VICIA FABA ROOT DEVELOPMENT

VICIA FABA ROOT DEVELOPMENT: A PARTIAL ANALYSIS WITH 5-AMINOURACIL

by

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SCOPE AND CONTENTS:

Experiments described in this study were carried out in order to (a) extend previous studies on the analysis of the response to 5-aminouracil to small primordia and primary roots of Vicia faba and (b) to determine whether treatment with deoxynucleosides would alter the cell's response to 5-aminouracil, either in DNA synthesis or at the S - G_2 transition block.

The present study reveals that the population heterogeneity, which was found to exist in lateral roots, just emerged roots, very large primordia, and large primordia, is also present in primary roots and small primordia; also, that the ratio of fast cycling cells to slow cycling cells

remains constant throughout root development.

All root meristems of V. faba exhibited the same general pattern of recovery after exposure to 5-aminouracil, i.e. the initial drop in mitotic index was followed by an increase to a value above controls. Variations between the different developmental stages were seen in (a) the time of onset of recovery, (b) the time of the appearance of the peak of mitoses, and (c) the degree of synchronization of recovery.

Data from post-5-aminouracil nucleoside treatments revealed that the nucleosides were unable to produce a reversal of the 5-aminouracil-induced block at the S - G_2 transition, but were able to increase the rate of DNA synthesis and thus alter one of the effects of 5-aminouracil on cell cycle kinetics.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (continued)

INTRODUCTION

In a seed of Vicia faba only one root is present and it develops, as a seed germinates, into the primary root. Secondary, or lateral, roots originate, as primordia, in the pericycle and endodermis. The primordia and the laterals, once they have emerged through the epidermis of the primary root, grow perpendicular to the long axis of the primary root. Growth of a lateral root is due to cell proliferation and cell elongation; however, it also involves many physiological changes in the proliferating cells.

A primordium is initiated opposite a xylem pole. About 24 pericycle cells are stimulated to divide (Davidson, 1961, 1965) and form a primordium, which is first evident as a small bump. Divisions occur periclinally and anticlinally and the primordium increases in length and diameter. Primordial cells can complete a cell cycle in 12 hours (MacLeod and Davidson, 1968) and the primordium grows rapidly; it consists of approximately 500 cells in 2 to 3 days. At this time polarity is established within the primordium and subsequent growth is mainly along one axis. Within a further 24 hours the primordium has 1,500 cells or more and is classed as a large primordium.

By 5 days after initiation, the primordium is very

large and lies just beneath the root epidermis. Just before emergence of the primordium, cell elongation begins and the primordium consists of both dividing and differentiating cells. After the primordium breaks through the epidermis, it is classed as a just emerged root and subsequent cell division and cell elongation produce a lateral root.

The meristems of V. faba are complex and consist of two sub-populations of proliferating cells: (a) fast cycling cells, which constitute 75 to 80% of the cells in division at any one time and, in lateral roots, make up approximately 50% of the total cell population; and (b) slow cycling cells, which make up 20 to 25% of the cells in division and are about 35% of the total cell population. Mean cycle time of fast cycling cells is 14 hours in lateral roots (Webster and Davidson, 1968), 12 hours in small primordia (MacLeod and Davidson, 1968), approximately 12 hours in large primordia, and 17 hours in primary roots (Davidson, 1971). In addition to the proliferating cells, primary and lateral roots also have non-dividing cells; these appear not to be present in the primordia. The differences in normal mitotic index in small primordia (about 12), large primordia (about 10), laterals and primary roots(about 8) reflect changes in cell cycle duration and the relative frequencies of dividing and non-dividing cells.

The morphological changes occurring in primordia have been correlated with physiological changes in the

meristematic cells. Different stages of primordial growth differ in the response to indole-acetic acid (IAA), colchicine, 5-fluorodeoxyuridine (FUdR), and 5-aminouracil (5-AU); they also differ in their ability to incorporate ^{3}H-TdR (see review by Davidson, 1971). It is the differences in response to treatments with 5-AU, exhibited by the various developmental stages, that are to be reported here.

The response to 5-AU has been studied using both short, i.e. 1 to 6 hours, and continuous treatments. Continuous treatment of whole roots of V. faba with 5-AU caused a reduction in the mitotic index. The decrease in mitotic index occurred sooner and to a greater degree in lateral roots than in large and very large primordia. Also, recovery in the presence of 5-AU occurred sooner in the primordia than in the lateral roots. With shorter exposures to 5-AU, at concentrations varying from 250 to 1,500 parts per million, the recovery differed in the lateral roots, just emerged roots, very large primordia, and large primordia (Socher, 1970).

5-AU, a pyrimidine analogue, was shown by Smith et al. (1965) to reduce the mitotic index of the lateral root meristem of Vicia faba to zero after a 24 hour treatment. After removal of the root from the analogue a partially synchronous peak of mitoses was observed. Duncan and Woods (1953) reported a similar suppression of mitotic activity in Allium cepa after a 24 hour treatment with 5-AU. The decreased

mitotic activity was accompanied by a reduced rate of elongation of the root, which they found could be overcome by the addition of folic acid and thymine to the 5-AU. They postulated that the 5-AU interferes with thymine synthesis and folic acid metabolism. If this is the case, then there should be an abundance of cells in the 2C condition, which is what they found spectrophotometrically.

Later workers found that a partially synchronous peak of mitoses occurred if Vicia or Allium were grown in 5-AU for extended periods of time (Jakob and Trosko, 1964; Mattingly, 1965; Wagenaar, 1966). It was also demonstrated that DNA synthesis actually continues in the presence of 5-AU, although it is slowed to 60 to 75% of its normal rate, producing a longer S period (Jakob and Trosko, 1964; Prensky and Smith, 1965). The increase in the duration of S could not produce the partially synchronous peak of mitoses; it was, therefore, postulated that cells must be blocked at some other point in interphase.

Mattingly (1966) has demonstrated that cells proceed from G_1 into S at a normal rate for the first 8 hours of 5-AU treatment; since this is a longer period of time than G_1, cells must also be leaving mitosis at a normal rate. Therefore, 5-AU has no effect on the duration of mitosis or G_1 and the block must lie elsewhere.

Wolff and Luippold (unpublished, 1964, quoted by Mattingly in "Cell Synchrony"), on the basis of a post-labeling

5-AU treatment showed that few labeled cells divided 5 to 9 hours after the beginning of 5-AU treatment and they concluded that 5-AU blocks cells in G_2. Socher and Davidson (1971) have demonstrated that cells in G_2, when given 5-AU, proceed at a normal rate through G_2 and into division. In fast cycling cells of V. faba the mean duration of G_2 + mitosis/2 is 4.3 hours (Webster and Davidson, 1968) and these cells constitute 75 to 80% of the cells in mitosis at any one time. After 5 hours in 5-AU, the mitotic index in lateral roots is about 20% of the control value and it was suggested that (a) the cells in division after 6 hours in 5-AU were slow cycling cells and (b) 5-AU blocks cells at the S - G_2 transition (Socher and Davidson, 1970).

On the basis that 5-AU blocks cells at a particular point in the cell cycle without affecting other stages, 5-AU can be used as a tool in cell population analyses (Socher and Davidson, 1970, 1971).

Various workers have reported that thymidine, thymidylic acid, folic acid, thymine, and cytidine, when given with 5-AU or immediately after 5-AU antagonizes its effects or produces a faster recovery (Duncan and Woods, 1953; Jakob and Trosko, 1964; Prensky and Smith, 1965; Wagenaar, 1966). In most instances these compounds were given in concentrations equimolar to the 5-AU solution, i.e. 1.1×10^{-5} to 4.0×10^{-3} M; at these concentrations the compounds alter normal cell cycle kinetics and the results, as a consequence, are sometimes

difficult to interpret. Similarly, the concentration or duration of treatments with 5-AU has often been greater than that capable of inhibiting mitosis and inducing a subsequent synchrony of cell division.

5-AU has been used to determine the duration of G_2 in various stages of lateral root development of V. faba (Socher and Davidson, 1970, 1971), i.e. lateral roots, just emerged roots, very large primordia, and large primordia. The study reported here extends previous studies and describes the response of small primordia and primary root meristems to 5-AU. The effect on meristems inhibited with 5-AU of treatment with a mixture of deoxynucleosides was also determined. It was found that mitotic activity was not immediately restored, suggesting that the metabolism of precursors of DNA is not the cause of the 5-AU block of the cells at the S - G_2 transition point. The results also show that even with short treatments of 3 hours, primordia at different stages of development respond differently to 5-AU and they confirm that proliferating cells undergo, while still mitotically active, significant physiological changes.

MATERIALS AND METHODS

Germination and culturing of beans.

Vicia faba L. seeds were soaked in distilled water for 24 hours. The testas were removed and the beans were planted in moist sand that had previously been washed and sterilized. After 3 days, at 20°C., the primary roots were approximately 5 centimetres long. The germinated seeds were removed from the sand, washed, and suspended in plexiglass tanks containing one-half strength Hoagland's solution (culture medium). The culture medium was changed every 24 hours. The beans were grown and treated at 20°C. ± 1C.° in the dark.

Treatments with 5-AU and/or a nucleoside solution were begun after the lateral roots had emerged, though in one experiment the beans were treated before the lateral roots had emerged. This was a preliminary experiment to determine if there was a significant difference between the results of a 6 hour and a 3 hour treatment with 5-AU. After treatment, the beans were washed and transferred back to the culture medium for recovery. Fixations of whole roots were made at varying times after treatments; the times are referred to in the individual experimental procedures.

Treatments.

Beans were treated with solutions of 5-AU, 500 p.p.m. (3.93×10^{-3} M), for 3 or 6 hours. Some beans were also treated with solutions of nucleosides containing deoxyadenosine (AdR), deoxycytosine (CdR), deoxyguanosine (GdR), and deoxythymidine (TdR). The nucleoside solution contained either 10^{-7} M or 10^{-6} M amounts of each nucleoside. Nucleosides were bought from Nutritional Biochemicals Corporation and from Mann Research Laboratories. Treatments with the nucleosides were for 1 hour or 3 hours. Treatments of 1 hour duration were used on roots previously treated with 5-AU for 3 or 6 hours and on untreated roots. The 3 hour nucleoside exposure occurred simultaneously with a 5-AU treatment.

Fixation and staining.

Whole roots were fixed in a chilled solution of absolute ethanol - glacial acetic acid (3:1 by volume), with a few drops of formalin added.

Roots were prepared for staining by first washing for 2 hours in 3 changes of water, then hydrolyzing for 9 minutes with 1N HCl at 60°C. The roots were stained with Feulgen's reagant for 1 to 1½ hours. Permanent squash preparations were then made using one meristem per slide; with small primordia, 2 or 3 meristems were used for each preparation.

Determination of mitotic index.

The mitotic index for lateral roots, just emerged roots, very large primordia, and large primordia was determined in the following way: the number of mitoses in a 1,000 cell sample was recorded from each of 5 slides per root type. Each mitotic index is based on 5,000 cells, unless otherwise indicated.

The mitotic index for primary roots was determined by recording the number of mitoses in five 1,000 cell samples taken, at random, from 2 or 3 roots.

The mitotic index for small primordia was determined by recording the number of mitoses in five 500 cell samples taken from 5 meristems.

Response to 5-AU and nucleosides was studied in the following meristems:

Primary roots. The first root to appear from the germinating seed, growing directly downwards, containing 2,000 to 3,000 meristematic cells.

Lateral roots, Roots that are 1 centimetre or more in length and have emerged from the primary root.

Just emerged roots. Roots that have just broken through the epidermis of the primary root.

Very large primordia. Conical-shaped meristem found just beneath the epidermis of the primary root.

Large primordia. Rounded meristems found under the epidermis, smaller than the very large primordia, consisting

of more than 1,500 cells.

Small primordia. A slight bulge in the pericycle, consisting of approximately 500 cells.

Figure 1. Diagram of whole root to illustrate the meristems studied.

a lateral root meristem

b just emerged root meristem

c very large primordium

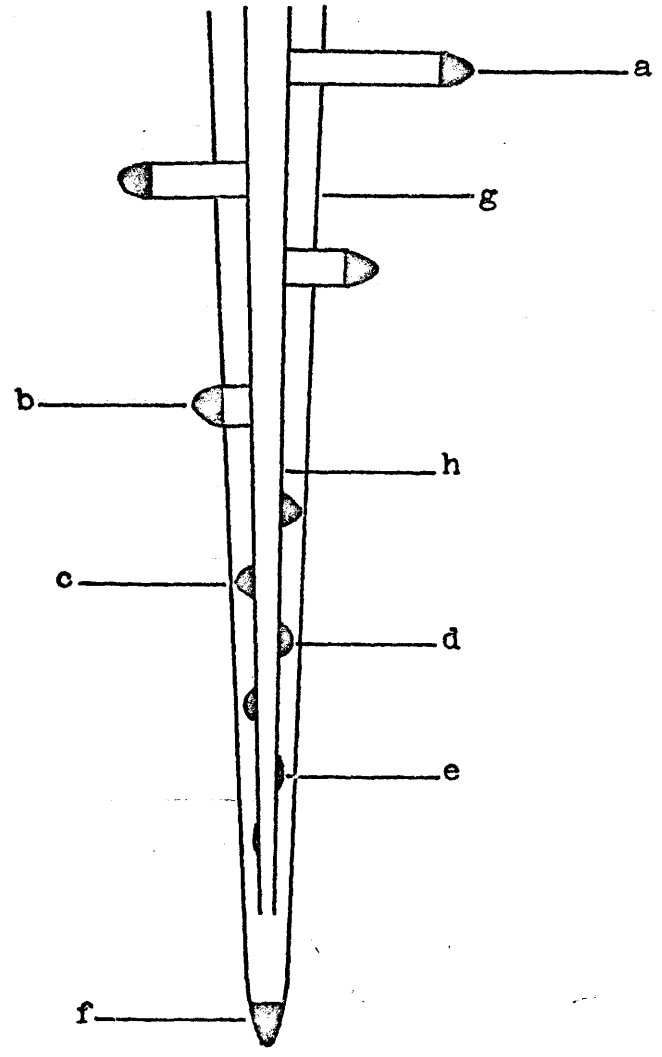
d large primordium

e small primordium

f primary root meristem

g epidermis

h pericycle



RESULTS

A. 5-aminouracil for 6 hours: post-treatment with nucleosides (10^{-7} M).

Growing primary roots that had developed lateral roots and primordia were treated with 500 p.p.m. 5-AU for 6 hours. Half of the roots were rinsed, replaced in culture tanks, and allowed to recover; the remaining roots were transferred to a solution of nucleosides, AdR, CdR, GdR, and TdR, each at a concentration of 10^{-7} M. After 1 hour these roots were rinsed and returned to the culture tanks to recover. Whole roots were fixed immediately after the end of the 5-AU treatment; and at 1, 6, 9, 12, and 15 hours fixations were made of roots treated with 5-AU or 5-AU and the nucleoside solution.

Primary roots. Immediately after the 5-AU treatment, the mitotic index of the primary root meristem was 0.82. During the first 9 hours of recovery the mitotic index fluctuated between 0.20 and 0.82. This fluctuation was found in both sets of primary and lateral roots (Tables 1a and 2a); this suggests that the mitotic index remains at a consistently low level for several hours after the end of the 5-AU treatment. If an increase in mitotic index had occurred between two fixations, 3 hours apart, it means that (a) the

degree of synchrony must be very high and (b) the duration of mitosis must be less than 3 hours. It seems unlikely that such a rise in mitotic index could have occurred and been missed since at later times, 12 and 15 hours, cells entering mitosis are not in absolute synchrony. It is concluded, therefore, that the low mitotic indices do represent a low level of mitotic activity for at least 9 hours from the end of a 6 hour treatment with 5-AU.

Some cells in mitosis were present in all meristems during the first 9 hours of recovery; these are cells in which the duration of G_2 is longer than the period of treatment and recovery in the roots examined at a particular time. From other experiments it is known that the slow cycling cells of V. faba have a G_2 duration in excess of 12 hours (Webster and Davidson, 1968).

Twelve hours after the end of the 5-AU treatment the mitotic index increased slightly (Table 1a), due mainly to a rise in the number of prophases; it is 12 per 1,000 cells at 12 hours compared with 2.7 per 1,000 cells at 9 hours. These are the first cells to overcome the block imposed by 5-AU. Within a further 3 hours the meristem of the primary roots showed a partially synchronized peak of mitoses; mitotic index equals 10.44. This increase, from 1.44 at 12 hours, is due mainly to a large increase in the number of prophases but metaphases, anaphases, and telophases are all much more frequent than at 12 hours (Table 1a). It appears

that a rise in prophase numbers is followed in 3 hours, or less, by a rise in the frequency of all mitotic stages. This argues against any suggestion that a rise in mitotic index is due to an accumulation of cells in any one stage of mitosis.

The level of mitotic activity remains low for at least 9 hours after the end of a 6 hour treatment and then an increase to a partially synchronized peak of mitoses at 15 hours shows that primary root meristems respond in a similar way to lateral roots (Socher and Davidson, 1971). The response of primary and lateral roots is, however, not identical, as is shown later.

Lateral roots. The mitotic index is low in 5-AU treated lateral root meristems for 9 hours and then begins to rise. After a 12 hour period of recovery it has risen to 17.68 (Table 1a) and by 15 hours the mitotic index is 23.94. At both 12 and 15 hours all stages of mitosis are much higher than they are at 9 hours, showing that cells entering mitosis are not delayed in prophase but progress at about the normal rate to metaphase and subsequent phases of mitosis.

The increase in mitotic index is not only higher in lateral roots than in the primary root meristem by 15 hours, 23.94 compared with 10.44 (Table 1a), but it also begins earlier. It is 17.68 at 12 hours in laterals but only 1.44 in the primary root. In their rate of recovery, the two types of meristems show signs of an inherent physiological difference.

Just emerged roots. Although this stage is similar, anatomically, to the laterals in that it has broken through the epidermis of the primary root, it can be regarded as a distinct stage in the development of the lateral root. Just emerged roots are those with only the meristem protruding past the epidermis. Anatomically and physiologically, the just emerged meristem is a transitional stage between a very large primordium and a lateral root. An example is seen in its response to 5-AU.

The meristem of the just emerged roots showed a low mitotic index for the first 6 hours of recovery (Table 1a), similar to the lateral and primary roots. At 9 hours of recovery the mitotic index had reached 11.94; the increase in the mitotic index was due not only to a large increase in the number of prophases, but also metaphases, anaphases, and telophases. By 12 hours the number of prophases and metaphases is lower than at 9 hours and anaphases and telophases have increased slightly in number. The change in the frequency of the various mitotic stages indicates that the cells move through mitosis; there is no evidence that 5-AU delays progress through division stages. At 15 hours the mitotic index is 3.34, the wave of cells that entered mitosis together has now passed on into interphase.

Since a just emerged root has broken through the primary root epidermis there should be no physical barrier, such as primary root tissue, that would prevent the entry

of 5-AU. But, although the just emerged root has broken through the epidermis, it does not show the same recovery pattern as the laterals. The just emerged meristem does not reach the same high peak of mitotic synchrony as do the laterals. The peak of divisions occurs 3 hours earlier in the just emerged roots as does the beginning of recovery. On the basis of these differences in recovery from 5-AU treatment, it is concluded that the just emerged and lateral roots are physiologically different from each other.

Very large primordia. At the end of the 5-AU treatment the mitotic index was 1.96; this decreased to 1.34 at 1 hour. By 6 hours the number of prophases had increased to 41 per 1,000 cells compared with 9 per 1,000 cells at 1 hour. Some cells have begun to recover from the effects of 5-AU and come into division in less than 6 hours, but the majority of cells are not seen in division until 9 hours, when the mitotic index is 17.24 (Table 1b). The change in the frequencies of the different stages of mitosis at 9 and 12 hours verifies the conclusion drawn earlier that cells are not held in mitosis, but move through at a normal or near normal rate.

At 15 hours the mitotic index has decreased to 3.10, i.e. below the normal level of 10 to 11 (Socher and Davidson, 1970), suggesting that nearly all the fast cycling cells that were blocked by 5-AU and subsequently proceeded to a partially synchronous division have completed one division. The

metabolic block imposed by 5-AU does not completely synchronize the population of cells in recovery; the variability of G_2 + mitosis/2 still exists, otherwise the mitotic index would be very high at one fixation only, when the synchronized population divided; this is not seen.

If the recovery curve for the just emerged roots is compared with that for the laterals and very large primordia (Figures 2ii, 2iii, and 2iv) it is obvious that the just emerged root resembles the very large primordia much more than the laterals in its recovery from the 5-AU treatment. In fact, the just emerged root may be, physiologically, a very large primordium that has broken through the epidermis, rather than a small lateral.

Large primordia. The recovery of the large primordia for the first 6 hours is almost identical to that for very large primordia (Table 1b); the frequencies of the stages of mitosis are also very nearly identical. The partially synchronous peak of mitoses at 9 hours is slightly lower, 12.72 compared with 17.24 in very large primordia, but again the frequencies of mitotic stages are the same. On the basis of time of onset of recovery from 5-AU treatment and degree of synchrony, very large primordia and large primordia appear to be physiologically identical. This is also indicated by the results from the roots fixed at 12 and 15 hours.

The mitotic index of the post synchronous peak is 4.33,

higher than would be expected if it was made up entirely of slow cycling cells (20% of the population of cells would produce a mitotic index of approximately 2). Since some of the cells of the primordia are known to have a cycle time of 12 hours, the 15 hour value could consist of cells that are undergoing their second division since the end of the 5-AU treatment. The mitotic index during the first 6 hours of recovery in very large primordia, large primordia, and small primordia are not as low as in other stages of development and since the ratio of fast to slow cycling cells remains constant, the higher mitotic index must be made up of cells that have finished G_2 and are now in division.

Small primordia. The mitotic index in small primordia immediately after the 6 hour treatment with 5-AU is 1.56 (Table 1b). On the basis of the response shown by laterals (Socher and Davidson, 1971) it is concluded that even in small primordia there is a sub-population of cells that is slow cycling. A rough estimate based on a mitotic index of 11 in untreated small primordia (Davidson, MacLeod, and Webster, 1968) is that slow cycling cells make up approximately 14% of the population in division under normal conditions. This is a slightly lower value than that found for primordia at later stages of development, i.e. approximately 18%, but it is of about the same order of magnitude. The 5-AU treatment shows, therefore, that the small primordia are heterogeneous for cell cycle duration; even at the initiation of primordial

development the population of cells exhibits a physiological differentiation of mitotically active cells.

Since the proportion of fast to slow cycling cells remains constant throughout the development of the lateral root, whatever mechanism controls the movement of cells from the fast cycling to the slow cycling sub-population must be present in all stages of root development.

One hour after the end of treatment the mitotic index is 2.96. This is slightly higher than the mitotic indices in very large and large primordia after 1 hour of recovery (Table 1b). A similar increase in mitotic index in small primordia is seen after a 1 hour treatment with nucleosides (Table 2b) and it appears that some cells in the small primordia have overcome the block imposed by 5-AU and are moving into mitosis. By 6 hours a noticeable recovery can be seen, mitotic index is 4.57; similar values are found in large and very large primordia at this time (Table 1b). A partially synchronized peak of mitoses occurs after 12 hours of recovery, mitotic index equals 16.12, and this is 3 hours later than the peak in large and very large primordia. However, recovery had begun by 9 hours, when mitotic index equaled 11.30; in small primordia it appears that the degree of synchronization induced by 5-AU is less marked than in large primordia and synchronization is spread over 3 hours.

From these results it is concluded that a 6 hour treatment with 5-AU, 500 p.p.m., causes the mitotic index of

the meristem to drop to a level equivalent to the mitotic index that would be found if the only cells in division were from the slow cycling sub-population, i.e. 15 to 20% of the control values. The mitotic index remains at this low level for less than 1 hour to more than 12 hours, depending upon the developmental stage of the root. When recovery from the effects of 5-AU was seen it was in the form of a partially synchronous peak of mitoses occurring between 9 and 15 hours after the end of treatment; the time taken for recovery varies with the developmental stage examined.

All stages examined have the same general pattern of recovery; they differ in certain specific details as follows:

1. Mitotic index. The extent and time of recovery are related, to some extent, to the mitotic index seen immediately after treatment with 5-AU. This value is different in different meristems, for example, 0.82 to 1.04 in primary, lateral, and just emerged roots and 1.56 to 2.42 in very large, large, and small primordia. But control values differ in the different meristems and the crucial point is that the percent reduction of the mitotic index by 5-AU is approximately the same in all stages.

2. Ability to recover. All meristems have the ability to recover from the effects of 5-AU since at some time after treatment there is an increase in the number of cells entering mitosis. The ability to recover varies from stage to stage; this is shown by the cumulative mitotic indices for the six

different stages examined (Table 3). By 6 hours, the meristems fall into 3 classes: (i) cumulative mitotic index is less than 2.0 (primary and lateral roots), (ii) cumulative mitotic index is 3.24 (just emerged roots), and (iii) cumulative mitotic index is greater than 8.0 (primordia). Even allowing for the higher control mitotic indices of the primordia, it is clear that they begin to recover sooner than other stages.

After 9 hours, 3 classes are still evident; they comprise the same meristems. But just emerged roots are beginning to resemble primordia. By 12 hours only primary roots show little or no sign of recovery and at 15 hours all stages have recovered or are recovering (Table 3).

3. Degree of synchrony. The highest mitotic index achieved in recovery is a measure of the degree of synchrony present in a population after treatment with 5-AU. In the present experiment lateral roots show the greatest degree of synchronization, mitotic index is 23.94 at 15 hours. At 12 and 15 hours, in laterals, more than 40% of all cells undergo mitosis (Table 1a), so although synchrony is good, it is not absolute and a residual variation in the time of recovery is present. The standard deviation of the mitotic index in lateral roots reveals the degree of the variation in recovery. At 12 and 15 hours the standard deviation is 8.91 and 10.02; these values are greater than those for the peak mitotic index in any type of meristem (Tables 1a and 1b).

In just emerged roots and all primordia, the maximum

mitotic index is lower than in laterals and ranges from 11.94 to 17.24. Taking the sum of the values at 9 and 12 hours, the total mitotic index is 20.34 to 27.42; this value is lower than the 41.54 of the laterals. The standard deviations of mitotic indices are lower in just emerged roots and distinctly lower in the primordia than in the lateral roots, indicating that at each fixation the primordial meristems were less variable than those of laterals. But the spread of the recovery curves (Figure 2) shows that in primordia the mitotic index increases over a longer period, i.e. the degree of synchrony is less than in lateral roots.

The conclusion from the data presented in this section is that primordia are physiologically different from other meristems of V. faba. The just emerged meristem is unlike the lateral root in its response to 5-AU even though it has broken through the epidermis and is, in fact, a transition stage between very large primordia and laterals, both anatomically and physiologically. Primary and lateral roots resemble one another more than they do other stages, but even they differ from one another in the rate of recovery from 5-AU.

The responses of the different meristems have been considered here in some detail. The overall pattern of response is similar in all treatments and therefore will not be described repeatedly, only the differences will be pointed out.

Legend. The following abbreviations are used in the tables included in this study.

t - time after end of treatment, in hours

I - the number of interphase cells

P - the number of cells in prophase

M - the number of cells in metaphase

A - the number of cells in anaphase

T - the number of cells in telophase

M.I. - mitotic index

S.D. - standard deviation of the mitotic index

Table 1a. 5-aminouracil, 500 p.p.m., 6 hour treatment followed by 15 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Primary	0	4959	22	10	5	4	0.82	0.24
roots	1	4953	23	11	8	5	0.94	0.22
	6	4990	7	2	1	0	0.20	0.02
	9	2991	8	0	0	1	0.30	0.17
	12	4928	61	4	7	0	1.44	0.87
	15	4478	367	90	54	11	10.44	3.68
Lateral	0	4948	37	8	5	2	1.04	0.33
roots	1	4985	8	2	4	1	0.30	0.20
	6	4997	1	1	1	0	0.06	0.05
	9	4988	7	1	2	2	0.24	0.21
	12	4116	744	61	64	15	17.68	8.91
	15	3808	759	212	154	72	23.94	10.02
Just	0	4950	29	4	14	3	0.98	0.61
emerged	1	4940	39	7	10	4	1.20	0.84
roots	6	4947	46	3	4	0	1.06	0.83
	9	4446	359	99	76	20	11.94	4.02
	12	4512	271	94	87	36	9.76	6.25
	15	4833	98	34	20	15	3.34	0.60

Table 1b. 5-aminouracil, 500 p.p.m., 6 hour treatment followed by 15 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Very	0	4902	54	17	19	8	1.96	0.85
large	1	4933	46	5	13	3	1.34	0.79
primordia	6	4759	205	17	17	2	4.82	1.66
	9	4148	603	112	111	26	17.24	4.29
	12	4507	303	58	82	50	9.86	1.38
	15	2907	55	14	13	11	3.10	1.01
Large	0	4883	74	20	15	8	2.42	0.75
primordia	1	4901	55	18	20	6	1.98	0.76
	6	4755	203	17	21	4	4.90	1.11
	9	4372	469	72	76	11	12.72	2.05
	12	4619	214	59	68	40	7.62	2.22
	15	4870	79	15	24	12	4.33	1.34
Small	0	2461	25	6	4	4	1.56	0.74
primordia	1	2426	51	10	10	3	2.96	0.74
	6	1917	62	6	12	3	4.57	1.55
	9	887	90	10	13	0	11.30	2.40
	12	2097	283	52	54	14	16.12	5.36
	15	2317	94	45	27	17	7.32	4.29

Table 2a. 5-aminouracil, 500 p.p.m., 6 hour treatment followed by a 1 hour treatment in a solution of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine (10^{-7} M each), followed by 14 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Primary	0	4959	22	10	5	4	0.82	0.24
roots	1	4979	13	2	4	2	0.42	0.14
	6	2994	5	0	1	0	0.20	0.04
	9	4972	18	5	5	0	0.56	0.22
	12	4827	134	27	12	0	3.46	3.14
	15	2291	480	117	78	34	23.63	1.16
Lateral	0	4948	37	8	5	2	1.04	0.33
roots	1	4986	10	1	3	0	0.28	0.17
	6	4996	2	1	1	0	0.08	0.07
	9	4975	20	4	1	0	0.52	0.17
	12	4454	429	55	44	18	10.92	9.52
	15	4049	577	147	157	70	19.02	5.45
Just	0	4902	29	4	14	3	0.98	0.61
emerged	1	4986	11	2	1	0	0.28	0.17
roots	6	4933	54	7	6	0	1.34	1.38
	9	2651	249	51	35	14	11.63	4.22
	12	4231	412	155	172	30	15.38	4.67
	15	2886	63	18	24	9	3.80	0.65

Table 2b. 5-aminouracil, 500 p.p.m., 6 hour treatment followed by a 1 hour treatment in a solution of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine (10^{-7} M each), followed by 14 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Very	0	4902	54	17	19	8	1.96	0.95
large	1	4950	33	4	10	3	1.00	0.22
primordia	6	4790	159	17	23	11	4.50	2.35
	9	4203	565	104	101	27	17.62	1.71
	12	3370	380	104	108	38	15.36	0.28
	15	4886	70	16	15	13	2.28	1.09
Large	0	4883	74	20	15	8	2.42	0.75
primordia	1	4932	45	8	12	3	1.36	0.20
	6	4790	163	27	14	6	5.06	2.17
	9	4246	544	89	89	32	19.08	1.67
	12	4166	493	144	143	54	16.68	4.17
	15	2929	44	11	9	7	2.36	0.34
Small	0	2461	25	6	4	4	1.56	0.74
primordia	1	2443	33	12	11	1	2.28	1.13
	6	935	49	7	8	1	6.50	1.27
	9	1237	197	29	32	5	17.53	5.00
	12	1688	205	43	47	17	15.60	10.06
	15	1442	33	13	7	5	3.86	1.33

Figure 2. A comparison of the rates of recovery of meristems after a 6 hour exposure to 500 p.p.m. 5-AU or a 6 hour exposure to 500 p.p.m. 5-AU followed by a 1 hour exposure to a solution of nucleosides (AdR, CdR, GdR, and TdR) at a concentration of 10^{-7} M.

.———. 5-AU

.– – – –. 5-AU followed by nucleosides

(i) Primary roots

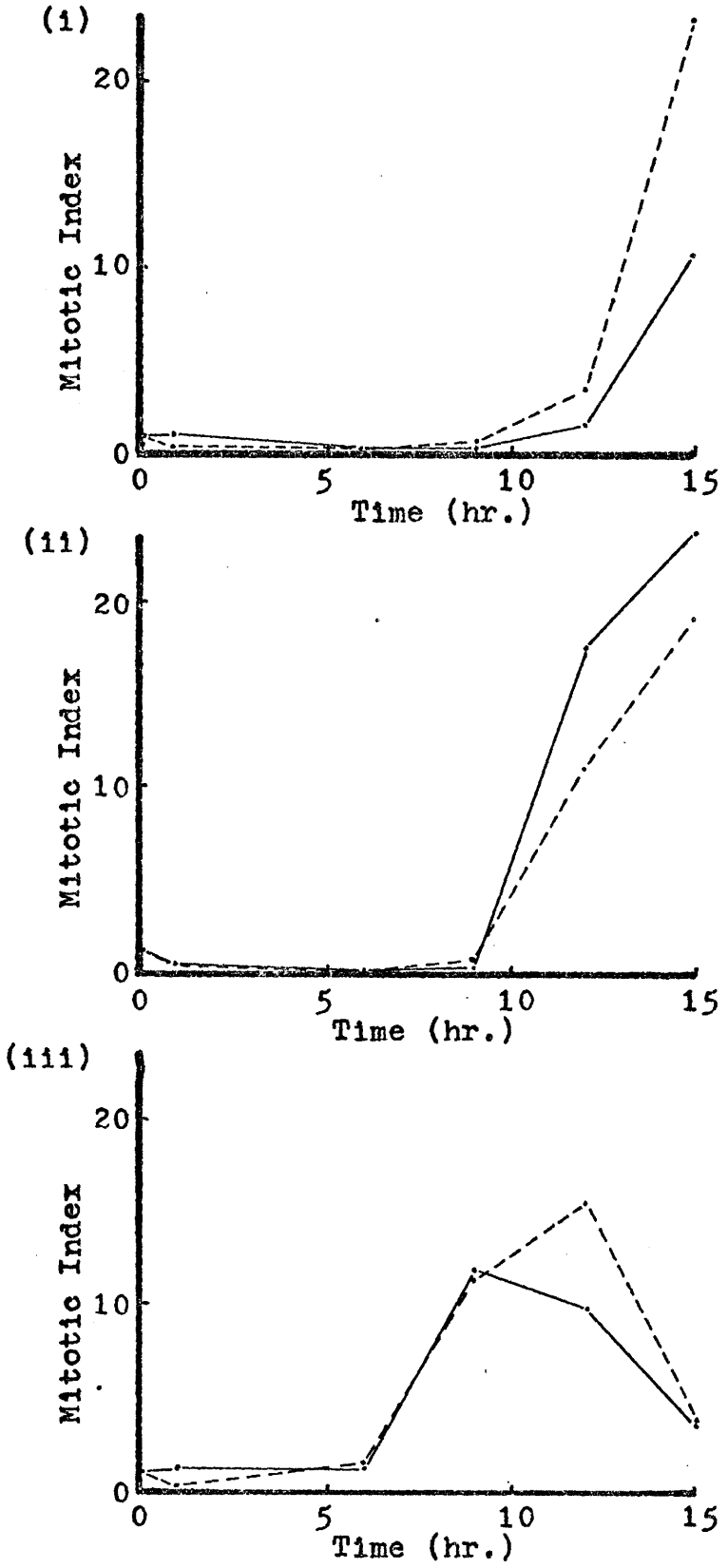
(ii) Lateral roots

(iii) Just emerged roots

(iv) Very large primordia

(v) Large primordia

(vi) Small primordia


(i)
Mitotic Index
20
10
0
0
5
10
15
Time (hr.)
(ii)
Mitotic Index
20
10
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Time (hr.)
(iii)
Mitotic Index
20
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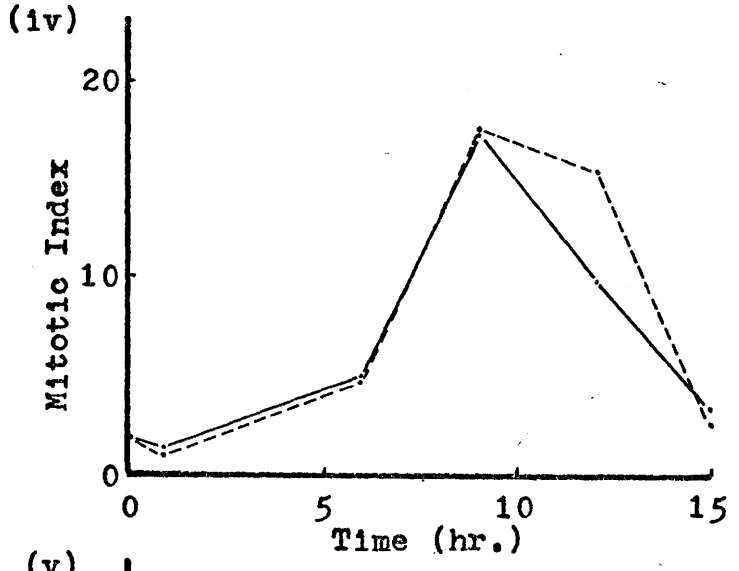
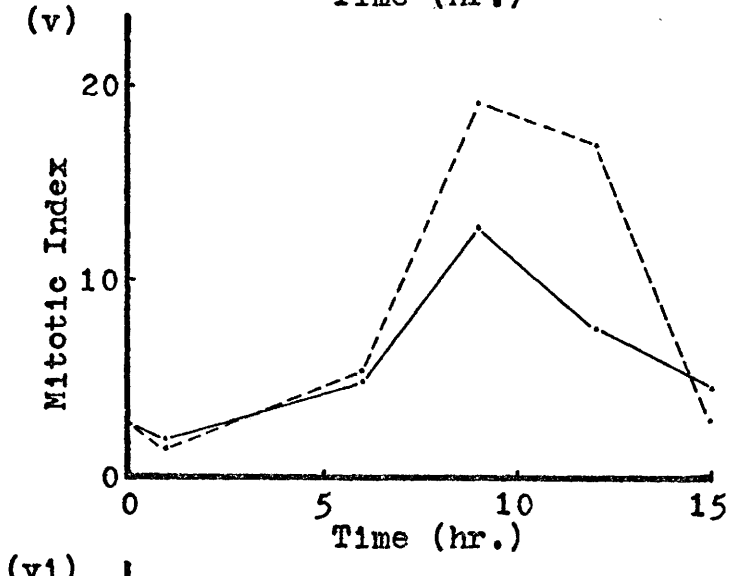
10
15
Time (hr.)


(iv)
Mitotic Index
20
10
0
0
5
10
15
Time (hr.)


(v)
Mitotic Index
20
10
0
0
5
10
15
Time (hr.)

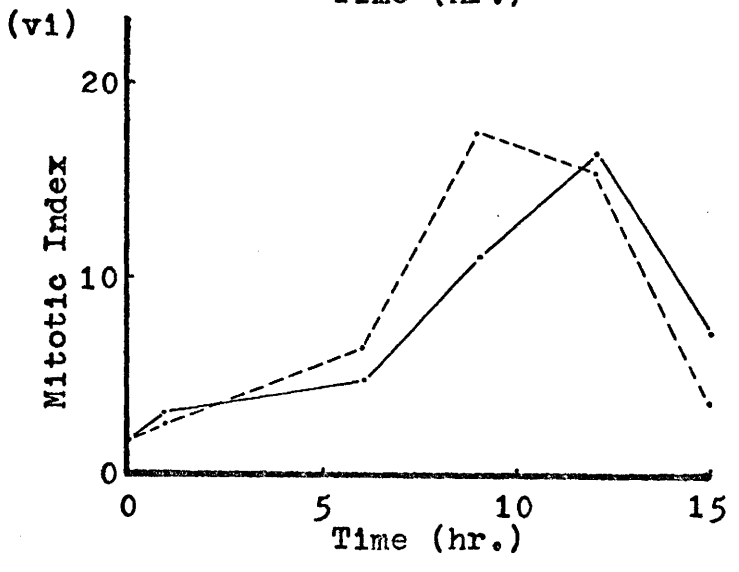

(vi)
Mitotic Index
20
10
0
0
5
10
15
Time (hr.)

Table 3. Cumulative mitotic indices for root meristems after a 6 hour exposure to 5-AU, 500 p.p.m..

	cumulative mitotic indices			
region	0 - 6 hr.	0 - 9 hr.	0 - 12 hr.	0 - 15 hr.
Primary roots	1.96	2.26	3.70	14.14
Lateral roots	1.40	1.64	19.32	43.26
Just emerged roots	3.24	15.18	24.94	28.28
Very large primordia	8.12	25.36	35.22	38.32
Large primordia	9.30	22.02	29.64	33.97
Small primordia	9.09	20.39	36.51	43.83

Table 4. Cumulative mitotic indices for root meristems after a 6 hour exposure to 5-AU, 500 p.p.m. followed by a 1 hour exposure to nucleosides.

	cumulative mitotic indices			
region	0 - 6 hr.	0 - 9 hr.	0 - 12 hr.	0 - 15 hr.
Primary roots	1.44	2.00	5.46	29.09
Lateral roots	1.40	1.92	12.84	31.86
Just emerged roots	2.60	14.23	29.61	33.41
Very large primordia	7.46	25.08	40.44	42.72
Large primordia	8.84	27.92	44.60	46.96
Small primordia	10.34	27.87	43.47	47.33

Roots treated with 5-AU and then with a solution containing AdR, CdR, GdR, and TdR for 1 hour were also examined (Tables 2a and 2b). The response of the meristems is most clearly shown by comparing the curves of mitotic indices (Figure 2). At the concentration used (10^{-7} M) the solution of nucleosides failed to stimulate any of the meristems to begin recovery at an earlier time, i.e. the mitotic index does not begin to rise earlier with nucleosides than without them. However, the cumulative mitotic indices (Table 4) reveal that by 12 hours more cells have divided in primordia treated with 5-AU and nucleosides than with 5-AU only (Tables 3 and 4). And by 15 hours, only lateral roots have not been stimulated by treatment with the nucleosides. In general, this stimulation is achieved without an increase in standard deviation of the mitotic index (Tables 2a and 2b), though see small primordia at 12 hours, standard deviation is 10.06. Lateral roots, on the other hand, also have large standard deviations at 12 and 15 hours. The five laterals, treated with 5-AU only, are increasing in mitotic index at different times, producing a large standard deviation.

B. 5-aminouracil for 3 hours: post-treatment with nucleosides (10^{-7} M).

The procedure employed in this experiment was similar to that in the previous section, but with the following changes: (1) the primary roots treated had not yet produced

laterals, (ii) a 3 hour exposure to 500 p.p.m. 5-AU was used instead of 6 hours, and (iii) fixations of whole roots were made at 6, 8, 10, 12, and 14 hours after the end of 5-AU treatment. The duration of the treatment with 5-AU was reduced to 3 hours in order to determine whether the ability of the meristems to respond to the nucleosides was influenced by the severity of the 5-AU treatment.

Primary roots. After 8 hours of recovery, the mitotic index is very low, 0.62 (Table 5). The mitotic index is approximately the same as is seen 6 or 9 hours after a 6 hour exposure to 5-AU; therefore, the cells seen in division at this time must be slow cycling cells. A 3 hour exposure appears to be as effective as a 6 hour exposure to 5-AU in reducing the mitotic index initially. By 10 hours, the meristem has begun to recover; the prophase frequency has increased from 4 per 1,000 cells at 8 hours to 25 per 1,000 cells at 10 hours. A peak of partially synchronous mitoses occurs at 12 hours, the mitotic index is 21.64. By 14 hours the prophase frequency has decreased and the metaphase, anaphase, and telophase frequencies have increased; the wave of cells that entered into mitosis together is now moving into interphase.

The pattern of recovery shown by the primary root meristem has been extended to the post-peak portion of recovery. Recovery shows the same general pattern as was seen for the other developmental stages after a 6 hour exposure to 5-AU.

Very large primordia. This stage showed little variation in the mitotic index after a 3 hour exposure to 5-AU; the mitotic index varied between 6.08 and 8.98 (Table 5). The mitotic index is highest at 8 hours, but it is not nearly as high as 8 hours after a 6 hour exposure to 5-AU; a peak of partially synchronous mitoses is absent. The mitotic index 6 hours after the end of treatment is higher than after a 6 hour exposure, 8.82 compared with 4.82. The very large primordia show very little fluctuation in the mitotic index and it must be concluded that the 3 hour exposure to 5-AU has had little effect in synchronizing the population.

Large primordia. The pattern of change in the mitotic index is similar to that seen after a 6 hour treatment with 5-AU, i.e. a fall followed by a partially synchronized peak. However, it differs in its overall extent. The mitotic index does not fall below 5.58 and the peak is spread over 4 hours.

A 3 hour treatment with 5-AU is, therefore, also effective in blocking cells and preventing them proceeding to mitosis. As with a 6 hour treatment, partial synchrony of division is found. This weaker treatment has one unique feature, however; it reveals a real difference between very large primordia and large primordia. The former show little effect whereas the latter respond. This is demonstrated by the cumulative mitotic indices, for very large primordia it increases by regular increments, but in large primordia it increases in an irregular fashion and, finally, is higher than

Table 5. 5-aminouracil, 500 p.p.m., 3 hour treatment followed by 14 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Primary	6	4999	1	0	0	0	0.02	
roots	8	4969	20	2	9	0	0.62	0.48
	10	4858	126	10	6	0	2.84	1.22
	12	3918	834	138	86	24	21.64	3.11
	14	4086	458	198	198	60	18.28	3.93
Very	6	4559	319	48	54	20	8.82	0.60
large	8	4551	290	67	64	28	8.98	1.60
primordia	10	4636	247	47	44	26	7.28	1.94
	12	4696	190	50	28	36	6.08	2.10
	14	4646	221	60	44	29	7.08	1.06
Large	6	4690	224	27	49	10	6.20	1.33
primordia	8	4358	420	103	96	23	12.84	2.64
	10	4270	475	121	107	27	14.60	3.43
	12	4603	223	74	77	23	7.94	1.70
	14	4721	172	41	47	19	5.58	1.36

Table 6. 5-aminouracil, 500 p.p.m., 3 hour treatment followed by a 1 hour treatment in a solution of deoxythymidine, deoxyguanosine, deoxyadenosine, and deoxycytosine (10^{-7} M each), followed by a 13 hour recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Primary	6	4987	2	6	4	1	0.26	0.17
root	8	4994	4	0	2	0	0.12	0.13
	10	4935	50	8	7	0	1.30	1.79
	12	4228	654	70	45	3	15.24	2.46
	14	4331	368	131	123	47	13.38	1.96
Very	6	4671	222	41	49	17	6.58	1.08
large	8	4409	389	102	73	27	11.82	2.43
primordia	10	4523	309	78	54	36	9.54	2.46
	12	4637	215	63	64	21	7.26	1.47
	14	4705	177	43	45	30	5.90	0.60
Large	6	4772	169	32	24	3	4.56	1.89
primordia	8	4416	391	100	75	18	11.68	1.76
	10	4384	419	99	77	21	11.92	2.87
	12	4448	280	100	117	55	11.04	1.70
	14	4724	168	49	47	12	5.52	1.28

Figure 3. A comparison of the rates of recovery of meristems after a 3 hour exposure to 500 p.p.m. 5-AU or a 3 hour exposure to 500 p.p.m. 5-AU followed by a 1 hour exposure to a solution of nucleosides (AdR, CdR, GdR, and TdR) at a concentration of 10^{-7} M.

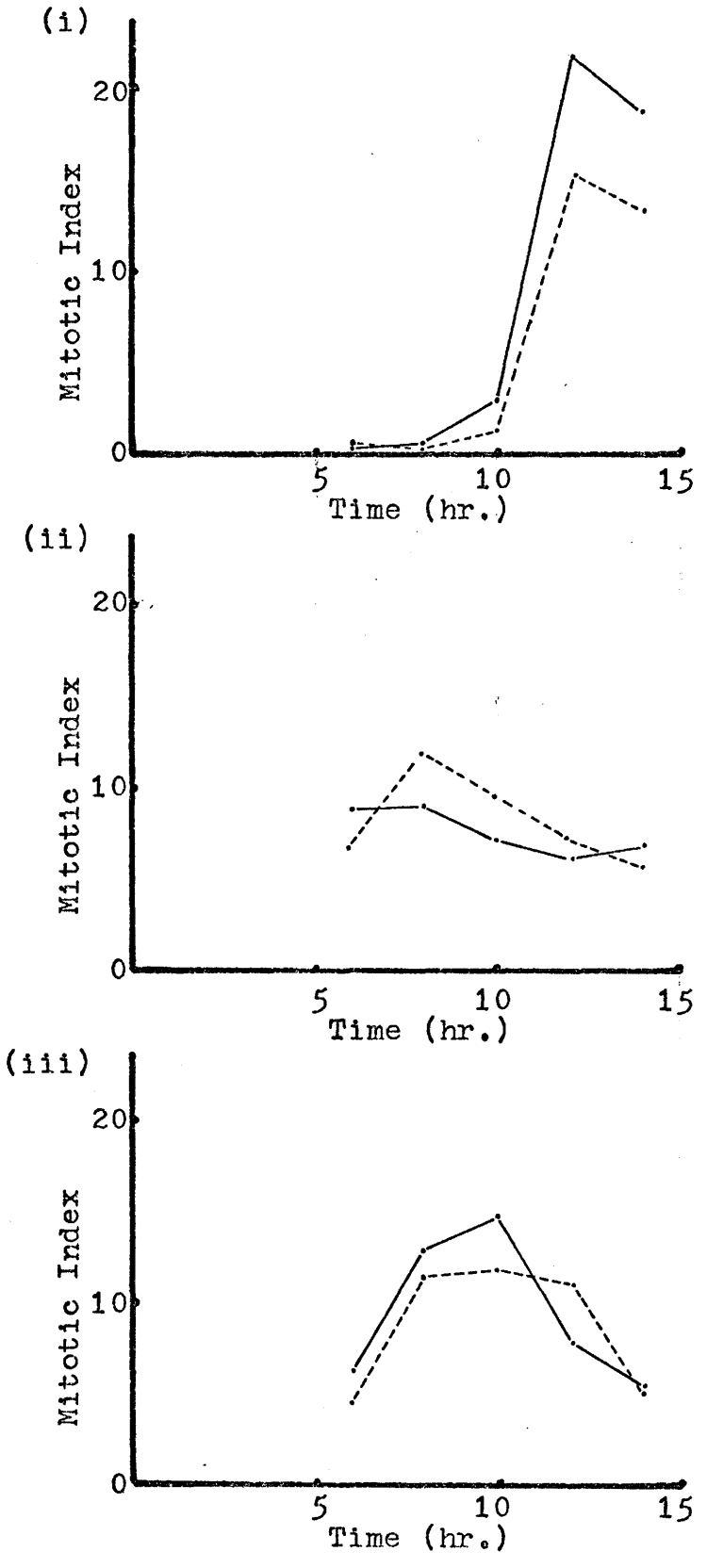
•———• 5-AU

•– – – –• 5-AU followed by nucleosides

(i) Primary roots

(ii) Very large primordia

(iii) Large primordia


(i)
Mitotic Index
20
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Time (hr.)
(ii)
Mitotic Index
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10
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15
Time (hr.)
(iii)
Mitotic Index
20
10
0
5
10
15
Time (hr.)

Table 7. Cumulative mitotic indices for root meristems after a 3 hour exposure to 5-AU, 500 p.p.m..

	cumulative mitotic indices				
region	0 - 6 hr.	0 - 8 hr.	0 - 10 hr.	0 - 12 hr.	0 - 14 hr.
Primary roots	0.02	0.64	3.48	25.12	43.40
Very large primordia	8.82	17.80	25.08	31.16	38.24
Large primordia	6.20	19.04	33.64	41.58	47.16

Table 8. Cumulative mitotic indices for root meristems after a 3 hour exposure to 5-AU, 500 p.p.m., followed by a 1 hour exposure to nucleosides.

	cumulative mitotic indices				
region	0 - 6 hr.	0 - 8 hr.	0 - 10 hr.	0 - 12 hr.	0 - 14 hr.
Primary roots	0.26	0.38	1.68	16.92	30.30
Very large primordia	6.58	18.40	27.94	35.20	41.10
Large primordia	4.56	16.24	28.16	39.20	44.72

in very large primordia, as if some of the cells delayed in the period 0 to 6 hours have entered mitosis and contributed to the total cumulative mitotic index (Table 7).

Roots treated with 5-AU and a solution of nucleosides did not begin to recover sooner than roots treated with 5-AU alone (Figure 3). Thus, when used after a 3 hour, rather than a 6 hour, treatment faster recovery is not induced. In primary roots it even appears that the nucleosides are delaying recovery to some extent. The cumulative mitotic indices after 5-AU and nucleosides is only 30.30 by 14 hours (Table 8). compared with 43.40 after 5-AU alone (Table 7).

C. 5-aminouracil for 3 hours: post-treatment with nucleosides (10^{-6} M).

Since a 10^{-7} M solution of nucleosides did not induce faster recovery of mitotic activity it was decided to test the effect of a higher concentration. Roots were grown in the usual way and treated with 500 p.p.m. 5-AU for 3 hours; some were treated with a 10^{-6} M solution of nucleosides for 1 hour, after 5-AU. Fixations were made 6, 8, 10, 12, and 14 hours after the end of the 5-AU treatment. Additional developmental stages were examined compared with the previous section.

Primary roots. Recovery from the effects of 5-AU begins approximately 10 hours after the end of the 3 hour exposure. There is a difference of approximately 2 hours in the time of onset of recovery after a 3 hour and a 6 hour

exposure to 5-AU; roots exposed for the shorter time begin recovery sooner. The peak of partially synchronous mitoses occurs at 14 hours, mitotic index equals 14.76; this is sooner than was seen in primary roots treated for 6 hours with 5-AU.

The effects of a 3 hour treatment with 5-AU lasts for a shorter time than a 6 hour treatment. This is clearly shown by the cumulative mitotic indices; it has reached 17.70 12 hours after a 3 hour treatment (Table 11) compared with 3.70, 12 hours after a 6 hour treatment (Table 3). The difference is even more striking within a further 2 to 3 hours, i.e. 32.46 by 14 hours after a 3 hour treatment and only 14.14 by 15 hours after 6 hours in 5-AU (Tables 3 and 11).

Lateral roots. Recovery begins after 8 hours and reaches a partially synchronous peak of mitoses at 12 hours (Table 9a). The onset of recovery and the peak of mitoses occur sooner than after a 6 hour treatment. The peak of mitoses is not as high as after a 6 hour treatment, 16.68 compared with 23.94; this is to be expected since a 6 hour treatment should accumulate a greater number of cells at the S - G_2 transition, and cause a higher mitotic index upon recovery.

Just emerged roots. At 6 hours the just emerged roots show a mitotic index of 7.28, a value much higher than seen after the 6 hour treatment, and near the control mitotic index of 8 to 9. Since at 8 hours the mitotic index is 10.24 it may be that the 6 and 8 hour values are really a low peak

of partially synchronized divisions. This is also suggested by the high prophase value at 8 hours and the subsequent fall in mitotic index after 8 hours (Table 9a).

The pattern of recovery shown by the just emerged roots differs from the laterals in time of onset of recovery, time of peak, and height of peak.

Very large, large, and small primordia. The primordia show a pattern of recovery that is similar to one another (Table 9b), but different from the other stages of development. The mitotic indices at 6 hours are 7.38 to 8.20, a level nearly twice that seen at 6 hours after the end of a 6 hour exposure to 5-AU. Recovery, based on the high frequencies of prophases, has begun before the 6 hour fixation. The peaks of mitoses occur between 8 and 10 hours in the primordia, with mitotic indices between 14.78 and 21.60 (Table 9b). The small primordia show a peak of mitoses slightly later than the very large and large primordia, as they did after the 6 hour treatment.

Examination of the cumulative mitotic indices (Table 11) reveals that the roots show two types of response 6 hours after a 3 hour exposure to 5-AU: (i) primary and lateral roots with mitotic indices less than 2.0 and (ii) just emerged roots, very large primordia, large primordia, and small primordia with mitotic indices greater than 7.0. The just emerged root, which was seen to be in a class separate from the other stages of development after a 6 hour treatment with

5-AU, is in the same class as the primordia after a 3 hour treatment with 5-AU, demonstrating its physiological similarity to prior stages of development, though anatomically it is more similar to the lateral root.

Meristems at all stages of development respond to the shorter 5-AU treatment in a similar way to their response to 6 hours of 5-AU. The time of onset of recovery is earlier after the shorter treatment and in the case of the primary and lateral roots, the peaks also occur earlier. Just emerged roots and primordia begin recovery sooner after the shorter treatment, but the time of the appearance of the peaks of division corresponds to the peaks of division after the 6 hour treatment. There is a greater variability in the rate of recovery of the cells found in these stages of development after the shorter treatment; these four stages appear, on the basis of their response to 5-AU, to form one physiological class of meristems.

Previously it was seen that treating growing roots with a solution of nucleosides immediately after a 6 hour treatment of 5-AU brought more cells into division during the recovery period (section A), although recovery did not begin sooner. If the cumulative mitotic indices are examined for roots treated with 5-AU (Table 11) or with 5-AU and nucleosides (Table 12) it is clear that between 0 and 14 hours of recovery a response to the nucleoside treatment is seen in several types of meristems. The only two to show

Table 9b. 5-aminouracil, 500 p.p.m., 3 hour treatment followed by 14 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Very	6	4631	257	46	51	15	7.38	1.70
large	8	4261	566	76	87	10	14.78	2.62
primordia	10	4563	277	72	56	32	8.74	0.78
	12	4583	251	67	78	21	8.34	1.26
	14	4727	181	47	32	13	5.46	0.84
Large	6	4610	283	47	50	10	7.80	1.48
primordia	8	4229	580	70	93	28	15.42	2.21
	10	4214	538	95	108	45	15.52	2.66
	12	4677	186	55	70	12	6.46	1.44
	14	4766	147	32	41	14	4.68	1.28
Small	6	2295	150	19	32	4	8.20	2.14
primordia	8	2123	292	39	40	6	15.08	6.41
	10	2060	271	69	76	24	21.60	7.38
	12	2320	95	36	36	13	7.56	4.71
	14	2375	81	17	21	6	5.00	1.72

Table 9a. 5-aminouracil, 500 p.p.m., 3 hour treatment followed by 14 hours of recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Primary	6	4920	51	15	13	1	1.60	1.05
roots	8	4934	55	4	7	0	1.32	0.57
	10	4903	74	11	12	0	1.94	0.50
	12	4358	412	127	93	10	12.84	1.42
	14	4262	433	138	139	28	14.76	0.50
Lateral	6	4974	20	5	1	0	0.52	0.28
roots	8	4987	11	2	0	0	0.26	0.20
	10	4693	245	33	21	8	6.14	2.83
	12	4166	575	126	117	16	16.68	1.00
	14	4410	305	105	137	43	11.80	0.97
Just	6	4636	244	65	46	9	7.28	0.94
emerged	8	4488	373	70	58	11	10.24	3.02
roots	10	4581	243	83	66	27	8.38	1.86
	12	4604	211	88	64	33	7.92	1.57
	14	4756	141	40	47	16	4.88	1.10

Table 10a. 5-aminouracil, 500 p.p.m., 3 hour treatment followed by a 1 hour treatment in a solution of deoxythymidine, deoxyguanosine, deoxyadenosine, and deoxycytosine (10^{-6} M each), followed by a 13 hour recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Primary	6	4980	9	5	4	2	0.40	0.14
roots	8	4886	110	2	2	0	2.28	1.54
	10	4893	89	9	7	2	2.14	1.04
	12	4076	612	148	150	14	18.48	1.90
	14	4211	458	160	153	18	15.68	0.88
Lateral	6	4993	4	1	2	0	0.14	0.10
roots	8	4624	314	30	24	8	7.52	3.23
	10	4332	569	59	31	9	13.36	5.12
	12	4102	609	129	146	14	17.96	5.93
	14	4660	239	74	103	24	8.80	2.63
Just	6	4694	233	31	38	4	6.12	2.31
emerged	8	4478	318	97	89	18	10.44	2.51
roots	10	4453	259	124	131	33	10.94	2.76
	12	4691	142	70	71	26	6.18	1.12
	14	4786	126	38	31	19	4.28	0.70

Table 10b. 5-aminouracil, 500 p.p.m., 3 hour treatment followed by a 1 hour treatment in a solution of deoxythymidine, deoxyguanosine, deoxyadenosine, and deoxycytosine (10^{-6} M each), followed by a 13 hour recovery: mitotic index and the frequencies of cells in interphase and the different stages of mitosis.

region	t	I	P	M	A	T	M.I.	S.D.
Very	6	4678	215	41	44	22	7.44	1.83
large	8	4007	674	112	155	52	19.86	5.19
primordia	10	4409	388	73	99	31	11.82	1.27
	12	4689	202	48	44	17	6.22	2.15
	14	4825	110	24	34	7	3.50	0.37
Large	6	4641	260	43	43	13	7.18	1.38
primordia	8	4204	554	102	119	21	15.92	2.73
	10	4289	458	106	117	30	13.18	4.53
	12	4718	178	31	62	11	5.64	1.51
	14	4850	106	12	27	5	3.00	0.86
Small	6	2324	127	20	28	1	7.04	2.74
primordia	8	2059	313	49	59	20	18.86	2.47
	10	2050	270	84	72	24	18.00	4.11
	12	2273	123	35	59	10	9.08	0.86
	14	2371	80	17	29	3	5.16	2.41

Figure 4. A comparison of the rates of recovery of meristems after a 3 hour exposure to 500 p.p.m. 5-AU or a 3 hour exposure to 500 p.p.m. 5-AU followed by a 1 hour exposure to a solution of nucleosides (AdR, CdR, GdR, and TdR) at a concentration of 10^{-6} M.

. . 5-AU

. . 5-AU followed by nucleosides

(i) Primary roots

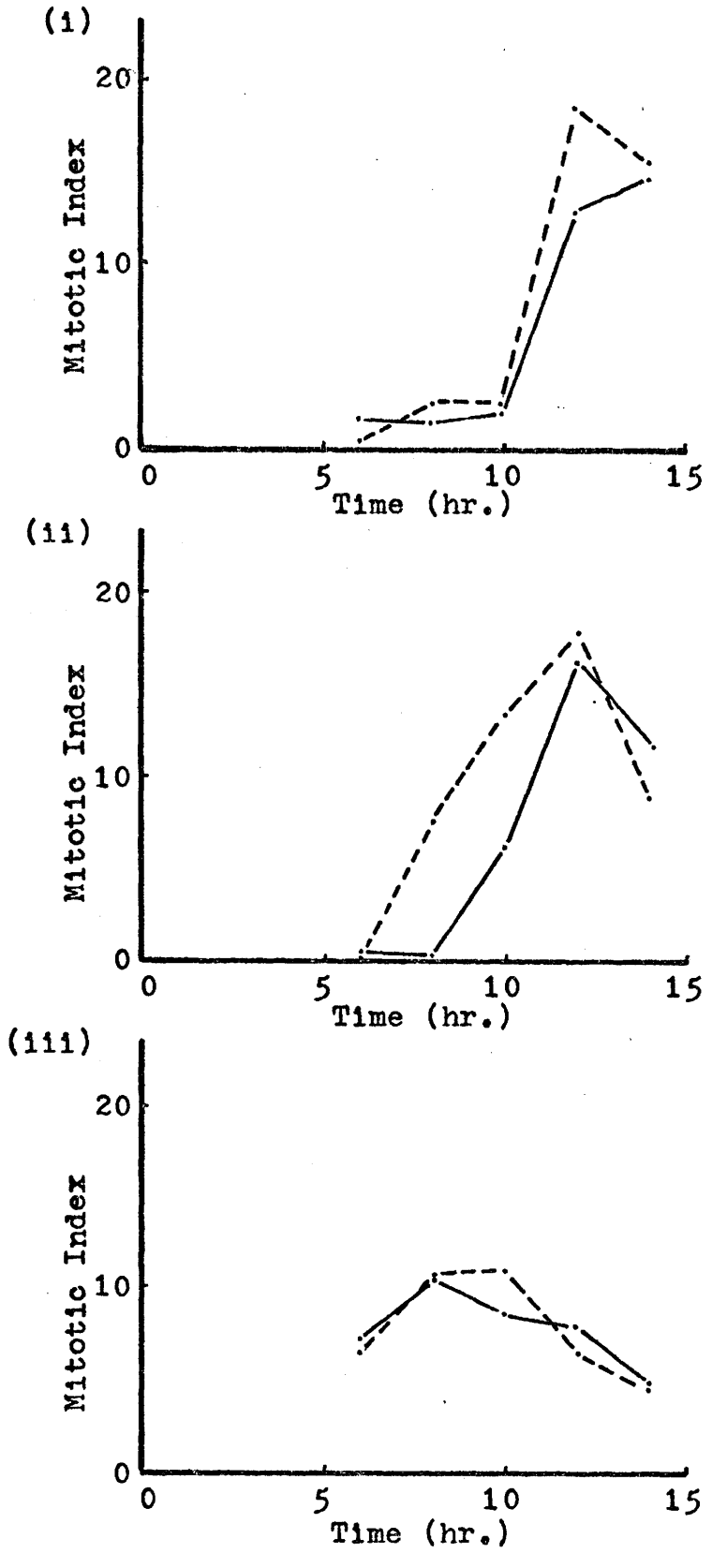
(ii) Lateral roots

(iii) Just emerged roots

(iv) Very large primordia

(v) Large primordia

(vi) Small primordia


(i)
Mitotic Index
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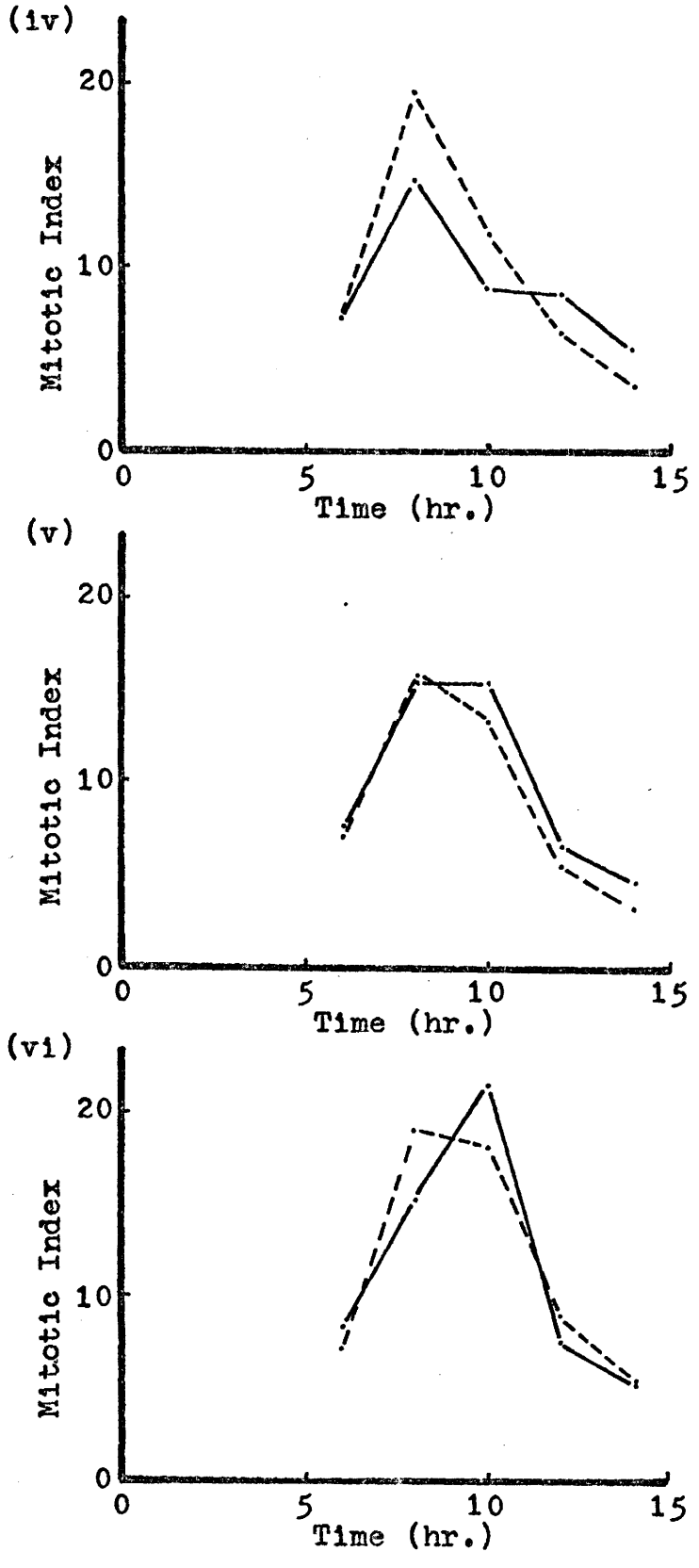
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Table 11. Cumulative mitotic indices for root meristems after a 3 hour exposure to 5-AU, 500 p.p.m..

	cumulative mitotic indices				
region	0 - 6 hr.	0 - 8 hr.	0 - 10 hr.	0 - 12 hr.	0 - 14 hr.
Primary roots	1.60	2.92	4.86	17.70	32.46
Lateral roots	0.52	0.78	6.92	23.60	35.40
Just emerged roots	7.28	17.52	25.90	33.82	38.70
Very large primordia	7.38	22.16	30.90	39.24	44.70
Large primordia	7.80	23.22	38.74	45.20	49.88
Small primordia	8.20	23.28	44.88	52.44	57.44

Table 12. Cumulative mitotic indices for root meristems after a 3 hour exposure to 5-AU, 500 p.p.m. followed by a 1 hour exposure to nucleosides.

	cumulative mitotic indices				
region	0 - 6 hr.	0 - 8 hr.	0 - 10 hr.	0 - 12 hr.	0 - 14 hr.
Primary roots	0.40	2.68	4.82	23.30	38.98
Lateral roots	0.14	7.66	21.02	38.98	47.78
Just emerged roots	6.12	16.56	27.50	33.68	37.96
Very large primordia	7.44	27.30	39.12	45.34	48.84
Large primordia	7.18	23.10	36.28	41.92	44.92
Small primordia	7.04	25.90	43.90	52.98	58.14

significant differences are the primary and lateral roots; in primary roots there has been a 20.4% rise in the cumulative mitotic index and in laterals the increase was 34.9%. Other meristems appear to be unaffected by the nucleoside treatment. With the exception of the lateral roots, none of the stages showed an earlier onset of recovery; this is similar to the result reported in section A. The laterals, however, did show a significant difference in the time of onset of recovery (Figure 4); treatment with nucleosides resulted in cells entering division 2 hours sooner than cells not treated with nucleosides. In view of this result, lateral roots were treated with a solution containing both 5-AU and nucleosides in order to determine whether the presence of the nucleosides would alter the response to 5-AU.

D. Treatment with 5-aminouracil and nucleosides.

Primary roots with fully emerged laterals, 1 centimetre or more in length, were exposed for 3 hours to a solution containing 500 p.p.m. 5-AU and AdR, CdR, GdR, and TdR, each at a concentration of 10^{-6} M. Roots were fixed 6, 8, 10, 12, and 14 hours after the end of treatment. Other roots were treated with a solution of nucleosides (10^{-6} M each) for 1 hour. Fixations were made every 2 hours from 6 to 14 hours. These were timed from the beginning of treatment so that they corresponded with the fixation times of roots treated with 5-AU and then with nucleosides for 1 hour (see previous sections).

Lateral roots showed no response to a 1 hour treatment with nucleosides (Table 13). Their mitotic indices overlap with control values (Figure 5). The average mitotic index of treated lateral roots was 5.92; in controls it was 5.84. The delay of 6 to 14 hours before roots were examined may seem long, but as shown in previous sections, any effect of the nucleosides on the mitotic index is never seen earlier than 8 to 10 hours after the beginning of treatment. The lack of response of roots treated only with nucleosides shows that they alone do not induce changes in cell cycle kinetics and that whatever their effect in 5-AU treated roots it is expressed only after a 5-AU treatment.

Lateral roots that were treated with 5-AU and nucleosides simulatneously showed a pattern of recovery (Table 13) almost identical to that shown by the lateral roots after 5-AU alone (Table 9a). The pattern of recovery does not resemble that seen with 5-AU followed by nucleosides (Table 10a). Nucleosides present at the same time as 5-AU do not prevent 5-AU imposing a block on the progress of cells through the cell cycle; they do not bring cells back into division sooner and they do not induce more cells to divide during the period of the experiment. In this treatment the duration of nucleoside exposure is 3 hours, three times longer than in previous treatments. It is no more effective than a 1 hour exposure. Whatever the mechanism of action of 5-AU, it is not affected by the presence of the nucleosides.

Table 13. Mitotic index and the frequencies of cells in interphase and the different stages of mitosis of lateral root meristems.

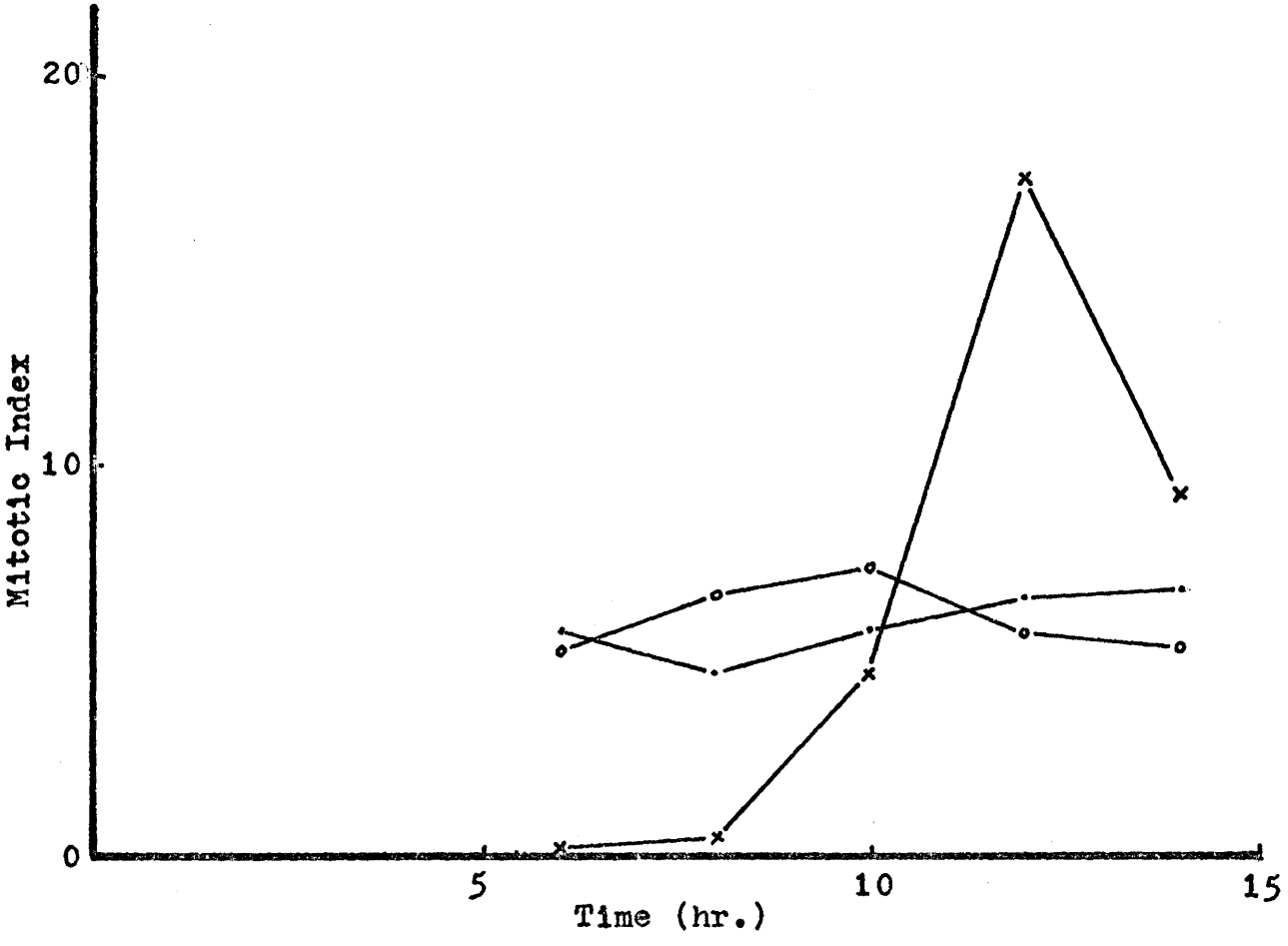
t	I	P	M	A	T	M.I.	S.D.
(a)	control						
6	4716	155	44	57	28	5.68	0.73
8	4764	110	62	47	17	4.58	1.36
10	4712	149	50	65	24	5.72	0.76
12	4677	173	73	46	31	6.46	1.06
14	4663	173	71	66	27	6.74	0.59
(b)	nucleosides, time is from the beginning of exposure						
6	4747	139	48	41	25	5.06	1.17
8	4669	181	65	64	21	6.62	1.25
10	4649	179	82	61	37	7.18	0.71
12	4735	162	31	52	20	5.70	1.13
14	4747	131	51	52	19	5.06	1.31
(c)	5-AU and nucleosides, time is from the end of exposure						
6	4991	6	0	1	2	0.18	
8	4978	16	1	4	1	0.44	0.14
10	4772	187	32	9	0	4.56	3.80
12	4135	503	172	154	36	17.30	4.82
14	4547	253	86	85	29	9.06	4.84

Figure 5. Mitotic index of lateral root meristems plotted against time, from the end of the combined 5-AU and nucleosides treatment.

x———x 5-AU (500 p.p.m.) and nucleosides (10^{-6} M each) for 3 hours prior to time = 0 hours

•———• nucleosides (10^{-6} M each) for 1 hour, from time = 0 hours to time = 1 hour

·———· control



DISCUSSION

The object of the experiments described here was, first, to extend the analysis of the response to 5-AU to small primordia and primary roots, in order to coorelate change in response with developmental stage and, secondly, to determine whether the effects of 5-AU on DNA synthesis or in blocking cells at the S - G_2 transition could be reversed by treatment with deoxynucleosides. The rationale behind this latter treatment was that a reversal of 5-AU induced changes, by the deoxynucleosides, would provide a clue to the possible point of action of 5-AU in nucleic acid metabolism.

Root development.

A growing root system of V. faba consists of a primary root, lateral roots, and the developing primordia that eventually emerge as lateral roots. Primary roots, lateral roots, just emerged roots, very large primordia, large primordia, and small primordia exhibit two sub-populations of cells. On the basis of cell cycle time, there are fast and slow cycling cells; the fast cycling sub-population has a cycle time of approximately 12 to 14 hours and the slow cycling sub-population has a cycle time of more than 30 hours. The ratio of fast to slow cycling cells is constant in all the developmental stages, which means that fast

cycling cells must move, in a regular fashion, from that population and undergo a change in cycle duration. In primordia all cells are proliferating and they are either fast or slow cycling; fast cycling cells; therefore, can change only into slow cycling cells. But in primary and lateral roots and in just emerging primordia non-dividing cells are also present and in these meristems this non-proliferating population may receive some of the fast cycling cells that undergo a change in cycle duration.

The more or less constant ratio of fast to slow cycling cells, together with the presence, in just emerged, lateral, and primary root meristems of a differentiating, i.e. non-dividing, population, suggests that some mechanism controls the change in cell cycle duration and that this mechanism is operating in all meristems. The fast and slow cycling sub-populations are not spatially discrete; this suggests that the control mechanism must work within individual cell lineages and it emphasizes that the proliferating cells that make up the meristem constitute a physiologically complex population. However, it is a dynamic system and, in root morphogenesis, the proliferating cells undergo a number of specific physiological changes. One of the experimental methods available for studying these changes is the use of compounds such as 5-AU.

Five-aminouracil treatments.

The experimental disruption of their functions snd

structure has been a useful tool in studies of cells. Temporary disruptions of cellular metabolism is one method of analysing cellular function and it can be achieved by treatments with anti-metabolites. These compounds resemble naturally occurring cell constituents but they either prevent normal reactions or they lead to the formation of abnormal products. In studies of nuclear function anti-metabolites, or analogues of the natural bases or nucleosides of DNA and RNA, have been widely used. One group of analogues includes derivatives of uracil with a substituent group in the 5' position, for example, 5-fluorouracil (5-FU) and 5-aminouracil (5-AU). These compounds are analogues of both uracil, which occurs in RNA, and of thymine, which occurs in DNA.

Five-aminouracil depresses the rate of DNA synthesis. This results in a fall in mitotic index. When recovery occurs, a partially synchronized burst of mitoses is seen; this cannot be due entirely to an effect of 5-AU on cells in S. Partial synchronization of cells in mitosis indicates that the relative rates at which different cells progress through S, from S into G_2, and then into mitosis have changed after treatment with 5-AU. If relative rates had remained the same, and all cells were slowed down equally, recovery would consist of a gradual return to normal levels of mitotic activity. This is not what is observed and some degree of synchronization is seen even after short treatments with low concentrations of 5-AU. It has been proposed, from this

evidence that (a) cells are blocked at the S - G_2 transition and (b) the duration of this block is dose dependent. The peaks in mitotic activity reported here (Tables 1a, 1b, 5, 9a, and 9b) support the postulated block at some stage prior to G_2. Previous workers have used high concentrations of 5-AU, often in excess of 750 p.p.m., and long treatments that often extended over one or more complete cell cycles (see Introduction). The disadvantage of these treatments is that they blur the sequence, in time, of the response of the proliferating cells to 5-AU. Treatments for 3 or 6 hours yield less dramatic results, but they still reveal a clear response to 5-AU.

A fall in mitotic index was found in all meristems treated with 5-AU and by 6 hours it was always low. Thus, the compound must have reached even cells in primordia in effective concentrations. Recovery, after treatment, reveals some differences between various developmental stages.

The greatest degree of synchrony occurs in meristems with the highest mitotic indices. Lateral roots exhibited the greatest degree of synchrony upon recovery; they showed the highest mitotic index of the stages examined and also the narrowest range of recovery time. In these respects, they are similar to the primary roots. Just emerged roots, very large primordia, large primordia, and small primordia exhibited lower mitotic indices and the curves of recovery were spread over a greater period of time. The variation in

the ability of cells to recover from 5-AU is greatest in the small primordia and becomes less as the root matures. The natural variation in the duration of G_2 + mitosis/2 is retained in all stages after exposure to 5-AU.

Primary roots took longest to recover from the effects of 5-AU, both in onset of recovery and time to reach the peak of mitoses. Lateral roots, while exhibiting a nearly identical recovery curve, began recovery and reached a peak approximately 3 hours earlier. Just emerged roots and primordia show signs of recovery 9 hours or less after the end of 5-AU exposure, 3 hours earlier than lateral roots.

If 5-AU imposes a definite block at the S -G_2 transition, then a 6 hour treatment should accumulate twice as many cells as a 3 hour treatment. In lateral roots, after a 6 hour exposure to 5-AU, the peak of mitoses is represented by a mitotic index of 23.94, compared with a mitotic index of only 16.68 after a 3 hour exposure. The former is approximately 15% above control values, while the latter is approximately 8% above control values. Therefore, a 6 hour treatment with 5-AU accumulates twice as many cells at the block as does a 3 hour treatment. This comparison cannot be made with other stages of development since just emerged roots and primordia do not exhibit the same degree of synchrony seen in the lateral roots.

The differences in the response of different meristems to 5-AU has two aspects. First, since the drop in mitotic

index occurs in all meristems, 5-AU must reach all cells in concentrations that induce the block at the S - G_2 transition. Secondly, recovery of mitotic activity occurs at different times and rates in different meristems and this suggests either that the concentration of 5-AU needed to reduce the rate of DNA synthesis differs in different meristems, or that the amount of 5-AU reaching primordia is less effective in terms of reducing DNA synthesis than in blocking cells at the S - G_2 transition. However, the just emerged roots, which are outside the primary root epidermis and are exposed to 5-AU to the same extent as lateral roots, are less affected by 5-AU than primary or lateral roots. This strongly suggests that there is a physiological difference between just emerged roots, primary roots, and lateral roots. And by comparison of just emerged roots with primordia, it is suggested that they also differ physiologically from primary and lateral roots and that their response is not due solely to less effective penetration by the analogue.

Additional evidence that 5-AU reaches primordia in effective concentration comes from studies of chromosome breakage. In experiments not described here it was found that micronuclei were less frequent in just emerged roots than in laterals (3.25 compared with 10.25 per 5,000 cells) but more frequent than in large primordia (3.25 compared with 1.75 per 5,000 cells). These data were not described in detail because the frequencies of micronuclei were

extremely low. However, chromatid breakage and reunion has also been found in large primordia; approximately 20% of metaphases showed a chromatid exchange (Davidson, unpublished). 5-AU is, it seems, reaching the nuclei. The differences in response of different primordia are evidence of inherent physiological variations.

Response to nucleosides.

Treatment with a solution of nucleosides induced a more rapid recovery from the effects of 5-AU only in lateral roots (Figure 4) and only when 10^{-6} M nucleosides followed a 3 hour treatment with 5-AU. The nucleosides do not reverse or compensate for whatever change is induced by 5-AU, thus it appears that the effect of 5-AU is not due to a disturbance in the metabolism of DNA precursors. Nevertheless, post-treatment with a nucleoside solution results in an increase in the number of cells entering mitosis; the cumulative mitotic indices were higher, for several meristems, following treatment with nucleosides than after 5-AU alone. From this result it is tentatively suggested that while the nucleosides are ineffective in reversing the block at the S - G_2 transition they reverse, at least to some extent, the inhibition of DNA synthesis.

SUMMARY

1. 5-AU induced mitotic inhibition and subsequent partial synchronization of mitoses, upon recovery, in primary roots, lateral roots, just emerged roots, very large primordia, large primordia, and small primordia of V. faba.

2. The 5-AU treatment showed that the small primordia and primary root are heterogeneous for cell cycle duration and that the ratio of the sub-populations is relatively constant during root morphogenesis.

3. The just emerged root is unlike the lateral root in its response to 5-AU even though it has broken through the primary root epidermis, and, in fact, resembles more closely the very large primordium in its response to 5-AU. The just emerged root may be regarded, anatomically and physiologically, as a transition stage between the very large primordium and the lateral root.

4. The inability of exogenous nucleosides to cause cells to overcome the 5-AU-induced block, when exposure is to 5-AU and nucleosides simultaneously, suggests that a disruption of the metabolism of precursors of DNA is not the cause of the 5-AU block of the cells at the S - G_2 transition. But, although nucleosides do not cause a reversal of the 5-AU induced block, they are capable of causing more cells to divide, when given as a post 5-AU treatment, during the

peak of partially synchronous divisions, suggesting that they can increase the rate of DNA synthesis in cells recovering from exposures to 5-AU, by supplying necessary DNA precursors.

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