusion which we wish to draw from these studies on wheat seedlings is that thymidine can and does act as an effective inducer of thymidine kinase. On the assumption that this conclusion is correct, the question concerning the inducibility of thymidine kinase in relation to the cell cycle may be approached experimentally.

Studies with Lily Microspores

In certain respects the microspores of lily are a material of choice for studying the question of inducibility and stage of cell development. The interphase between the completion of meiosis and the beginning of DNA synthesis is about 20 to 22 days.

During this interval various synthetic events are occurring, but thymidine kinase activity is found at appreciable levels for only one day. If uniform conditions of growth are maintained, the time of enzyme appearance is readily predictable (1). Moreover, such appearance is due to a *de novo* synthesis of enzyme protein (1). Since about 5 per cent of the G1 interval is associated with enzyme formation, one may readily inquire as to whether the enzyme can be induced during the remaining 95 per cent. The available data provide no indication, since under normal conditions the appearance of enzyme is associated with the appearance of a pool of deoxyribosides containing thymidine (9). Basically, the design of the experiments to be reported was to administer thymidine to microspores at various stages of interphase and to measure the

TABLE I

Transformation and Retention of Exogenously Supplied Thymidine by Microspores
and Somatic Tissues of Cultured Lily Anthers

Fraction	Bud length (mm)				
	23-25	25-30	35-40	40-45	45-55
Somatic tissues					
Ethanol-soluble	18,145	15,390	3,210	2,668	3,444
Acid-soluble	4,400	42,909	10,182	26,400	20,000
Lipid	1,118	1,000	1,069	2,398	6,068
RNA	20	55	45	70	60
DNA	25	79	—	75	126
Protein	280	218	264	140	—
Total	23,988	59,651	14,770	31,751	29,698
Microspores					
Thymidine	1,497	1,636	1,231	1,365	1,284
Acid-soluble	160	145	145	480	1,440
Lipid	18	49	139	72	126
RNA	—	—	—	—	—
DNA	160	49	—	—	220
Protein	—	—	—	—	—
Total	1,835	1,879	1,515	1,917	3,070

All values are expressed as $\mu\mu$moles thymidine incorporated by microspores or somatic tissues from the equivalent of 10 anthers, which had been exposed to a concentration of 400 μg thymidine per ml for 4 days. Details of the procedure are discussed in the text. The "ethanol-soluble" fraction is obtained by extraction with 70 per cent ethanol. This extractant removes thymidine and thymidylates quantitatively from these tissues. Under microspores the fraction is listed as thymidine because the values were obtained after chromatographic resolution of the fraction. Eighty per cent or more of this fraction consisted of thymidine; the remaining radioactivity was therefore neglected in computing totals. The components of the ethanol-soluble fraction in the somatic tissues were not resolved by chromatography.

thymidine kinase content after given intervals of exposure.

Groups of anthers were excised at different developmental stages and grown in the presence of thymidine for 4 to 6 days prior to removal and isolation of the microspores. The length of exposure was considered to be ample for kinase induction, since previous studies had shown that the entire process of enzyme formation occurs within 12 hours (1). A broad range of thymidine concentrations was tested (0.025 to 10 mg/ml) because of the relatively sluggish uptake of solutes by excised anthers and also because of the probability that much of the thymidine absorbed might be transformed by the anther tissues (11). This latter point will be returned to later. Microspores were tested for inducibility from just after completion of meiosis until the period of DNA synthesis, an inter-

val of approximately 22 days. The responses of the microspores in early interphase to thymidine exposure are not documented because they were uniform and identical with those of the later but still early stages reported in Fig. 4. No appreciable thymidine kinase activity could be detected in these microspores irrespective of the time of exposure or concentration of thymidine used. For the 60 anthers removed from buds with lengths between 24 and 40 mm which were tested, the highest activity recorded was 240, a value which may be seen to be small by referring to Fig. 4.

A more detailed summary of the results obtained from studies of microspores removed from buds 40 to 60 mm in length is given in Fig. 4. To orient the reader, the time intervals corresponding to bud lengths are provided in the abscissae. Bud length is used to identify the developmental stage

at the time of removing the anthers from the plant, whereas time is used to measure development in the cultured systems. The interconvertibility of the two parameters has been previously discussed (1). The points on the curves are the values of thymidine kinase activity in the microspores at the termination of the incubation period. Thus, if a set of anthers was grown for 4 days in the presence of thymidine and the kinase activity is recorded at the equivalent of 45 mm bud length, this means that the anther set had been excised from the plant 4 days earlier at a bud length of 40 mm. Control values were obtained from anthers treated identically except for the omission of thymidine from the medium. In plotting the data, no indication is given as to whether the exposure was for 4 or 6 days, since no differences in response were observed. The critical factor was the time at which the cultured anthers were removed; thymidine concentration also showed little effect on the characteristic response. This characteristic response is obvious on inspection of the figure. No increase in thymidine kinase activity was found in any of the microspores removed from anthers below the equivalent of a bud length of 49 mm. On the other hand, whereas the control cultures showed the typical single peak of thymidine kinase activity at 52 mm, the interval between 49 and 52 mm—a span of about 2 days—is filled with high activities from those sets of anthers which had been treated for 4 to 6 days with thymidine at concentrations of 0.05 to 5.0 mg/ml. On the assumption that the thymidine is absorbed more or less equally by microspores at all stages of development, the conclusion to be drawn from this group of experiments is that exogenously supplied thymidine is an effective stimulant to thymidine kinase formation only during a very restricted portion of the cell cycle, a portion which is somewhat greater than that encompassing the normal appearance of thymidine kinase activity.

A more direct test for the validity of this conclusion could be obtained by culturing the microspores themselves in the absence of their otherwise neighboring somatic cells. Thus far, however, the techniques for doing so have proved inadequate. Inasmuch as the results obtained were based on cultures of whole anthers, two major forms of regulation could be advanced to explain the restricted action of thymidine. One form would be intercellular, the other intracellular. The first would require that biochemical processes out-

side the microspores control the availability of thymidine; this explanation would be compatible with earlier observations that the normally formed deoxyriboside pool first appears outside the microspores (9). The second explanation would require that the inducibility (not the induction) of thymidine kinase be independent of the concentration of thymidine within the microspores. It is possible to make a choice between these alternatives by measuring the intracellular concentrations of thymidine at various stages in the cell cycle after exposure of the anthers to thymidine. The results of such a set of experiments are provided in Table I. The bud lengths indicate the interval of development during which the cultured anthers were exposed to thymidine. Inducible microspores are therefore found in the last column. One general feature of the table has already been discussed in another context (10): the lack of any direct relationship between the metabolic activity of somatic tissues and that of the microspores. The variations in thymidine uptake and conversion in somatic tissues are not matched by the microspores. Leaving aside, however, the complex pathways along which thymidine may be metabolized (see Table II), the point of special interest in Table I is the fact that the amount of thymidine found in the microspores is more or less the same at all intervals of development. A differential

capacity for absorbing and retaining thymidine as the immediate cause of inducibility is therefore unlikely.

This conclusion is supported by the additional observation that high concentrations of thymidine have the same effect on enzyme formation in the microspores as they do in wheat embryos. At concentrations of 10 mg/ml in the medium, the highest activity recorded is appreciably lower than the peak value for the controls (Table II). The concentrations as stated are somewhat deceptive, since the total uptake by the entire anther was of the order of 4 per cent, and of this less than 10 per cent reached the microspores. If, however, the amount of thymidine reaching the microspores were limiting, one would expect that stimulation would be enhanced by increasing the concentration of thymidine supplied. This, clearly, does not occur. The highest values for thymidine kinase activity observed in microspores exposed to the two most concentrated solutions of thymidine were below those for the unexposed ones. Moreover, the peak thymidine kinase values bear no simple relationship to the concentration of thymidine in the medium. Most probably, the values observed reflect the time at which the microspores were removed from culture relative to the characteristic rising or descending curve in thymidine kinase activity. To demonstrate this particular point for each of the concentrations tested would have required about ten times as many anthers as were actually used, a total of approximately 3000. In view of the results obtained, such an experiment did not seem to be worth the undertaking.

DISCUSSION

The principal objective of this study was to determine whether inducibility of an enzyme in a po-

tentially inducible cell is solely a function of the presence of inducer. Superficially, the answer would appear to be clear. At least one enzyme, thymidine kinase, cannot be induced by thymidine except at a highly restricted interval of the cell cycle. This evidence implies the presence of some other regulatory mechanism which governs the effectiveness of the inducer-enzyme relationship so well documented in microorganisms. The relevance of such a mechanism to developmental phenomena is patent and needs no elaboration. With respect to the specific phenomenon studied here, the present study does not aim to encompass all those aspects which give it a configuration different from that commonly encountered in microorganisms. The tight temporal control of enzyme formation is matched by a tight temporal control of enzyme removal. A very limited insight into the mechanisms effecting a cessation of enzyme synthesis and removal of enzyme has been acquired in a number of experiments (2). The present study may add a little understanding to the early phase of the phenomenon—enzyme induction. Whether one may translate the apparent temporal restriction of inducibility in terms of changes at the chromosomal level remains to be seen. Speculations on this topic are not lacking. However, irrespective of the basis for the observed restriction, the results do strongly suggest that the kind of protein a cell may synthesize at a particular time in its life cycle is governed not only by the composition of its substrate pool but also by other factors which reflect the temporal control of cell development.

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