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Nuclear Cycle In Maize (zea Mays, L) Root Tips

Ram Sagar Verma

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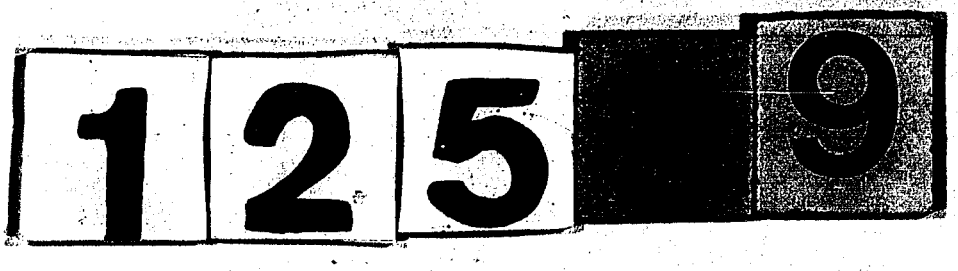

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NUCLEAR CYCLE IN MAIZE (*Zea mays* L.) ROOT TIPS

by

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Submitted in partial fulfillment of
the requirements for the degree of
Doctoral of Philosophy

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The University of Western Ontario,
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DEDICATION

With deepest gratitude, I dedicate this thesis to my mother, Moonga D. Verma

(1924 - 1950)

ABSTRACT

Estimates of the duration of the components of the nuclear cycle in cells from maize (Zea mays) root tips have been made from autoradiographic data, and a number of variables have been assayed. Of particular interest are the influence of the genotype, temperature, autopolyploidy and some 'mitotic poisons' - cycloheximide (0.001%) and chloramphenicol (0.03%). An estimate of the duration of each phase was obtained from a proportion method. A total of approximately 500,000 nuclei were classified.

Comparisons were made of the nuclear cycle component durations (singlecross hybrid 'Seneca 60') at 20, 25, 30 and 35°C. It was shown that the duration of all components (G_1, S, G_2, and Mitosis) of the nuclear cycle were shortened by an increase in temperature from 20 to 35°C. The duration of the nuclear cycle at 20, 25, 30, and 35°C was 16.5, 9.9, 7.0 and 4.4 hours, while the duration of mitosis was 2.7, 1.1, 0.8, and 0.4 hours respectively.

Derived autotriploid and autotetraploid stocks of inbred W23 demonstrated an essentially identical nuclear cycle. At 25°C, the total nuclear cycle was 11.2 to 11.5 hours and the DNA synthesis period (S) was 5.0 to

5.5 hours for each of 2N, 3N, and 4N stocks. It was concluded that the nuclear cycle was under specific genetic control and that the DNA synthesis period in autopolyploids was not dependent upon the amount of DNA present in a cell.

Primary radicles (Z. mays) were given a 30 minute pulse label with ^{3}H-TdR prior to a two hour incubation with cycloheximide (0.001%) or chloramphenicol (0.03%) at either 25 or 30°C. At 25°C, the duration of the total nuclear cycle was increased over the control value by a factor of 1.5 for cycloheximide and chloramphenicol, whereas the 30°C the increment factors were 1.7 and 1.8 respectively. There was a significant reduction in comparison to the control values in the mitotic index after treatment. This fall in mitotic index was interpreted to indicate that some interphase processes were delayed.

The nuclear cycle was compared among several stocks and it was shown that there was no significant differences among them. At 25°C, the nuclear cycle was 10 to 11 hours and mitosis was 0.8 to 1.3 hours in all stocks. The S period accounted for approximately 50

percent of the nuclear cycle; of the remainder, G_2 was longer than G_1 or mitosis in all stocks.

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R. S. Verma

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CHAPTER 1

INTRODUCTION

Following the rediscovery of Mendel's work in 1900, the corn plant (*Zea mays*) became a favored subject for cytogenetic investigations. The discovery in 1933 of the usefulness in cytogenetic research of the polytene chromosomes in the salivary gland cells of *Drosophila melanogaster*, the recognition of the value of heteroploidy and polyploidy as cytogenetic tools, and the use of maize in cytogenetic studies have provided additional avenues of approach to an understanding of the chromosome in cell division as well as in heredity. The cytogenetist is indebted to Brink, Burnham, Emerson, Jones, Longley, McClintock, Randolph, Rhoades, Stadler, and others by whose painstaking and arduous labors maize genetics has been brought to its present advanced stage.

Besides having several desirable features, such as separation of the male and female inflorescences which makes unnecessary the tedious emasculation required for controlled pollination, and the abundance of mutants, maize is an excellent material for manipulating a specific chromosome or a whole genome.

It would appear from the literature that only certain problems of corn cytogenetics have been investigated. For example, Clowes (1965) published data on the duration of each phase of the nuclear cycle in four regions of the root meristem cells of Zea mays. Later, estimates of the nuclear cycle in Z. mays root tips have been reported by several workers employing the squash technique (Dougall, 1970; Douglas, 1971; Evans and Rees, 1971; Jain and Gupta, 1971).

The study reported in this thesis includes the discussion of several variables which might affect the duration of the nuclear cycle and its component phases. Of particular interest are the influence of genotype, temperature, euploidy, and some 'mitotic poisons', cycloheximide (0.001%) and chloramphenicol (0.03%). A further aim of this study was to clarify whether or not a relationship exists between the DNA content and the duration of the S period. This latter study was carried out by comparing the duration of the S period from three derived stocks of inbred W23 (diploid, autotriploid, and autotetraploid) of Z. mays.

CHAPTER 2

LITERATURE REVIEW

" ..... a theory may be true even though nobody believes it, and even though we have no reason for accepting it, or believing it is true; and another theory may be false, although we have comparatively good reasons for accepting it."

Karl R. Popper
Conjectures and Refutation, 1965.

2.1 General:

The story of the discovery of mitosis is interesting and well documented. Historical accounts will be found in the works of Strasburger (1880), Flemming (1879), Henneguy (1896) and others. A detailed and critical account is given by Mazia (1961).

The study of cell proliferation has been simplified in recent years by the use of isotopically labelled substances. One of the most useful labelling substances in this regard is tritium-labelled thymidine (^{3}H-TdR, Figure 2.1-1). The study of cell proliferation in blood and blood forming organs in mammals has been one of the primary areas of investigation. A listing of many of the earlier publications dealing with the use of ^{3}H-TdR in the study of cell proliferation may be seen in the bibliography

on autoradiography prepared by Lima-de-Faria (1959).

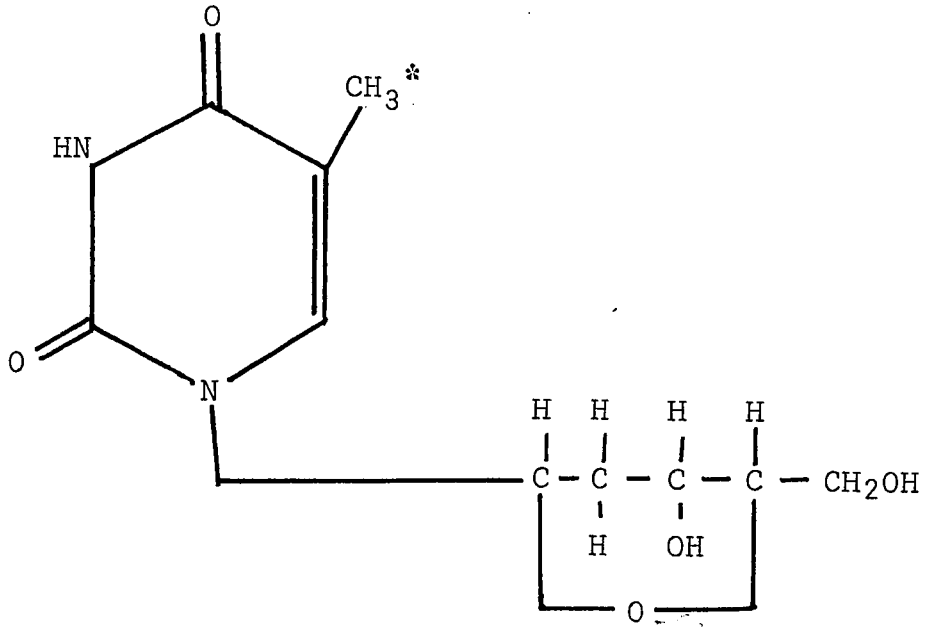


Fig. 2.1-1 Structural formula of ^{3}H-TdR (after Hall, 1962).

In 1953, Howard and Pelc proposed the composition of a nuclear cycle. They divided the interphase into three stages on the basis of results obtained in autoradiographic studies. They found that ^{32}P was incorporated into chromosomal DNA during the middle third of interphase (named the S period). Howard and Pelc estimated the duration of S to be about six hours and that of the period between the end of S and the beginning of the prophase (named the G_2 period) to be about eight hours in Vicia faba root tip cells. The duration of the interval (G_1) between telophase and S was calculated to be 12 hours.

After the pioneer work of Howard and Pelc (1953), a more precise approach was developed by Quastler and

Sherman (1959) and Wimber (1960) known as the 'pulse-labelling technique'. The rationale behind this method is as follows:

If ^{3}H-TdR is made available to an asynchronously dividing cell population such as is found in root meristems, presumably only those nuclei that are in the S period will incorporate the label. A representation of an asynchronously dividing population of cells is shown in Figure 2.1-2A. Wimber (1960) stated ... "As the cells that were in the S phase at the time of labeling advance through the cycle, there would be a period during which no labeled mitoses are seen. The duration of this period would correspond to G_2 on the figure. As the labeled cells reach mitosis, the number of labeled dividing cells progressively increases until 100% are labeled. This condition is maintained until the label block of cells progresses through mitosis. Theoretically, there would then be a period equal to the duration of G_1,G_2 and Mitosis during which no labeled dividing cells could be seen. Such a condition would prevail if the duration of the mitotic cycle and the various phases thereof were invariable in length and the labeling period could be regarded as instantaneous". However, the complete absence of variation from cell to cell in the duration of the component phases of the nuclear cycle is never found in proliferating tissue and the original block of labelled cells soon spreads as seen in Figures 2.1-2B and 2.1-2C (Wimber, 1960).

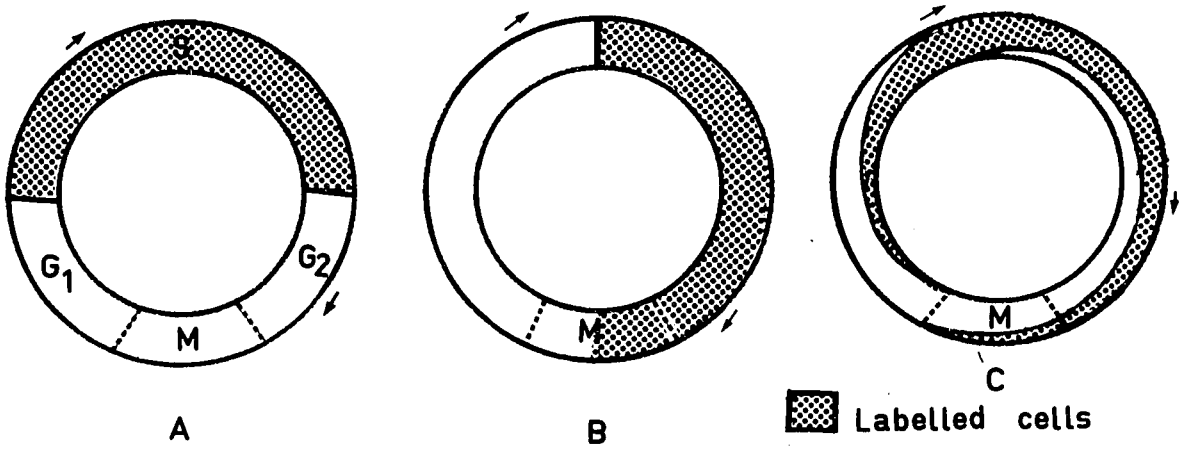


Fig. 2.1-2 A representation of an asynchronously dividing population of cells progressing through the nuclear cycle;

A. A cell population at time of a hypothetical instantaneous labelling with ^{3}H-TdR.
B. Shortly after labelling.
C. Entering the second division after labelling.
(after Wimber, 1960)

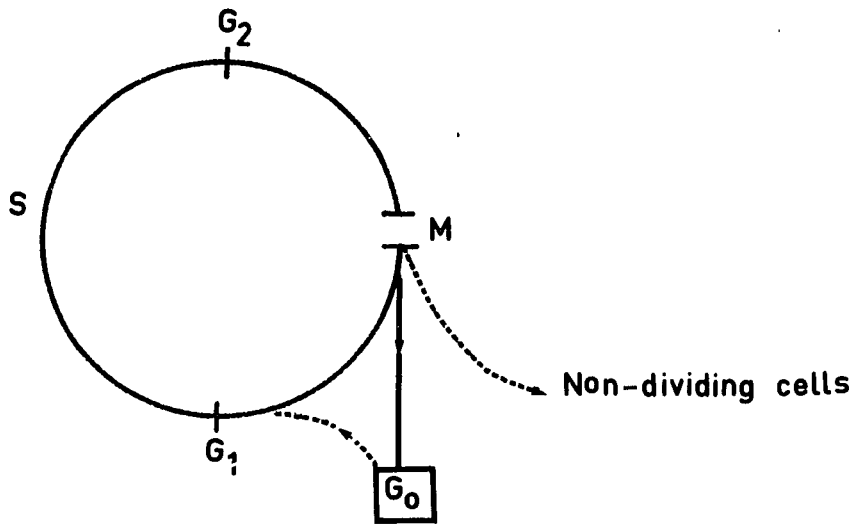


Fig. 2.1-3 The nuclear cycle (after Baserga, 1968).

Although the periods G_1, S, and G_2 are considered to be component phases of the interphase of dividing cells, they probably do not exist in the interphase of differentiated and/or mitotically inactive cells. Lajtha (1963) has suggested the name G_o for such a 'true' resting stage, which in contrast to the stages G_1, S, and G_2, is not part of a nuclear cycle. Some G_o cells can be triggered into division. If this happens, the progression of cells is usually through the $G_1 \rightarrow S \rightarrow G_2$ cycle before they divide (Patt and Quastler, 1963).

There is also a third group of cells which, after mitosis, permanently 'leave' the nuclear cycle and die without dividing again. This kind of cell has been called a nondividing cell by Baserga (1968) (Figure 2.1-3). Examples of this type of cell include: erythrocytes, polymorphonuclear leukocytes, and keratinizing cells. They usually 'leave' the nuclear cycle after completion of mitosis, although in some cases it may be possible that cells leave the cycle during the G_2 period (Frankfurt, 1967). However, a discussion of this third group of cells belongs more properly to the problem of cell differentiation, aspects of which have been illustrated by Papacontantinou (1967) in his review of lense cell differentiation.

The duration of the nuclear cycle found in various cell populations varies considerably depending on the environmental conditions. The references about the nuclear cycle in several animal tissues have been cited in review

papers by Baserga (1968) and Epifanova (1967). In addition, several investigations using plants have been published in the last 15 years; some of the more recent papers are summarized in Table 2.1-1. It is clear from Table 2.1-1, that about 85 to 90 percent of the total nuclear time is spent in interphase while the remaining 10 to 15 percent is occupied by mitosis. The S period usually requires the longest portion of the interphase.

2.2 Temperature:

There is an optimum temperature for every biological process. Cell division is a very complex biological process, consisting of a number of different reactions. Numerous studies have been performed in attempts to determine changes that may occur in the cell under different environmental conditions. A factor that changes almost continuously under natural conditions is the temperature, and it is one that influences cell proliferation. Therefore, the actual duration of the entire 'life cycle' of a cell depends on the temperature.

2.21 Root Meristem:

Studies with Pisum sativum roots demonstrated that the rate of root elongation increased consistently over a temperature range -2 to 29°C (Leitch, 1916). Other studies using different methods, indicated that the duration of the nuclear cycle decreased over the temperature range of 10 to

Table 2.1-1

Duration of the nuclear cycle in root tips of several plant species as determined by pulse labelling with ^{3}H-TdR.

Species	Temp-erature °C	Cycle duration (hrs.)					References
		G_1	S	G_2	Mitosis	Total	
Allium cepa	21	2.6	12.7	3.7	4.0	23.0	Matagne (1968)
A. cepa	24	1.5	6.5	2.4	2.3	12.8	Bryant(1969)
Bellevalia romana	25	6.0	7.0	6.3	2.7	21.0	Jona (1966)
Brassica juncea	22	2.2	6.9	2.3	1.3	12.7	Ayonoadu & Rees (1968)
Crepis capillaris	23	1.0	6.3	2.1	1.1	10.5	Abraham et al. (1968)
C. capillaris	23	1.6	5.1	2.2	1.5	10.4	Kuroiwa & Tanaka (1970)
Haplopappus gracilis	24	1.5	4.0	3.5	1.5	10.5	Sparvoli et al.(1966)
Helianthus annus	19	0.6	5.5	2.8	0.8	9.7	Todorova & Ronchi (1969)
Phalaris canariensis	23	2.7	3.4	6.8	1.6	14.5	Prasad & Godward (1965)
Pisum sativum	20	11.3	4.2	3.7	1.8	21.0	Van't Hof (1965a)
		8.7	4.3	3.2	1.8	18.0	
Spiranthes sinensis	23	4.0	9.0	6.0	3.0	22.0	Tanaka (1965)
Tradescantia paludosa	22	4.0	10.8	2.7	2.5	20.0	Wimber (1960)
T. paludosa	21	1.0	10.5	2.5	3.0	17.0	Wimber & Quastler (1963)
Triticale hexaploide	20	1.2	5.1	4.7	1.0	12.0	Kaltsikes (1971)
Vicia faba	19	12.0	6.0	8.0	4.0	30.0	Howard and Pelc (1953)*
V. faba	19	4.9	7.5	4.9	2.0	19.3	Evans &Scott (1964)
V. faba	19	5.3	6.0	5.2	1.5	18.0	Grant & Heslot (1964)
V. faba	20	3.6	8.3	2.8	1.9	16.6	MacLeod (1968)
V. faba	22	2.5	6.2	3.3	2.0	14.0	Webster & Davidson (1968)
Zea mays							
(a) Cap initial	18	-1.0	8.0	5.0	2.0	14.0	Clowes (1965)
(b) Quiescent centre(QC)		151.0	9.0	11.0	3.0	174.0	
(c) Stele just above QC		2.0	11.0	7.0	2.0	22.0	
(d) Stele 200 µ from QC		4.0	9.0	6.0	4.0	23.0	
Z. mays	28	-0.8	6.0	3.1	1.9	10.2	Douglas (1968)
Z. mays	22	-1.9	7.4	5.4	1.8	12.7	Dougall (1970)
		-1.7	8.3	4.6	1.8	13.0	
Z. mays	25	2.0	5.0	1.8	1.1	9.9	Verma (1970)
Z. mays	**	0.5	4.3	**	**	10.5	Evans & Rees (1971)

*Authors employed continuous labelling with ^{32}P.

**Not reported.

30°C (Van't Hof and Sparrow, 1963b). Brown (1951) has reported the mean durations of the nuclear cycle in root tips of P. sativum. The data are presented in Table 2.21-1.

TABLE 2.21-1

Nuclear cycle durations (hours) in P. sativum root tips at four temperatures (Brown, 1951)

Phase	Temperature			
	15°C	20°C	25°C	30°C
Interphase	26.6	17.0	14.5	13.3
Mitosis				
Prophase	2.1	1.3	0.9	0.7
Metaphase	0.4	0.2	0.2	0.2
Anaphase	0.1	0.1	0.05	0.04
Telophase	0.4	0.2	0.2	0.2
Subtotal	3.0	1.8	1.4	1.1
Total	29.6	18.8	15.9	14.4

It is clear from the data in Table 2.21-1 that all phases show a decrement with increasing temperature in the intact root of P. sativum over the range of temperatures used by Brown (1951). The ratios of durations at two temperatures suggested Q_{10} values of approximately 2.0.

The effect of temperature on the duration of the nuclear and its component phases in Vicia faba root meristem

cells has been investigated by Evans and Savage (1959). The values obtained are listed in Table 2.21-2.

TABLE 2.21-2

Nuclear cycle durations (hours) in V. faba root tips at four temperatures (Evans and Savage, 1959)

Phase	Temperature			
	3°C	10°C	19°C	25°C
Interphase	245.2	57.9	23.1	21.0
Mitosis				
Prophase	8.4	4.1	1.9	1.1
Metaphase	2.7	0.9	0.3	0.3
Ana + Telophase	3.7	1.3	0.9	0.5
Subtotal	14.8	6.3	3.1	1.9
Total	260.0	64.2	26.2	22.9
Mitotic Index (%)	4.0	7.2	8.9	6.8

Evans and Savage (1959) found that the relative changes in the duration of nuclear cycle differed with changes in temperature. The mitotic indices at the various temperatures were also recorded (Table 2.21-2). They suggested also that the rate of cell progress, or mitotic rate, through the nuclear cycle increased linearly with increase in temperature in the range 3 to 25°C.

Lopez-Saez et al. (1966) calculated the durations

of the total nuclear cycle and mitosis from meristematic root tip cells of Allium cepa. The values are listed in Table 2.21-3. They reported that there was no significant change in the relative duration of mitosis compared to the total nuclear cycle time under any of the conditions tested. In other words, about 13 percent of the nuclear cycle time was allocated to mitosis while the remaining 87 percent of the time was involved in interphase. Lopez-Saez et al. (1966) reported that at 0°C and 40°C, cells were not mitotically active.

TABLE 2.21-3

Nuclear cycle durations (hours) in A. cepa root tips at seven temperatures (Lopez-Saez et al. 1966)

	Temperature						
	5°C	10°C	15°C	20°C	25°C	30°C	35°C
Interphase	112.6	47.5	26.2	16.4	11.8	9.6	9.4
Mitosis	16.8	7.1	3.6	2.4	1.7	1.4	1.4
Total	129.4	56.6	29.8	18.8	13.5	11.0	10.8

Murin (1966) has reported the nuclear cycle at several temperatures in Vicia faba root tips and showed that the duration of the nuclear cycle and the component phase are dependent on the temperature. The higher temperatures cause shortening of the duration of the total

TABLE 2.21-4

Nuclear cycle durations (hours) in *V. faba* root tips at seven temperatures (Murin, 1966)

Phase	Temperature						
	3°C	8°C	13°C	20°C	25°C	30°C	35°C
Interphase	236.8	77.3	29.5	19.7	10.2	11.2	23.5
Mitosis							
Prophase	14.7	7.7	2.8	1.5	1.0	0.7	0.3
Metaphase	5.5	1.9	0.7	0.5	0.3	0.3	0.07
Anaphase	3.0	1.4	0.5	0.3	0.2	0.1	0.07
Telophase	3.9	2.0	0.7	0.4	0.2	0.1	0.05
Sub-total	27.2	13.0	4.3	2.3	1.7	1.2	0.5
Total	264.0	90.3	33.8	22.0	11.9	12.4	24.0

nuclear cycle (Table 2.21-4).

Van't Hof and Ying (1964b) have reported the time intervals for the total nuclear cycle in Pisum sativum root meristem cells (Table 2.21-5). The observations of Van't Hof and Ying also suggest that the rate of root growth increases linearly from 10 to 20°C.

TABLE 2.21-5

Nuclear cycle durations (hours) in P. sativum root tips at five temperatures (Van't Hof and Ying, 1964b)

Phase	Temperature				
	10°C	15°C	20°C	25°C	30°C
Interphase	29.5	18.0	10.2	8.6	8.9
Mitosis	4.5	4.0	1.8	1.4	1.1
Total	34.0	22.0	12.0	10.0	10.0

The duration of the nuclear cycle and its component phases in cells from root tips of Tradescantia paludosa has been reported by Wimber (1966). It should be noted from the Table 2.21-6 that the relative durations of the nuclear cycle did not change proportionately, i.e. a comparison between the nuclear cycle at 21°C and 13°C showed that at the lower temperature mitosis and G_2 were approximately tripled in duration while S period was doubled.

Also, it should be noted that the durations of the

TABLE 2.21-6

Nuclear cycle durations (hours) in T. paludosa root tips at three temperatures (Wimber, 1966)

Phase		Temperature		
		13°C	21°C	30°C
Interphase				
	G_1	15.4	5.8	2.4
	S	22.5	10.8	9.5
	G_2	8.3	2.5	2.4
	Sub-total	46.2	19.1	14.3
Mitosis				
	Prophase	4.1	1.3	1.3
	Metaphase	0.3	0.1	0.1
	Anaphase	0.2	0.1	0.1
	Telophase	0.5	0.2	0.2
	Sub-total	5.1	1.7	1.7
Total		51.3	20.8	16.0

subdivisions of mitosis are similar at 21 and 30°C, an observation which Wimber (1966) has suggested is indicative of a wide optimum temperature range for mitosis in T. paludosa root tip cells.

2.22 Tissue Culture:

Mackenzie et al. (1966) described the effect of temperature on nuclear cycle in Tetrahymena pyriformis HSM. Each component phase demonstrated a temperature dependency in

its duration similar to that of the nuclear cycle; G_1 + ½ D (D = Cytokinesis) accounted for approximately 44%, S for 28%, and G_2 + ½ D for approximately 28%.

The effect of changes in temperature on the duration of each phase of the nuclear cycle for human amnion cells in culture was reported by Sisken et al. (1965). All phases of the nuclear cycle responded quickly to changes in temperature. However, at temperatures below optimum, G_1 and metaphase were most sensitive and G_2 + Prophase and anaphase the least sensitive. At temperatures above the optimum, metaphase was again the most sensitive to increments, G_1, S, and G_2 + Prophase had moderate and similar sensitivities, and anaphase was reported to be completely insensitive.

In cultured blood cell lines from a leukemic mouse Watnabe and Okada (1967) reported the nuclear cycle durations at three temperatures, 31, 34, and 37°C. All the component phases of the nuclear cycle were affected. The most temperature sensitive stage was G_1, followed by S, the G_2 and Mitosis in that order. They suggested that the alteration of the total nuclear cycle time by temperature was attributed mainly to changes in the duration of G_1 and S stages. In HeLa cells, Rao and Engelberg (1965) made the contrasting observation that mitosis was the most temperature sensitive.

In summary, it is clear from the literature that changes in temperature are known to influence the nuclear cycle and its component phases. The effect of temperature

on the nuclear cycle has not been studied in Zea mays root meristem cells. In the present study, the duration of nuclear cycle and its component phases at 20, 25, 30 and 35°C in root meristem cells of Z. mays have been estimated.

2.3 Ploidy:

The hypothesis has been proposed that homologous sites on chromosomes may synthesize DNA at the same time. If the hypothesis is valid, one would expect that even with an increase in ploidy, the temporal pattern of DNA replication would remain the same. In theory, corresponding portions of homologous should replicate simultaneously, and the pattern of replication throughout the complement would be retained at various euploid levels. Thus increased euploidy level would not be expected to lengthen the time required for DNA synthesis provided that precusors and enzymes were not limiting factors.

Troy and Wimber (1968) have demonstrated that the S period duration is independent of the nuclear DNA content employing the diploid and autotetraploid stocks of Tradescantia paludosa, Lycopersicum esculentum, Ornithogalum virens, Cymbidium, and Ehrharta erecta. The S period durations obtained for the diploid and autotetraploid plants are presented in Table 2.3-1.

TABLE 2.3-1

Ploidy level, chromosome number, interphase nuclear volume and S period duration in root tips of several plants (Troy and Wimber, 1968)

Species	Ploidy level (N)	Chromosome number (n*)	Interphase nuclear (μ^3)	S period duration (hrs)
Cymbidium	2	20	-	7.1
	4	40	-	7.7
E. erecte	2	12	196.0	6.1
	4	24	392.0	6.2
L. esculentum	2	12	-	7.2
	4	24	-	7.6
T. paludosa	2	6	1050.2	10.7
	4	12	2150.4	10.4
O. virens	2	3	494.1	7.9
	4	6	1001.0	7.9

*chromosome number in gametophyte

The results presented by Troy and Wimber (1968) are not in agreement with those of Van't Hof and Sparrow (1963a). The latter authors indicated that a linear relationship existed between the interphase nuclear volume and the nuclear cycle time, and also between the DNA content per cell and the nuclear cycle time. However, the durations of the S period were not investigated by these latter workers.

In these experiments, the nuclear cycle time determinations were performed at 23°C. Their data are presented in Table 2.3-2.

It is apparent from the work of Prasad and Godward (1965) that there was no pronounced difference between the diploid Phalaris canariensis and the tetraploid P. minor with regard to the duration of the total nuclear cycle or mitosis. However, the S period in the tetraploid P. minor (6.4 hours) was twice the duration of the S period in the diploid P. canariensis (3.4 hours). The investigators concluded that nuclei having more DNA required more time for DNA synthesis than those nuclei with less DNA. This is in agreement with the hypothesis proposed earlier by Van't Hof and Sparrow (1963a).

The conclusion reached by Defendi and Manson (1963) indicated that the number of chromosomes had no bearing on the length of the S period. These authors tabulated results from the literature on the relationship of chromosome number and DNA synthesis and expressed the opinion that the duration of S is independent of the number of chromosomes.

The relationship between the amount of DNA per cell and the duration of the S period in three strains of Tetrahymena pyriformis (HSM, EU6000 and EU6002) was determined by Camerson and Stone (1964). Wells (1960) had indicated that two of these strains (EU6000 and EU6002) contained nearly twice as much as DNA as strain HSM. It

TABLE 2.3-2

Chromosome number, interphase nuclear volume, and nuclear cycle duration in root tips of several plants (Van't Hof and Sparrow, 1963a)

Species	Chromosome number (n*)	Interphase nuclear volume (μ^3)	Total nuclear cycle (hrs)
Helianthus annus	17	195	9
Pisum sativum	7	200	10
Tradescantia paludosa	6	640	18
Trillium erectum	5	1175	29
Tulipa kaufmanniana	12	800	23
Vicia faba	6	377	13

*chromosome number in gametophyte

appeared that under identical conditions, the duration of the S period was not a function of the amount of DNA in the nucleus nor was it closely related to the total nuclear cycle duration. The durations of S were 59, 59, and 58 minutes in HSM, EU6000, and EU6002 strains respectively. The total nuclear cycle time was about 137 minutes in all three strains.

Van't Hof (1966) reported the nuclear cycle durations in diploid and tetraploid cells of the same root meristem of Pisum sativum root tips. The nuclear cycle duration of tetraploid cells was less than diploid cells. At 21°C, the total nuclear cycle was 13 hours in tetraploid cells while in diploid cells it was 16 hours. Van't Hof (1966) concluded that the S period was not different in diploid and tetraploid cells, and that any difference in the total nuclear cycle duration between the diploid and tetraploid cells was the result of either an extended G_1 period in the diploid cells or shortened G_1 in the tetraploid cells.

Van't Hof (1966) offered three possible explanations for the different nuclear cycle durations of tetraploid and diploid cells: (1) colchicine shortened the duration of nuclear cycle of the affected cells; (2) colchicine and ^{3}H-TdR affected differentially the cell population (differing in cellular constituents); and (3) the β-rays emitted from tritium 'irradiated' the nucleus to such an extent that an increased cycle duration resulted.

Additional insight can be obtained from other investigations. The synthesis of DNA in haploid and diploid neurulae of Xenopus laevis has been studied by Graham (1966). The duration of the S phase in haploid and diploid cells was similar. Therefore, the duration of DNA synthesis was not changed by increased euploidy and the amount of DNA in the nucleus.

Friedberg and Davidson (1970) have reported the nuclear cycle in diploid and tetraploid cells of mixoploid meristems in Vicia faba. Their results showed that diploid and tetraploid cells have similar cycle time. Also, the S period was of similar duration in the two cell types. They suggested that a doubling of the amount of nuclear DNA does not result in an increase in either the duration of the S period or entire nuclear cycle.

The relative amount of nuclear DNA of root meristematic cells of two related diploid Avena species, A. strigosa and A. pilosa, which have different karyotypes, and an autotetraploid of one (A. strigosa) were measured. The result showed that the autotetraploid had the same S period duration as the diploid. There was no difference in total nuclear cycle of the three species (Yang and Dadson, 1970).

Thus, the literature provides conflicting views: on one hand, reports of a correlation between DNA content and the duration of DNA synthesis in both plants and animal tissues: nuclei having more DNA require more time for synthesis than those with less DNA; on the other hand, it has been

shown in some organisms that the duration of S period is independent of the nuclear DNA content.

2.4 Mitotic Poisons:

Study of the disruption of the nuclear cycle by various chemicals has been very popular for a considerable number of years and has given rise to a rather extensive literature. If one is interested in antimitotics, he may find himself involved in at least three questions: (1) the type of disruption; (2) the part of the nuclear cycle where reaction appears to take place; and (3) the biochemical nature of disruption. While it is a rare case where one might hope to answer all three questions with any degree of confidence, the questions themselves should be kept in mind. A number of efforts have been made in this direction. To quote Biesele (1958): "The great diversity of possible initial points of attack by mitotic poisons in the broad sense results from the complicated interlocking of the metabolic features of cell life". A corollary of this statement might suggest that different antimitotics acting in different ways may appear to have the same action if measured by only one criterion. It seems, therefore, highly desirable to measure antimitotic activity in a number of different ways.

Agents which prevent the nucleus from entering mitosis may inhibit the division of the cell. Similarly, although they may inhibit the formation of free chromatids

or daughter chromosomes, they do not necessarily inhibit chromosome reproduction (D'Amato, 1949a).

Usually the stage affected is interphase, or sometimes early prophase, which then is caused to revert to the interphase condition. When these "prophase inhibitors of mitosis" (D'Amato, 1949b) act on later stages of prophase, they often inhibit the normal progress of prophase to metaphase, an effect called "prophase poisoning" (D'Amato, 1952).

An agent which acts in G_1 or S would inhibit chromosome reproduction, whereas agents which produce their effect mainly in G_2 would affect only the formation of free chromatids since at this stage DNA has already been synthesized.

Since almost any drug will in some way or other influence cell division if applied at sufficiently high concentration and under appropriate experimental conditions, an attempt to review all the available literature on the subject would be beyond the scope of this thesis. Table 2.4-1 is a list of the effect of those chemicals which are known to influence certain phases of the nuclear cycle in root meristem cells of plants.

Studies on the effect of chloramphenicol on the nuclear cycle are not found in the literature. However, Ronchi and Buiatti (1967) and Buiatti and Ronchi (1969) have suggested this chemical might affect the G_2 period of the nuclear cycle.

Cycloheximide is now known to be an inhibitor of

TABLE 2.4-1

A partial list of mitotic poisons which affect the nuclear cycle in root meristem cells

Species	Mitotic Poisons	Phase affected	References
Allium cepa	Ethyl alcohol	S, G_2	Arcara and Ronchi (1967)
A. cepa	6-methylcoumarin	S	Nuti and Arcara (1967)
Vicia faba	Mitomycin C	Mitosis	Merze (1961)
V. faba	Nitromethyl-urethane	S, G_2	Grant and Heslot (1964)
V. faba	Maleic Hydrazide	S	Evans and Scott (1964)
V. faba	8-ethoxycaffein	G_2	Scott and Evans (1964)
V. faba	5-aminouracil	S	Jacob and Trosko (1965)
V. faba	Kinetin	G_1, S, G_2	MacLeod (1968)
V. faba	Trichlorophenoxy acetic acid	S, G_2	MacLeod (1969)
V. faba	5-aminouracil	S—G_2 Transition	Socher and Davidson (1971)
Zea mays	Mitomycin C	S, G_2	Lin and Walden (1971)
Z. mays	Hydroxylamine sulphate	S, G_2	Lin and Walden (1972)

protein synthesis in higher plant cells (Key, 1966, 1969; Waters and Dure, 1966) as well as other eukaryotic cells (Ennis and Lubin, 1964; Siegel and Sisler, 1965). This action on protein synthesis does not preclude the possibility of other effects within plant cells (MacDonald and Ellis, 1969).

In the slim mould, Physarum polycephalum, cycloheximide added between telophase and nucleolus dissolution in prophase completely blocked mitotis (Cummins et al., 1965). In mammalian cells, cycloheximide apparently does not effect mitosis but it has considerable effect on G_2 (Tobey et al., 1966; Verbin and Farber, 1967). In plant material, cycloheximide can block cells at different stages of mitosis, depending on when in the nuclear cycle the poison is added. According to Rose (1970), there may be protein synthesis requirements in prophase and telophase for completion of mitosis.

It is clear from the literature that more direct evidence is needed to interpret the action of chloramphenicol and cycloheximide on the nuclear cycle duration in plant cells. Therefore, in the present study, I have investigated the effect of these mitotic poisons on the duration of nuclear cycle and its component phase in Zea mays root tips.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Protocol:

The protocol followed is outlined in Figure 3.1-1. The flow chart lists all the major steps. Zea mays was used exclusively in this study.

3.2 Description of Material and its Preparation

3.21 Stocks and Pollination:

The maize stocks employed in the present investigation are listed in Table 3.21-1. Advanced generation seed was produced from all stocks except the singlecross hybrid 'Seneca 60' (su_1/su_1). The triploid W23 was obtained as the progeny of tetraploid W23 X diploid W23. The crosses using the tetraploid as the female parent were the more successful. Crosses, advanced generation seed production and the production of triploids were carried out in a nursery which was surrounded by plants from a colored aleurone marker stock. Colored kernels were eliminated as contaminants. To check the chromosome number of each stock, Chen's (1969) technique was employed. Representative karyotypes are presented in Figure 3.21-1.

FIG. 3.1-1: FLOW CHART OF EXPERIMENTAL PROTOCOL

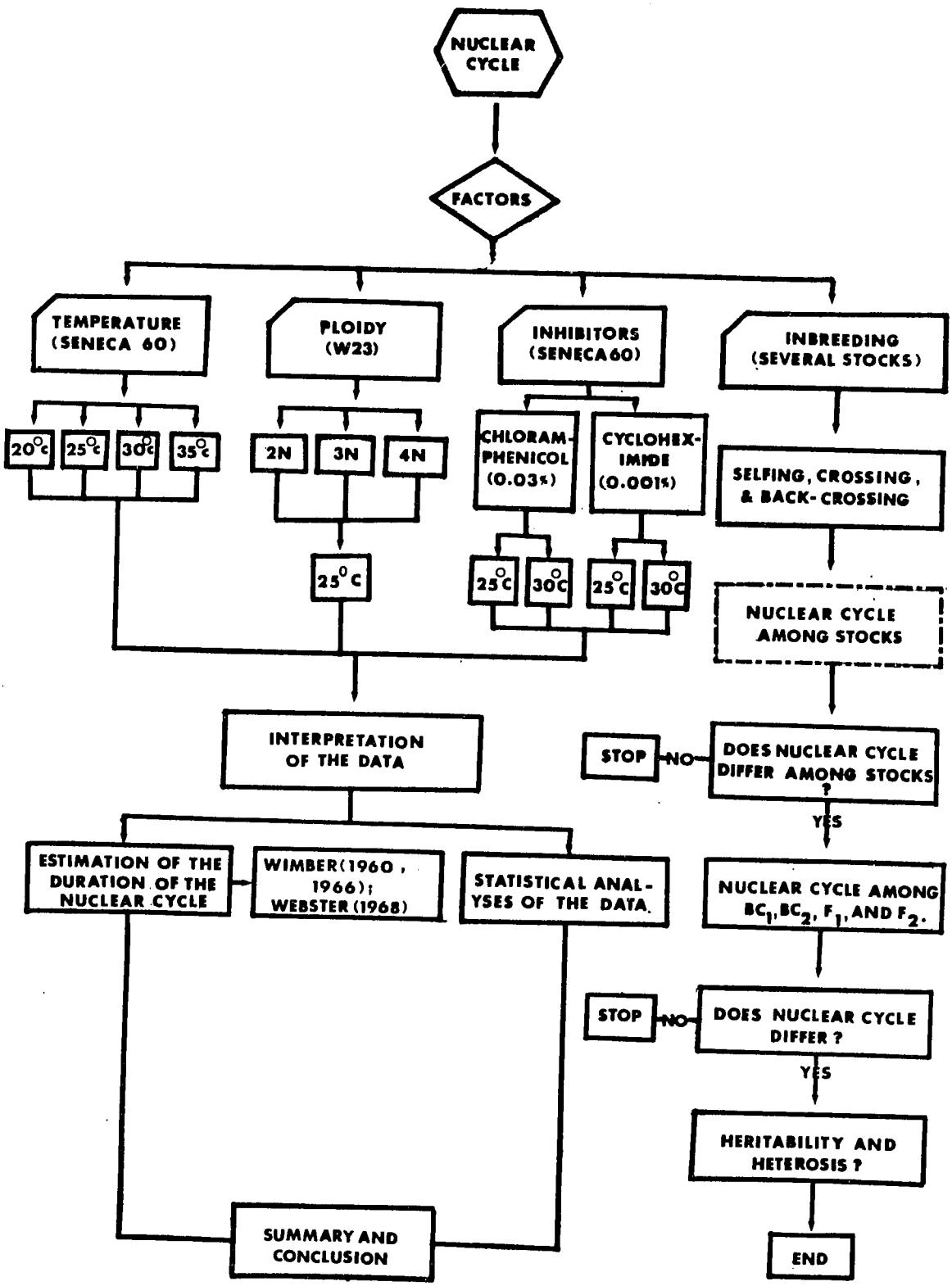


TABLE 3.21-1

Stock Information

Stock	Source
'Seneca 60'	Robson Seed Co., Hall New York
'W23'-2N	Maize Cooperative Stock Center, U.I.*
'W23'-3N	Maize Cytogenetics Laboratory, U.W.O.**
'W23'-4N	Maize Cooperative Stock Center, U.I.
Chromosome 3 tester	Maize Cytogenetics Laboratory, U.W.O.
Chromosome 9 tester	Maize Cytogenetics Laboratory, U.W.O.
Chromosome 10 tester	Maize Cytogenetics Laboratory, U.W.O.
'KYS'	Maize Cytogenetics Laboratory, U.W.O.
'Bothwell'	Maize Cytogenetics Laboratory, U.W.O.

*University of Illinois, Urbana, Illinois, U.S.A.

**University of Western Ontario, London, Canada

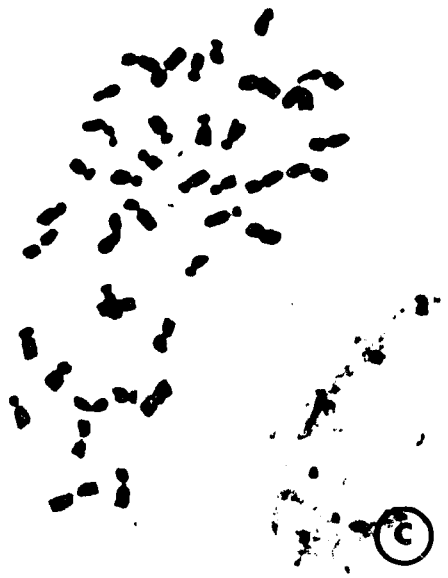


Fig. 3.21-1 Somatic metaphase chromosomes of Z. mays root tips (inbred W23):

A. Early metaphase, diploid.

B. Late metaphase, triploid.

C. Mid metaphase, tetraploid.

(X1400)

To produce the stocks required for the study, crosses were made in 1968 between several inbreds. The progeny of such crosses, and advanced generations, yielded the necessary F_1's, F_2's, BC_1's, and BC_2's stocks.

Preparation for the pollination was carried out using the established method of cutting silks and bagging tassels in late afternoon. The pollinations were performed during the following morning or early afternoon.

3.22 Temperature and Germination:

Seeds of the singlecross hybrid (su_1/su_1) 'Seneca 60' were employed for observing the root elongation and percent germination. All seeds pretreated with Arasan 75, following washing with distilled water, were germinated on moist filter paper in sterile Petri dishes in temperature controlled germination chambers. Each seed was placed so as to be approximately 1 cm from another. The temperature (20, 25, 30, or 35°C ± 0.5°C) was maintained under constant light (ca 8 ± 2 f.c.). For root elongation data each emergent primary root was measured on three consecutive days at 12 hour intervals with a flexible plastic ruler having millimeter subdivisions.

3.23 Cytological Preparation:

3.231 Pulse labelling and fixation:

Primary roots were raised under controlled environmental

conditions at a specific temperature. The attached primary roots (at least 3 cm in length) were placed for 30 minutes in a solution of ^{3}H-TdR, diluted with distilled water (1 µc/ml; specific activity 6.7 c/mM). ^{3}H-TdR was purchased from the New England Nuclear Corporation. The roots were placed in sterile Petri dishes and the solution of ^{3}H-TdR was poured into the Petri dishes.

At the end of the pulse exposure, the seedlings were washed thoroughly, placed on moistened filter paper in Petri dishes and returned to the germination chamber to resume growth. Root tips were excised and fixed in acetic alcohol (1:3) at two hour intervals at 20 and 25°C. At 30 and 35°C, the roots were fixed at one hour intervals. The fixative was prepared just prior to fixation in all cases. The roots were left in fixative overnight at room temperature. A minimum of five roots were sacrificed at each collection period. After one day, the roots were washed in 70 percent alcohol, and stored in the same fluid at 4°C until needed.

3.232 Stain and squash technique:

Hydrolysis was accomplished in 1 N HCl at 60°C for eight minutes. The roots were rinsed in distilled water for three to four changes, two-three minutes per change, and stained by the Feulgen technique. Roots were treated with 5 percent pectinase (Nutritional Biochemical Corporation) for at least two hours at room temperature,

and were transferred to 45 percent acetic acid for ten minutes to clear the cytoplasm. The root cap was removed by touching it to the surface of the filter paper and squashes of the terminal 1.5 ± 0.02 mm of the root meristem were prepared in 45 percent acetic acid. Slides were cleaned with "Dow Corning's" sight savers. Mayer's adhesive (Sharma and Sharma, 1965) was smeared directly on to a pre-cleaned slide before making squashes. Squash preparation were sealed with wax and kept in slide boxes at 4°C until needed. All slides were marked with a Dremel Electric Engraver.

3.24 Autoradiography:

3.241 Emulsion:

In preparation for dipping, the wax was removed from the sealed slide by cutting it away with a single-edge razor blade. The cover slips were removed by the dry ice technique (Conger and Fairchild, 1953) and the slides were rinsed in running distilled water for 15 to 20 minutes before air drying.

NTB2 (Eastman Kodak) emulsion was used in all experiments. The regular size of silvergrains, low background and high sensitivity of this emulsion made it excellent for our purposes. In a dark-room maintained at 17-18°C, a Kodak Wratten Safe light No. 2 was placed 3-4 feet above the work bench. On the bench, a water bath was

adjusted to 40°C. The original emulsion was diluted 1:1 with water in a 100 ml cylinder. This dilution was thin enough for nearly all the grains to be included in one focal plain under oil emulsion.

Melted emulsion was poured into a home made Borrel tube. The tube was filled with emulsion to a level such that the tissues on the slide were covered but the upper end of the slide was not covered. The tube was then placed in the water bath. Bubbles were rarely produced by this procedure and, if present, tended to disappear with time. A clear glass slide (without tissues) was dipped into the emulsion and the coat was examined before the safe light to find out if bubbles were present. If so, they were scooped out with a porcelain spoon.

Each slide was dipped for 1-2 seconds in the melted emulsion. After withdrawal of a slide from the emulsion, the excess was allowed to drain back into the container (i.e. Borrel tube) and the reverse side of the slide was wiped clean with soft tissue paper. As the slide was withdrawn, care was taken to keep it vertical. The slides were then allowed to dry in a vertical position with the top up and their base on tissue paper. Care was taken to assure that all of the slides were completely air dried before they were stored in the boxes.

3.242 Exposure:

The coated slides were stored in a double slide box

having two compartments. The bottom compartment was filled with silica gel (silica gel was wrapped in tissue paper) while the slides were in slots in the top compartment. There was plastic screen between compartments. The storage of slides in consecutive slots was found to be satisfactory. All emulsion coated surfaces were facing the same direction. The boxes were sealed with black adhesive tape. On each box the date of coating, identity of the experiment, and the date to be developed were recorded. The slides were exposed for 14 days at 4°C.

3.243 Processing of slides:

Processing solutions were kept in the dark room at 17-18°C. The exposed preparations were developed for two minutes in Dektol developer at 15°C and transferred to distilled water for 30 seconds. Following washing, fixation was carried out for three minutes in rapid fixer. The slides were then washed in running distilled water at 18°C for 15 - 20 minutes and dehydrated in an alcohol series and transferred to absolute alcohol. The autoradiographs were mounted by adding a drop of Euparal and covering the preparation with the coverslip.

The autoradiographic technique described above was employed for all the experiments.

3.25 Root Meristem:

The 1.5 ± 0.02 mm terminal roots were employed in

this study. The anatomy of the Z. mays root tip can be seen in longitudinal section of terminal root in Figure 3.25-1. A standard micro-technique was employed to prepare the section (Johansen, 1940; and Foster, 1934).

3.26 Autopolyploidy:

Inbred W23 was used in the diploid, triploid, and tetraploid form to ascertain if the amount or quantity of DNA influenced the nuclear cycle and, in particular, the S period. 25°C was chosen for the main study, inasmuch as our preliminary work indicated that 25°C provided good resolution of the cycle, i.e. it was neither telescoped nor elongated. Likewise, experimental error appeared to be minimal at 25°C.

3.27 Mitotic Poisons:

Cycloheximide and chloramphenicol were purchased from Sigma Chemical Company, St. Louis, Missouri. Preliminary experiments were performed to ascertain a suitable concentration for the main study. All treatments were carried out on attached 3 days old primary radicles of 'Seneca 60'. Several thousand kernels were germinated at 25 or 30°C for 48 to 84 hours in constant light. Following exposure to ^{3}H-TdR (30 minutes), the roots were thoroughly washed and transferred to cycloheximide (0.001%) or chloramphenicol (0.03%) for an additional two hours. After incubation in the treatment, the roots were thoroughly washed, and placed

Fig. 3.25-1 A. Median section of root apex of Z. mays.

Where:

E = Epidermis,

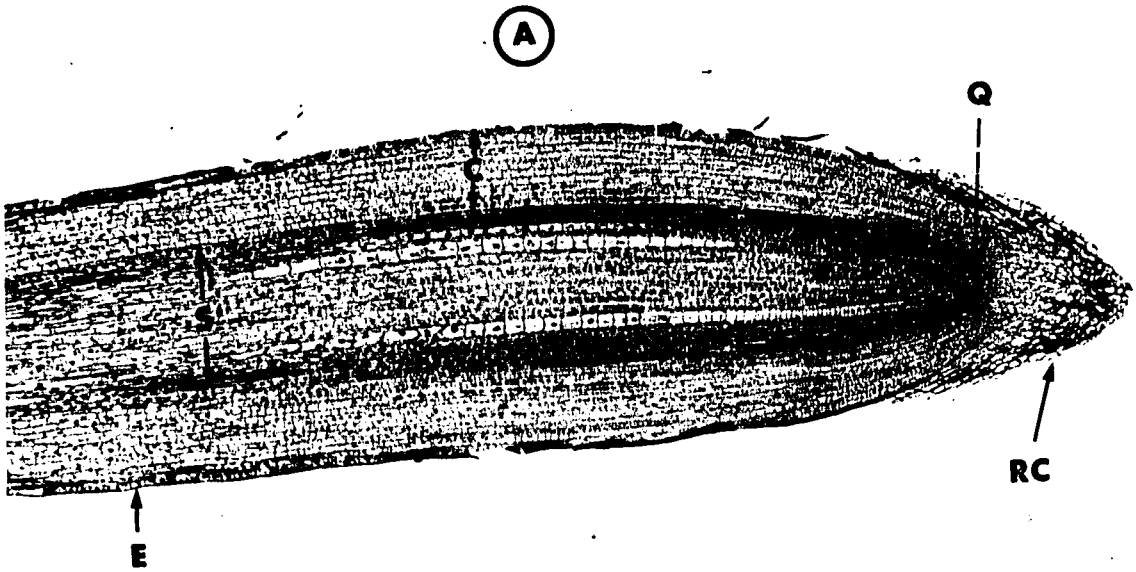
S = Stele,

C = Cortex,

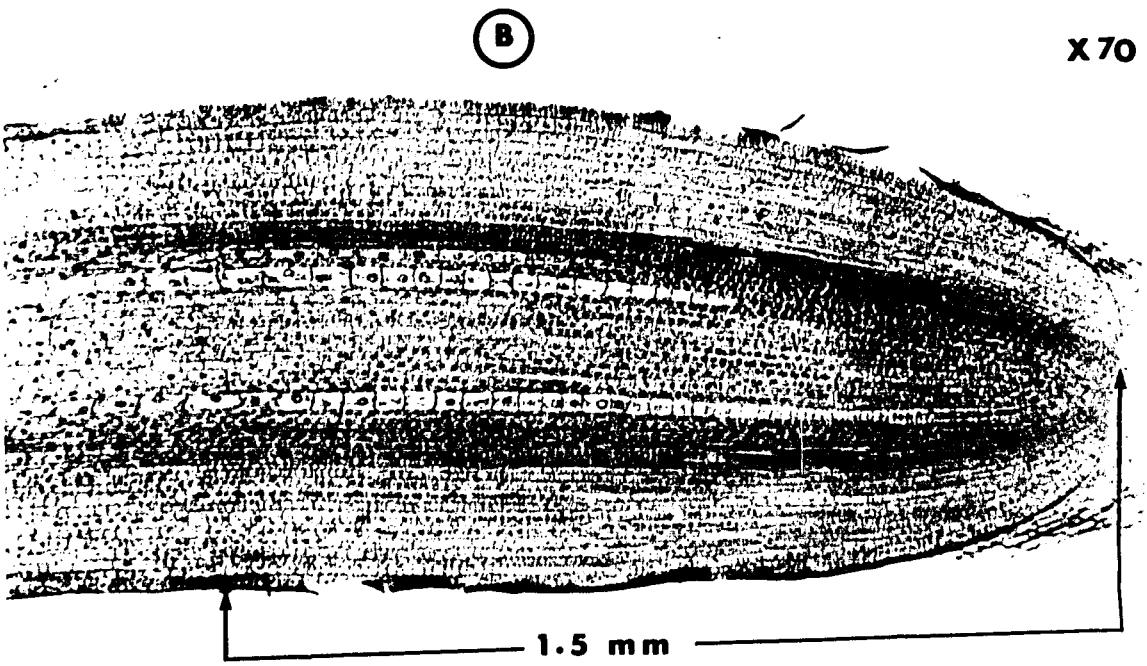
Q = Quiescent centre,

RC = Root cap.

B. Higher magnification of same root showing a 1.5 mm root section.



X45



X 70

on moistened filter paper in Petri dishes and returned to the germination chamber. Roots were collected at two hour intervals and processed according to the schedule.

3.28 Other Stocks:

The protocol followed to study the nuclear cycle and its component phases among different stocks is outlined in Figure 3.1-1. In an attempt to broaden the scope of this study, the nuclear cycle was determined at 25°C from a chromosome 9 tester stock carrying alleles yg_2 c sh_1 Bz wx, and a KYS inbred. These stocks were chosen as late maturity material to complement 'Seneca 60', and W23 as early, and medium maturity material respectively.

3.3 Data Collection:

Each slide was engraved with an identification including the treatment, replicate and experiment number. For scoring and data collection, the identification on each slide was covered by a paper label and the slides were recorded by a second party to yield a blind code unknown to the primary investigator. The slides were decoded jointly only after all the readings for an experiment had been completed.

The observations were made along a series of random transects taken across the slide. One edge of the coverslip was brought into the field of the objective and the slide was then moved along the same lateral line until

the second edge was brought into the view. The process was repeated with different transects until a total of approximately 1000 cells had been counted.

Throughout this investigation adherence to specific criteria for each stage was an absolute requirement. The criteria employed included (Brown, 1951):

Prophase, cells showing distinct chromosome threads within a nuclear membrane, and which were not associated with other cells in such a way as to suggest late telophase;

Metaphase, cells without a nuclear membrane and with distinct chromosomes either randomly distributed or on a metaphase plate;

Anaphase, cells without a nuclear membrane but with two groups of chromatids with free chromatid ends showing;

Telophase, cells having two distinct nuclei without loose chromatid ends and without a cell wall between them. All slides were examined with an Olympus microscope under oil emersion (X1000).

Five roots were sacrificed for each collection period. At least three slides (one root per slide) were utilized for counting in each collection period of each treatment in each experiment.

3.4 Estimation of the Nuclear Cycle and its Component Phases:

3.41 Method No. '1'

Two kinds of data were collected: (a) the frequency of various mitotic figures; and (b) their characterization as to possession of label. For an asynchronously dividing, uniform population of cells, the ideal curve as shown in Figure 3.41-1 (dotted line) would be obtained. This type of curve will be found when populations possess the following constraints (Quastler and Sherman, 1959; Wimber, 1960):

(1) The cycle is in a steady state.

(2) The cells are proliferating asynchronously.

(3) Phases of the nuclear cycle show no variation from cell to cell.

However, complete absence of variation in the duration of component phases of nuclear cycle from cell to cell is never found in proliferating cells. As a consequence of such variations, the 100% plateau of percent labelled prophase curve is shortened (Figure 3.41-1; solid line). Frequency histograms of the observed data were plotted as shown in Figure 3.41-2. The differences between observed and ideal curve are due to differential rates of progression of cells through the nuclear cycle.

The duration of the nuclear cycle can be estimated from the rhythmic appearance and disappearance of labelled prophases. In this method, the time interval between the 50% intercepts of the two successive ascending

Fig. 3.41-1 Hypothetical percent labelled prophase curve for ideal population (dotted lines). 'Observed' curve for ideal population (solid line); data for 'observed' curve from Figure 3.41-2.

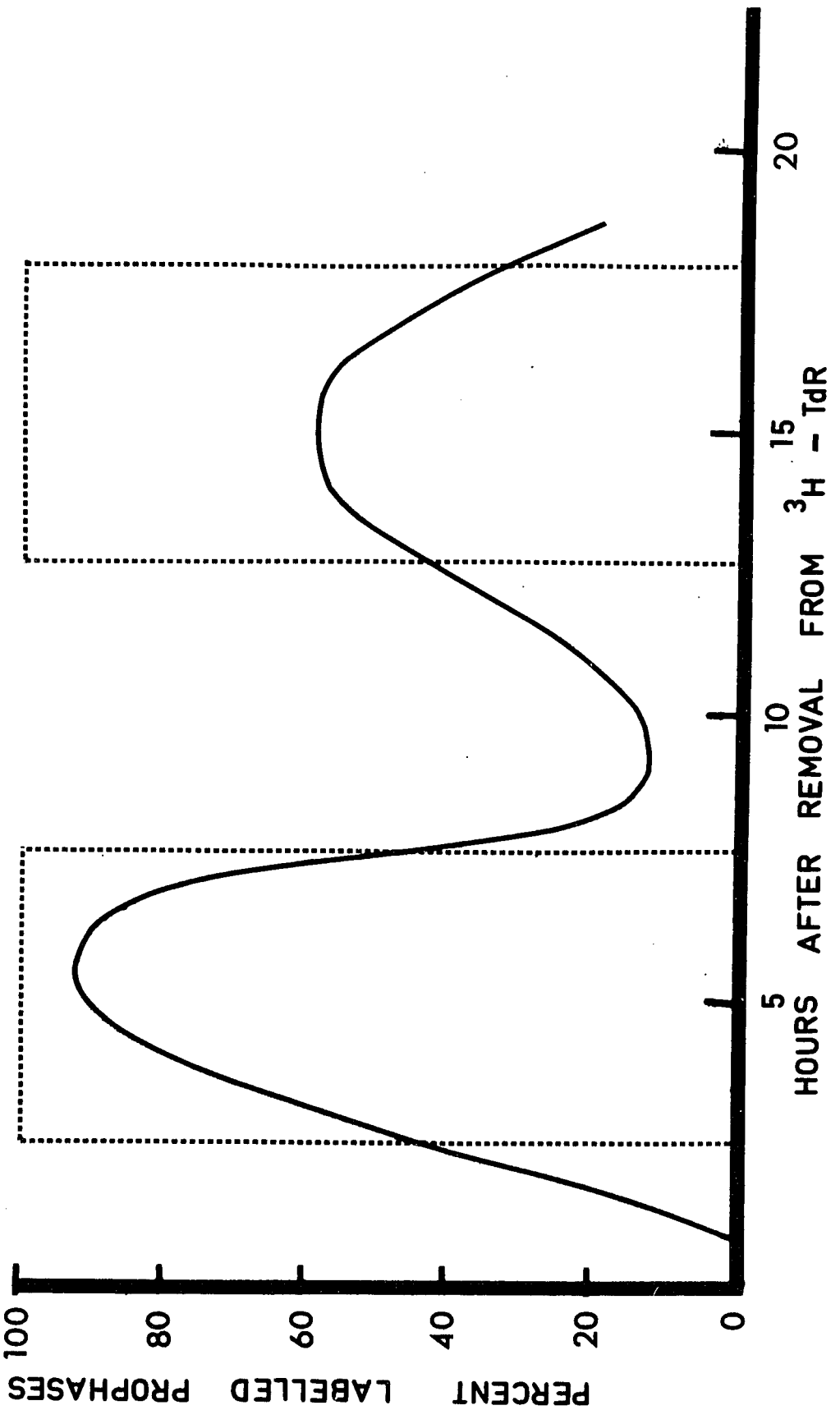

PERCENT LABELLED PROPHASES
0
20
40
60
80
100
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H - TdR

Fig. 3.41-2 Observed percent labelled prophase curve, following pulse labelling with ^{3}H-TdR for 30 minutes at 25°C in 'Seneca 60'.

Where:

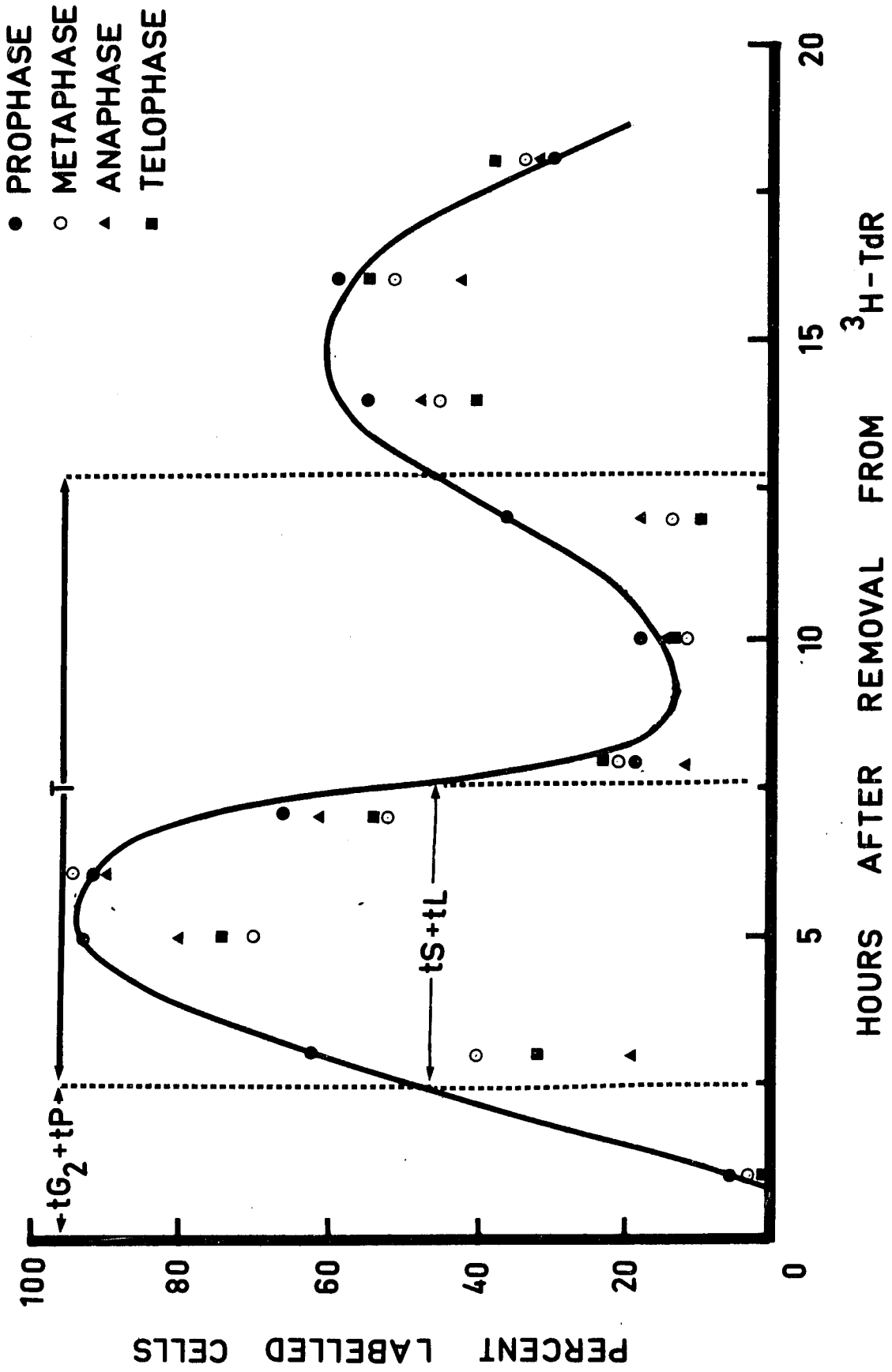
tG_2 = duration of the G_2 period,

tP = duration of prophase,

tS = duration of DNA synthesis period,

tL = incubation time (30 minutes) of ^{3}H-TdR,

T = total nuclear cycle.


PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
HOURS AFTER REMOVAL FROM ^{3}H-TdR
5
10
15
20
tG_2+tP
T
$tS+tL$

portions of the curve was used to estimate the total nuclear cycle duration as shown in Figure 3.41-2. The average duration of G_2 + Prophase was estimated as the distance between the origin and the 50% intercept of the first ascending portion of the curve. The duration of the S period was obtained by subtracting labelling time (i.e. 30 minutes) from the interval between the 50% intercept of the first ascending and descending portions of the curve of the labelled prophases (Figure 3.41-2).

In 1960, Wimber proposed to estimate the approximate duration of prophase by extrapolation from the metaphase data. The durations of metaphase, anaphase, and telophase were obtained by the relative frequency ratios of these phases to prophase (Wimber, 1960). Sparvoli et al., (1966) developed a more precise approach for estimating the duration of prophase, metaphase, anaphase, and telophase:

$$\frac{nS}{tS} = \frac{nP}{tP} = \frac{nM}{tM} = \frac{nA}{tA} = \frac{nT}{tT} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where:

nS = number of cells in the DNA synthesis period,
nP = number of cells in prophase,
nM = number of cells in metaphase,
nA = number of cells in anaphase,
nT = number of cells in telophase

tS = duration of the S period as estimated from curve,
tP = duration of prophase,
tM = duration of metaphase,
tA = duration of anaphase,
tT = duration of telophase.

Equation 1 was employed for estimating the duration of prophase, metaphase, anaphase and telophase. The durations of G_1 and G_2 were calculated from the following equations:

$$tG_2 = (G_2 + \text{Prophase}) - tP \quad \ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$tG_1 = T - (tS + tG_2 + \text{Mitosis}) \quad \ldots\ldots\ldots\ldots \quad (3)$$

Where:

tG_2 = duration of G_2,

$(G_2 + \text{Prophase})$ = duration of G_2 + Prophase as estimated from the curve,

tG_1 = duration of G_1,

T = duration of the total nuclear cycle.

3.42 Method No. '2'

Method No. '2' is a modification of the previous method in that G_2 + ½ Prophase is substituted for G_2 + Prophase (Figure 3.41-2). The explanation for this is as follows:

There is no fixed point which separates a particular phase of the nuclear cycle from another phase in an asynchronously dividing population of cells. Therefore, the distance between the origin and the 50% intercept of the first ascending portion of the curve was regarded as G_2 + ½ Prophase by Wimber (1966) and several other investigators. It is suggested that this latter method is a better approach for estimating tG_1 and tG_2.

3.43 Method No. '3'

In method '1' and '2' the nuclear cycle durations

are estimated from percent labelled prophase curves. In method '3', the percent labelled metaphase curve is also taken into consideration. The duration of G_2 + ½ Prophase is estimated in the similar manner as described in method '2'. The duration of G_2 + Prophase + ½ Metaphase is considered the value of the abscissa at which the intersection occurs of 50% of the ascending portion of the percent labelled metaphase curve. Accordingly, the duration of component phases of the nuclear cycle can be calculated as follows (Webster, 1968):

(G_2 + Prophase + ½ Metaphase) - (G_2 + ½ Prophase) =

$$\frac{tP + tM}{2} = A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Therefore:

$$tP + tM = 2A \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Where:

tP = duration of prophase,

tM = duration of metaphase.

The duration of metaphase alone can be obtained from equation 6.

$$tM = \frac{2A}{Z + 1} = B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

Where:

Z = The average nP/nM frequency ratio,

Therefore:

$$tP = (tP + tM) - B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

$$\text{Mitosis} = 2A \times C = F \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (8)$$

Where:

C = total mitotic cells / (nP + nM); frequency ratio.

Therefore:

$$tA + tT = F - 2A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (9)$$

Where:

tA = duration of anaphase

tT = duration of telophase

The durations of the S period and total nuclear cycle are calculated from percent labelled prophase and percent labelled metaphase curves as described by Webster (1968)

The estimates of the duration of other phases are obtained in a similar way that described in method '1'.

3.44 Method No. '4'

Gerecke (1970) introduced a new method for estimating tS. The advantage of his method is the fact that it allows for any variation of tS, tG_2, and Mitosis so that the constraints about the cell system under observation can be reduced. The percentage of labelled prophase seen at different times after removal from ^{3}H-TdR is called p(t) (Figure 3.44-1). The equations for estimating tS + ta (ta = incubation time of ^{3}H-TdR) are shown in Figure 3.44-1.

In addition, the area under the percent labelled prophase curve can be determined by the use of planimeter and that area will be an estimate of tS + ta (Gerecke, 1970b).

Fig. 3.44-1 Equations for the area under the curve:

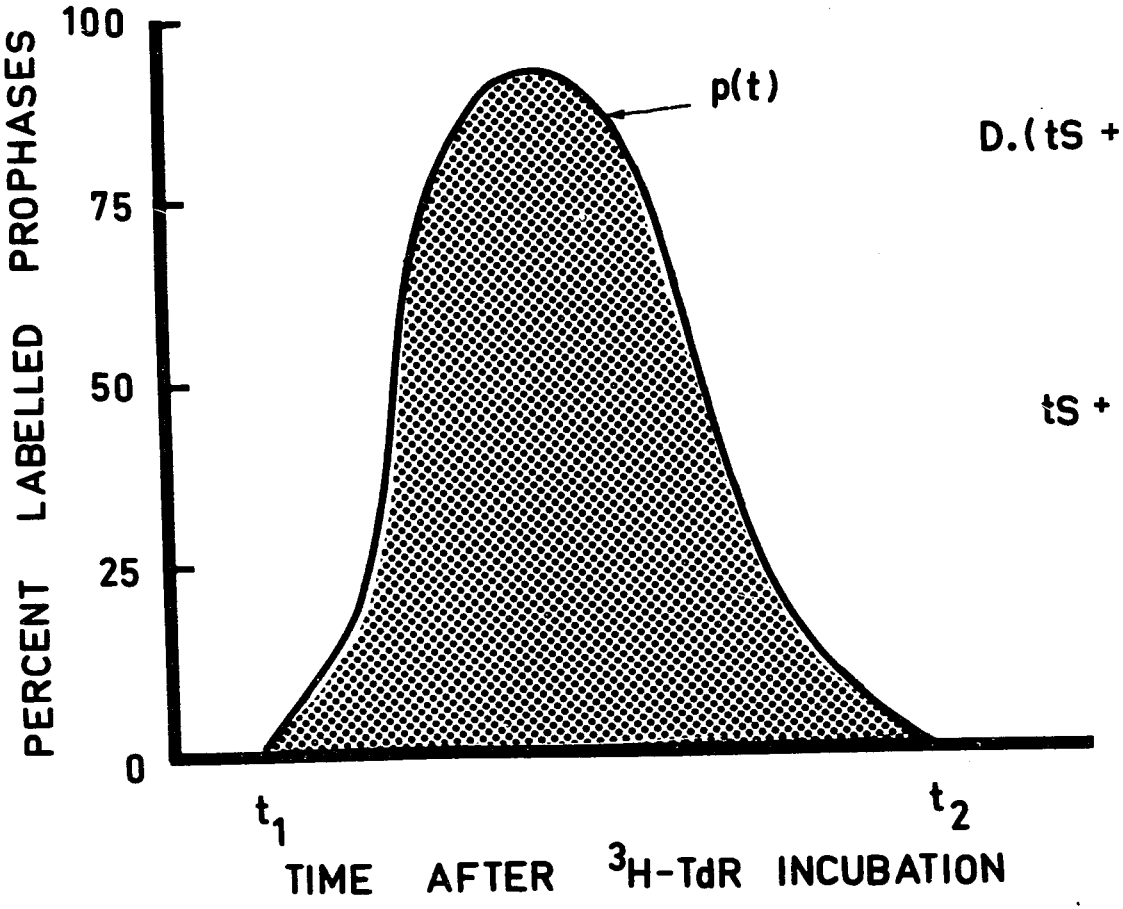
Where:

D = cells/unit time,

p(t) = The percentage of labelled cells seen at different times after ^{3}H-TdR incubation,

$$\int_{t_1}^{t_2} p(t).dt$$ = is the area under the curve.

(Gerecke; 1970a, 1970b)



$$D.(tS + ta) = D.\int_{t_1}^{t_2} p(t).dt \quad \text{.....(10)}$$

$$tS + ta = \int_{t_1}^{t_2} p(t).dt \quad \text{.....(11)}$$

The area under curve was calculated according to the equation (11) in Figure 3.44-1.

3.5 Biometrical Analysis:

The following statistics were employed:

3.51 Standard deviation of percent labelled cells for each hour of each treatment:

The standard deviation for the proportion of prophase, metaphase, anaphase, and telophase labelled cells for each point on the curve were computed by the following formula (Snedecor and Cochram, 1967; Sokal and Rohlf, 1968):

$$\text{Standard deviation for } p = \sqrt{\frac{pq}{n}} \quad X\ 100$$

Where:

p = proportion of labelled cells in particular stage;

q = (1-p), the proportion of unlabelled cells;

n = number of cells scored for each collection period.

3.52 Standard deviation for mean duration of phases in the nuclear cycle:

Probit regression analyses were performed to obtained weighted mean values and standard deviations appropriate to these means (Wimber, 1960; Finney, 1962; and Trucco, 1968).

3.53 Test of homogeneity of variances:

For testing the homogeneity of several variances an F_{max} test was performed; for comparing two variances an F-test was employed (Sokal and Rohlf, 1968).

3.54 Testing the equality of two mitotic indices:

A t_s-test was used to test the equality of two mitotic indices (Sokal and Rohlf, 1968):

$$t_s = \frac{\arcsin\sqrt{p_1} - \arcsin\sqrt{p_2}}{\sqrt{820.8\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

Where:

$df = \infty$

p_1 = mitotic index of group one,

p_2 = mitotic index of group two,

n_1 = total number of cells in group one,

n_2 = total number of cells in group two.

t_s is approximately distributed as Students t, and 820.8 is a constant representing the parametric variance of a distribution of arcsin transformations of percentages (Sokal and Rohlf, 1968).

3.55 Test of significance of means:

For testing the mean estimates of nuclear cycle components, Harvey's (1970) method was employed. This method is suggested for testing the means where the

variances are heterogeneous.

3.56 Confidence limits for each point on percent labelled curve:

The 95% confidence limits were calculated according to the formula (Sokal and Rohlf, 1968):

$$95\%\ \text{confidence limit} = \left(p \pm t_{0.05\,(\infty)} \sqrt{\frac{pq}{n}}\right) \times 100$$

Where:

p = proportion of labelled cells in a particular stage;

q = (1-p), the proportion of unlabelled cells;

n = number of cells scored for each collection period.

Biometrical analyses were done on the PDP-10 computer in FORTRAN IV language. The programs were written by the author.

CHAPTER 4

EXPERIMENTAL RESULTS

4.1 Temperature:

Preliminary experiments were performed to ascertain the percentage of germination and root elongation at 20, 25, 30, and 35°C in Zea mays ('Seneca 60'). The data are presented in Tables A*-1 and A-2. The root elongation increased with temperature. In brief, 100 percent of the seeds were germinated after 84 hours in the germination chamber at 20°C, while at 35°C, it took only 36 hours. The root lengths were 2.0, 4.5, 5.0 and 7.3 cm after 96 hours in the germination chamber at 20, 25, 30, and 35°C respectively (Table A-2).

The percent labelled prophase curves for roots grown at four temperatures are shown in Figures 3.41-2, 4.1-1, 4.1-2, and 4.1-3. The data for percent labelled metaphases, anaphases, and telophases are also plotted in the Figures. It can be seen from Figures 3.41-2, 4.1-1, 4.1-2, and 4.1-3 that the appearance of the ascending and descending slope (after removal from ^{3}H-TdR) was delayed as the

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*Appendix A

Fig. 4.1-1 The percent labelled mitotic cells found in root tips in 'Seneca 60' at 0 to 21 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 20°C. For standard deviations and confidence limits see Tables A-3 and A-7.

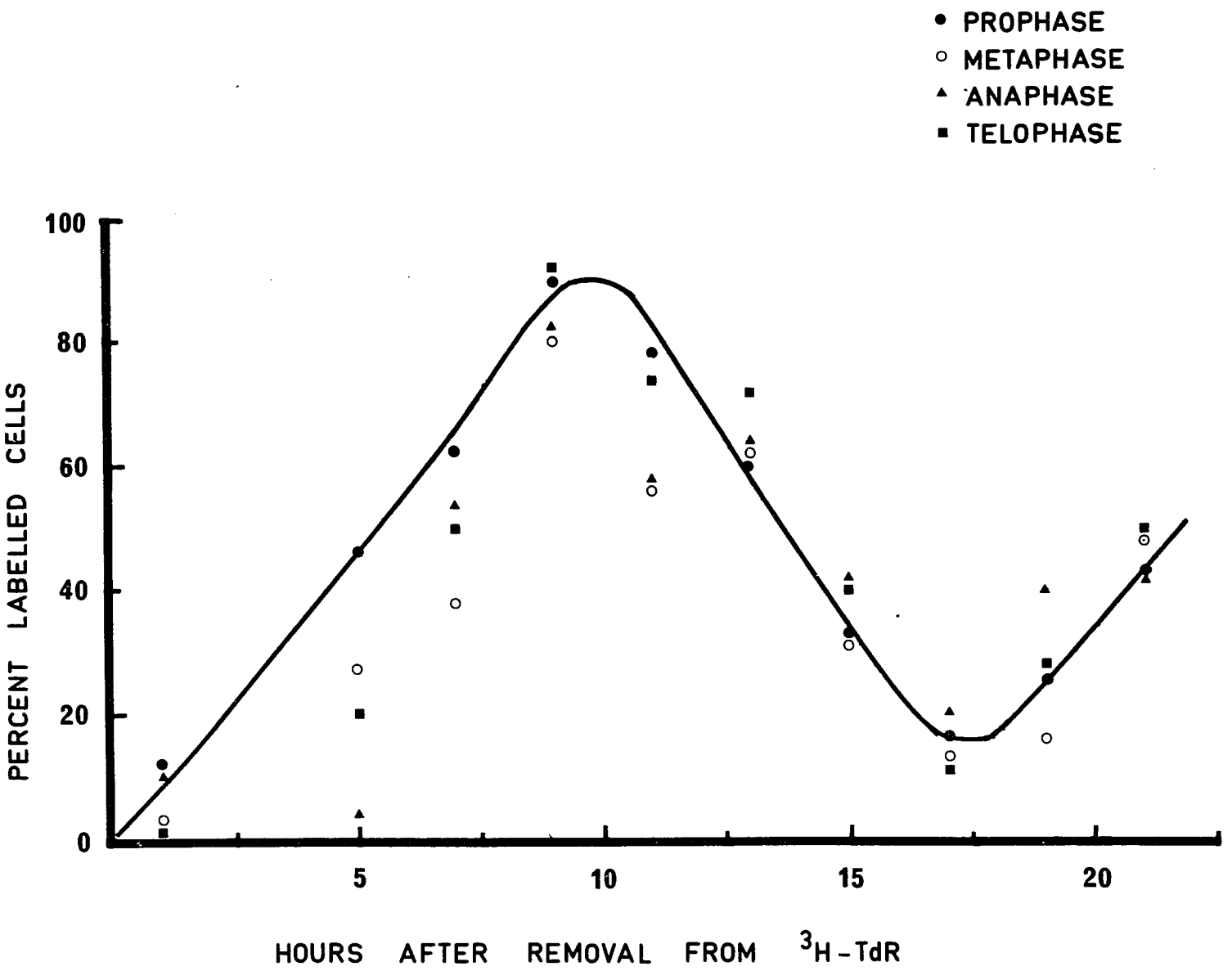

PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H-TdR

Fig. 4.1-2 The percent labelled mitotic cells found in root tips in 'Seneca 60' at 0 to 12 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 30°C. For standard deviations and confidence limits see Tables A-5 and A-9.

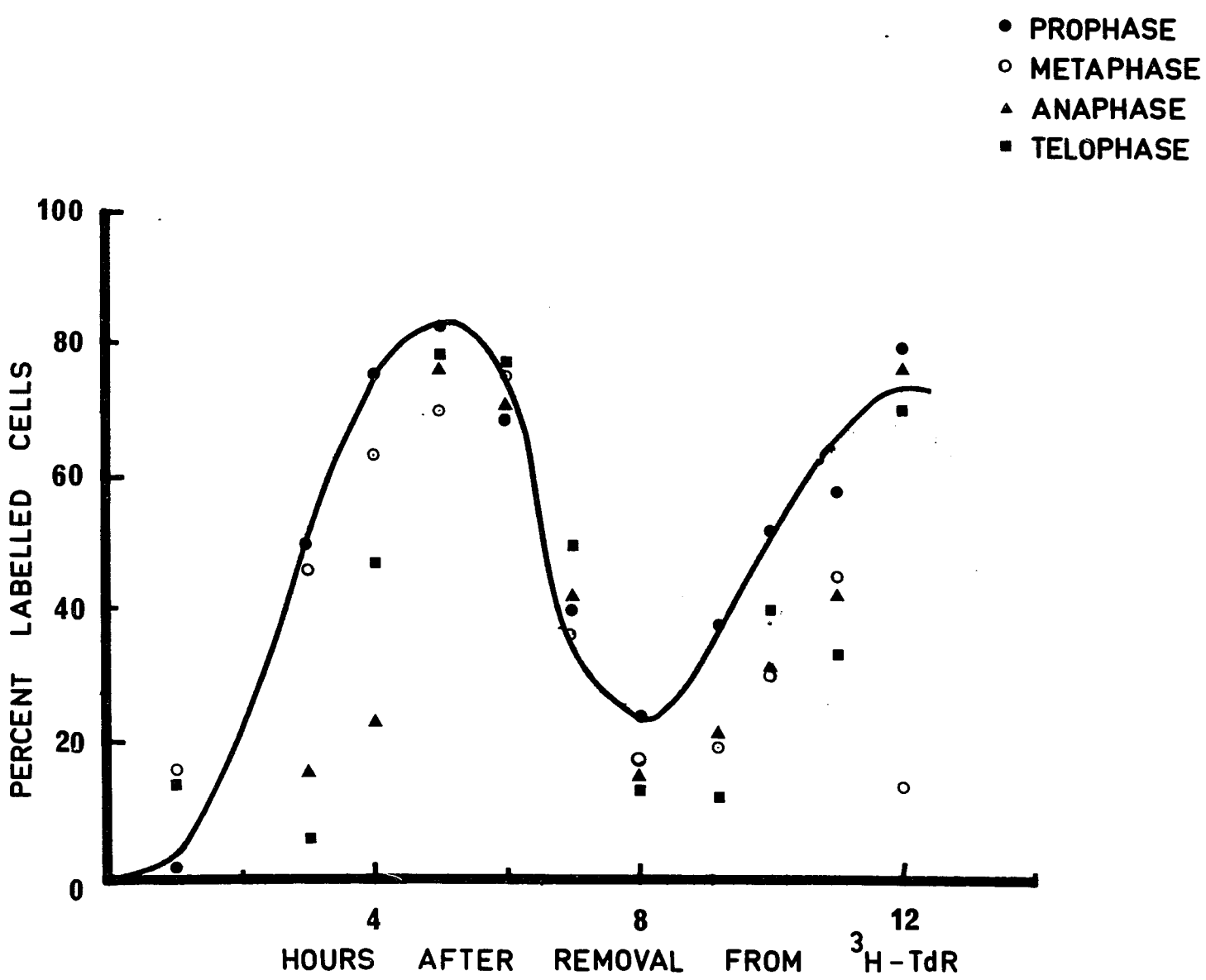
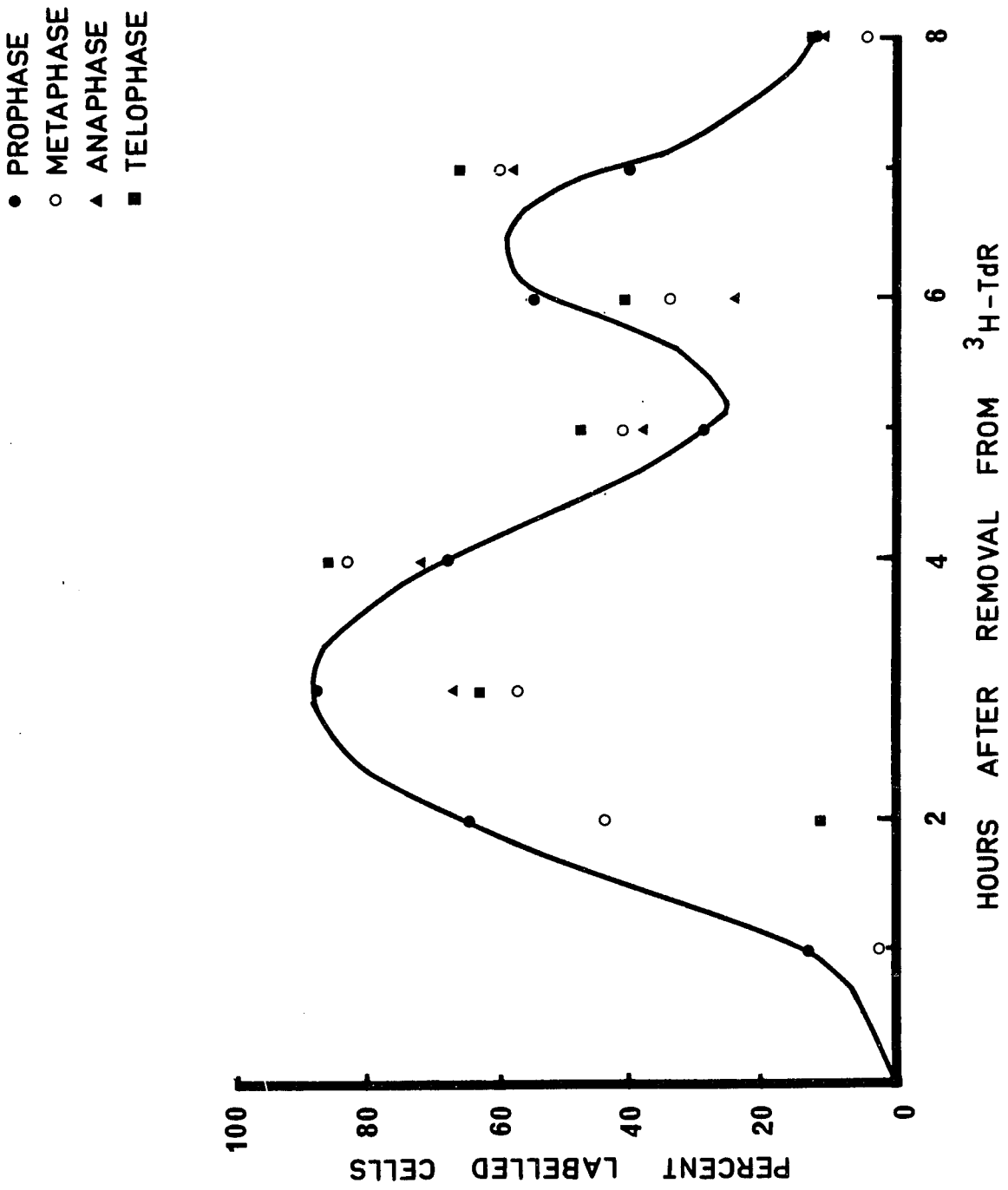

PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
100
80
60
40
20
0
PERCENT LABELLED CELLS
4
8
12
HOURS AFTER REMOVAL FROM ^{3}H-TdR

Fig. 4.1-3 The percent labelled mitotic cells found in root tips in 'Seneca 60' at 0 to 8 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 35°C. For standard deviations and confidence limits see Tables A-6 and A-10.


PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
0
20
40
60
80
100
2
4
6
8
HOURS AFTER REMOVAL FROM ^{3}H-TdR

temperature was lowered from 35 to 20 °C. In summary, the highest level (90%) of labelled prophase cells was reached at nine hours after removal from ^{3}H-TdR at 20 °C (Figure 4.1-1). Nearly all of the prophases were labelled at this time. A decline began after this and reached the lowest level (16%) at 17 hours. The percent labelled prophases increased to 43 percent at 21 hours at 20 °C.

At 25 °C (Figure 3.41-2), 92 percent prophase cells were found labelled after five and six hours of treatment with ^{3}H-TdR. The second peak was found between 14 and 16 hours. At 30 °C (Figure 4.1-2), the first peak was found at five hours as at 25 °C, but the second peak occurred at 11 hours.

At 35 °C (Figure 4.1-3), the first peak (88%) was found at three hours and the second peak was recorded at six hours.

The classification data are presented in Table A-11. A total of 124,000 nuclei were scored. At least 3000 nuclei were counted from each collection period in each experiment.

A compilation of the various cycle sub-divisions that are deducible from Figures 3.41-2, 4.1-1, 4.1-2, 4.1-3, and Table A-11 are found in Table 4.1-1. In the present study we have chosen three methods for estimating the nuclear cycle durations. All methods yield the same results for all stages except for G_1, because this is a derived value. Our description is based on the nuclear

TABLE 4.1-1

Estimates of the duration (hours) of the nuclear cycle in primary root tips of hybrid 'Seneca 60' at four temperatures*

Phase		Temperature			
		20°C	25°C	30°C	35°C
Interphase					
	G_1	2.5	1.7	0.4	0.1
	S	8.0	5.0	3.5	2.5
	G_2	3.3	2.1	2.3	1.4
	Sub-total	13.8	8.8	6.2	4.0
Mitosis					
	Prophase	1.34	0.57	0.40	0.22
	Metaphase	0.56	0.23	0.18	0.09
	Anaphase	0.16	0.06	0.04	0.02
	Telophase	0.62	0.24	0.21	0.10
	Sub-total	2.68	1.10	0.83	0.43
Total		16.5	9.9	7.0	4.4

*Durations are calculated by method '2'

cycle calculated by method '2'. The calculations from the other two methods are available (Appendicies A-12 and A-13).

Harvey's (1970) method was employed for testing the significance of means, where four temperatures have been considered as columns (each column has seven values; three for interphase and four for mitosis). There was a highly significant difference among columns indicating that the nuclear cycles differ at the four temperatures tested. Individual component phases cannot be tested by this method.

The standard deviations of the nuclear cycle and its component phases are presented in Table 4.1-2. The standard deviations did not remain constant at four temperatures. The variances of the nuclear cycle and its component phases can be found in Table A-14. The variances are heterogenous.

In Table 4.1-3, the estimates of the nuclear cycle are presented as percentages. It should be noted that the major part of the nuclear cycle is the DNA synthesis period (S) which requires about 50 percent of the cycle time. The major changes occurred in G_1 and G_2. In general, about 85 to 90 percent of the nuclear cycle is spent in interphase while the remaining 10 to 15 percent of the cycle is occupied by mitosis. All the stages of mitosis are temperature sensitive (Table 4.1-1). The estimates of the nuclear cycle and its component phases are represented graphically in Figures 4.1-4 and 4.1-5 (data from Table 4.1-1).

TABLE 4.1-2

Standard deviations (hours) of the nuclear cycle components in the primary root tips of 'Seneca 60' at four temperatures

Phase	Temperature			
	20°C	25°C	30°C	35°C
G_1 + Mitosis	0.39	0.31	0.21	0.48
S	0.15	0.22	0.33	0.18
G_2 + ½ Prophase	0.05	0.09	0.21	0.10
Total Nuclear Cycle	0.42	0.40	0.36	0.51

TABLE 4.1-3

Nuclear cycle durations as percentage in 'Seneca 60' at four temperatures*

Phase		Temperature			
		20°C	25°C	30°C	35°C
Interphase					
	G_1	15.1	17.3	5.3	1.8
	S	48.5	50.5	50.0	56.8
	G_2	20.2	21.2	32.7	31.7
	Sub-total	83.8	89.0	88.0	90.3
Mitosis					
	Prophase	8.1	5.7	5.7	5.0
	Metaphase	3.4	2.3	2.5	2.0
	Anaphase	0.9	0.6	0.6	0.5
	Telophase	3.8	2.4	3.2	2.2
	Sub-total	16.2	11.0	12.0	9.7

*Calculations are based on data from Table 4.1-1.

Fig. 4.1-4 Duration of the nuclear cycle and its component phase as a function of temperature.

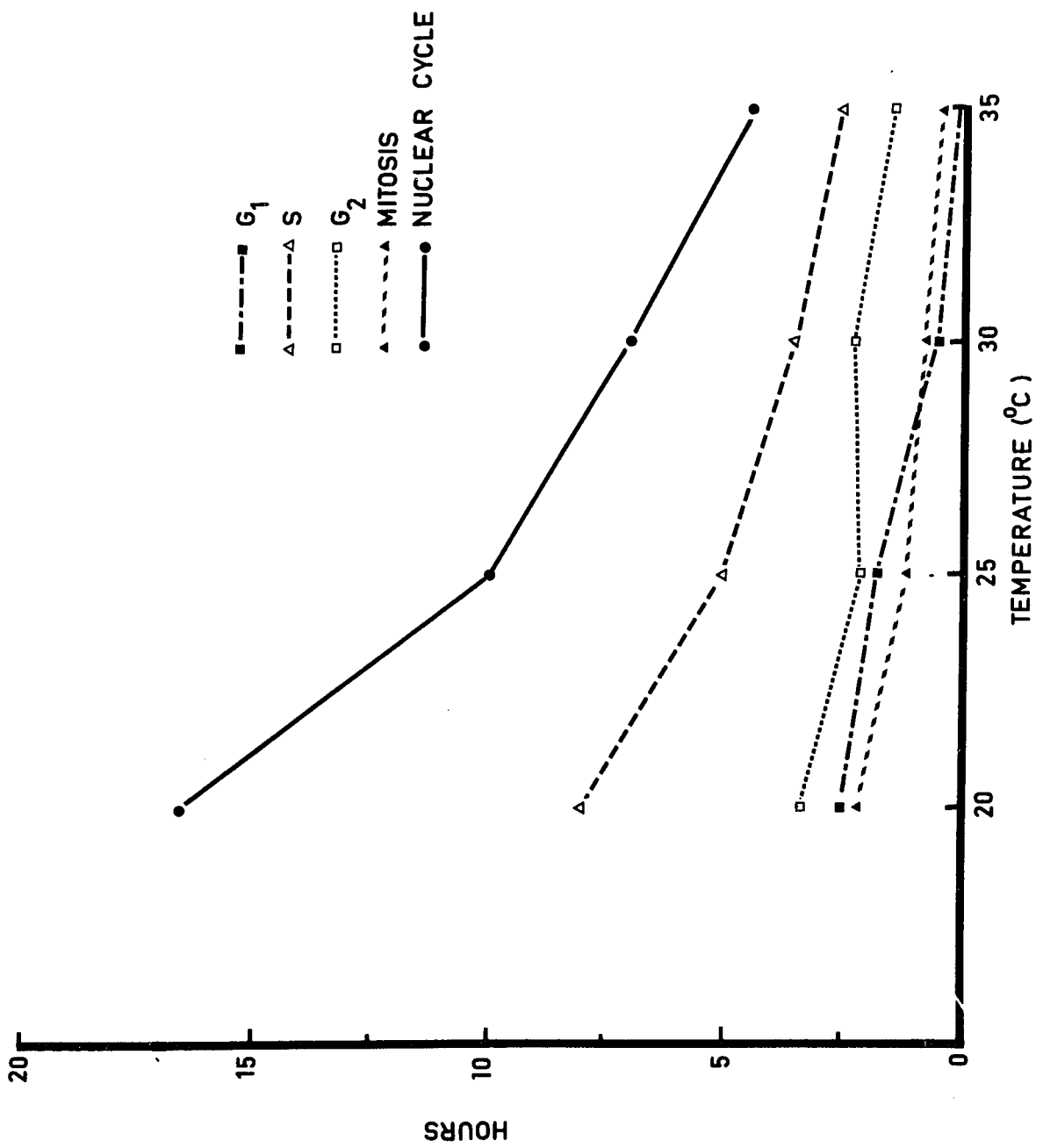
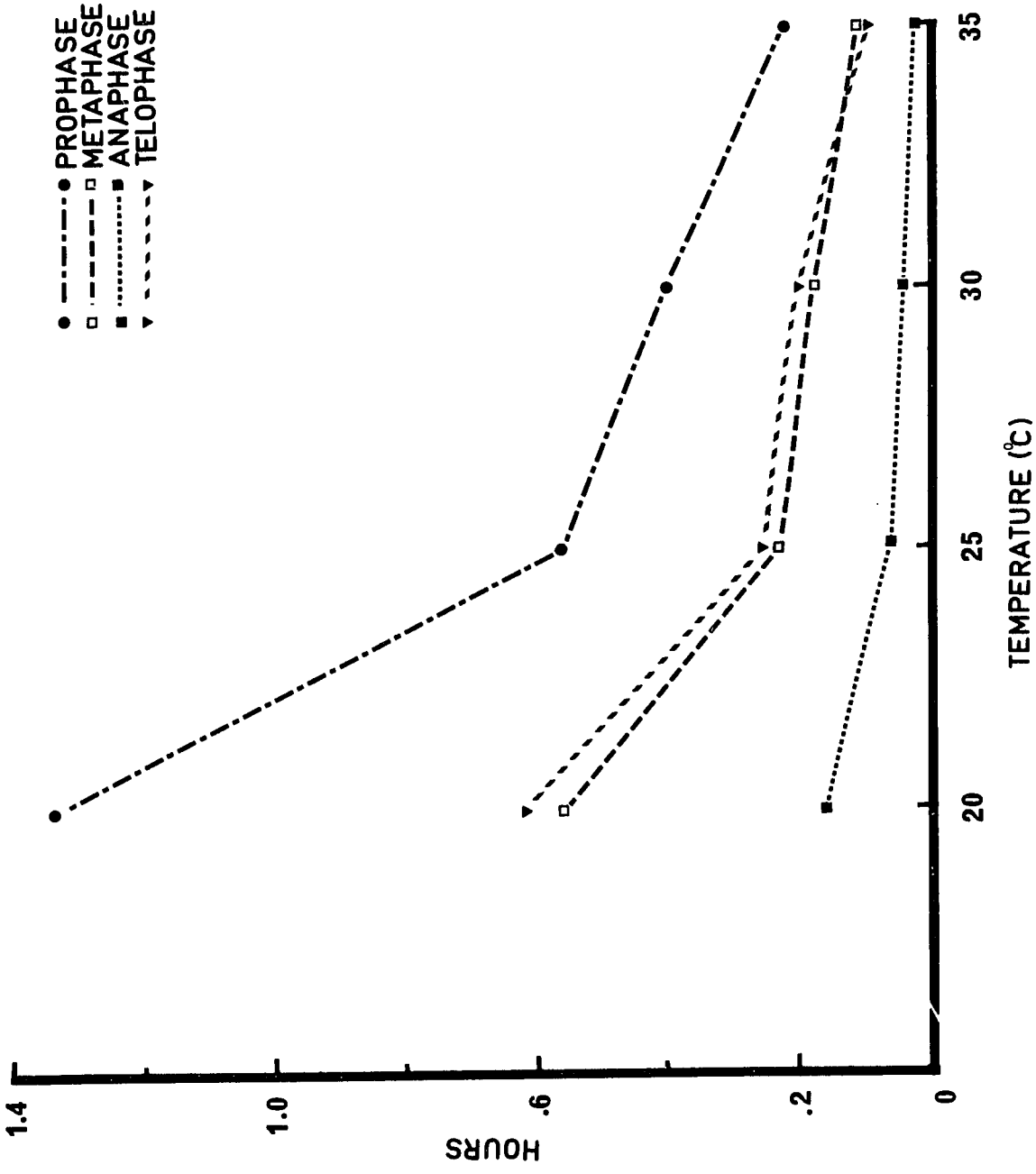

G1
S
G2
MITOSIS
NUCLEAR CYCLE
HOURS
0
5
10
15
20
20
25
30
35
TEMPERATURE (°C)

Fig. 4.1-5 Duration of mitosis as a function of temperature.


PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
HOURS
1.4
1.0
.6
.2
0
20
25
30
35
TEMPERATURE (°C)

Determinations were made of the mitotic indices (mitotic index is defined as the percentage of the cells in mitosis in a population of cells) from the 20, 25, 30, and 35°C experiments. The indices are shown in Figures 4.1-6 and 4.1-7. The mean mitotic indices at 20, 25, 30, and 35°C were 9.8 ± 0.30, 9.1 ± 0.29, 5.3 ± 0.28, and 4.9 ± 0.23 respectively. There is no significant difference between the mitotic indices at 20 and 25°C or between those at 30 and 35°C. However, the mitotic indices at 20 and 25°C do differ significantly from those at 30 and 35°C.

The curves in Figures 4.1-6 and 4.1-7 may be divided into two sections as follows:

(i) The first few hours, during which there is considerable fluctuation in the mitotic indices (this is believed to be due to sudden changes in environmental conditions, such as washing the roots after removal from ^{3}H-TdR and the effect of ^{3}H-TdR itself).

(ii) Later hours during which a plateau is established.

4.2 Autopolyploidy:

As described earlier, the nuclear cycles of an autotriploid stock and an autotetraploid stock of inbred W23 (diploid) were studied at 25°C. Somatic metaphase chromosomes of W23 diploid, autotriploid, and autotetraploid

Fig. 4.1-6 Mitotic Index, plotted against hours after removal from ^{3}H-TdR at 20°C (upper) and 25°C (lower). Standard deviations either side of the mean are indicated as a vertical bar.

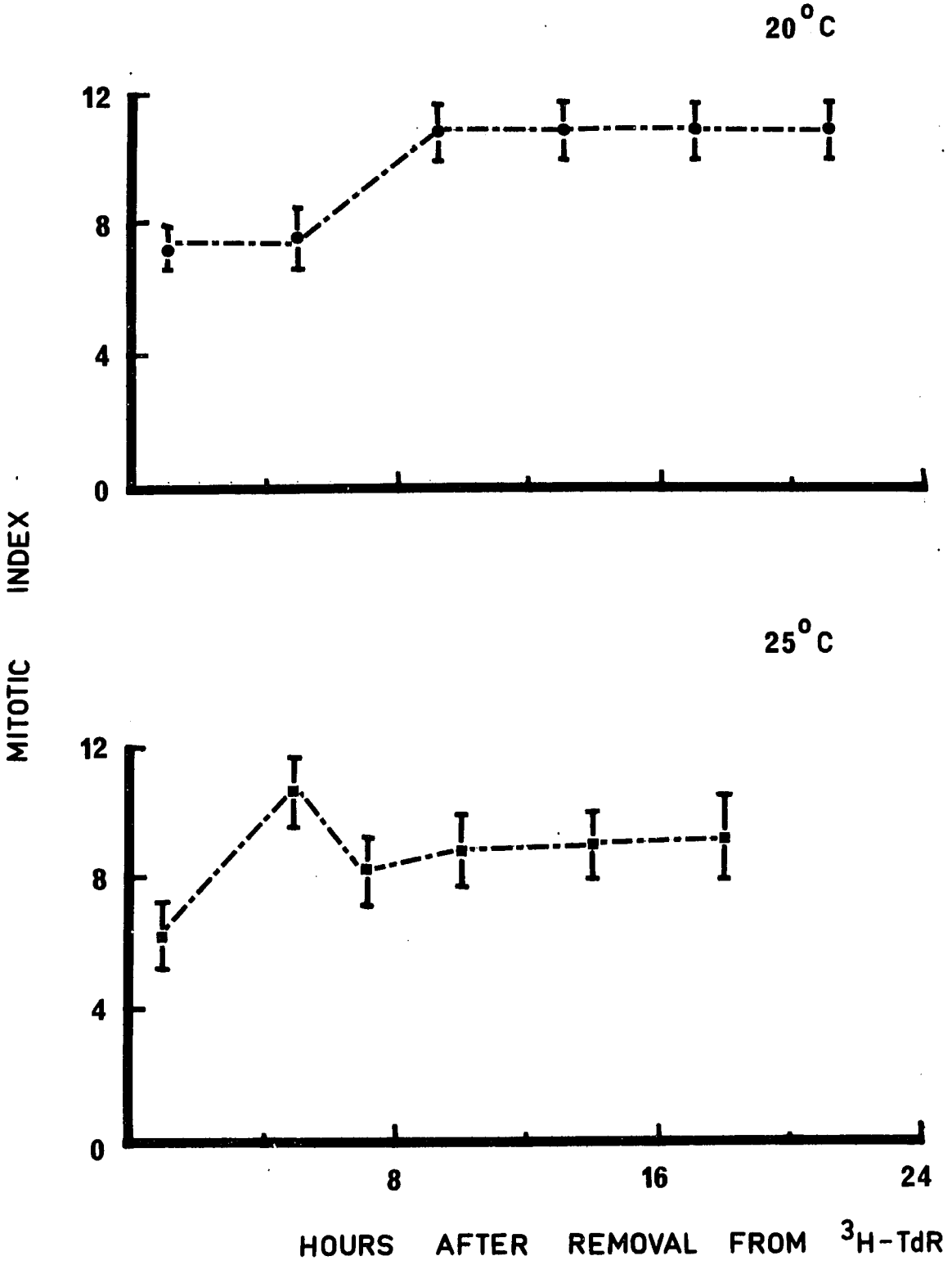
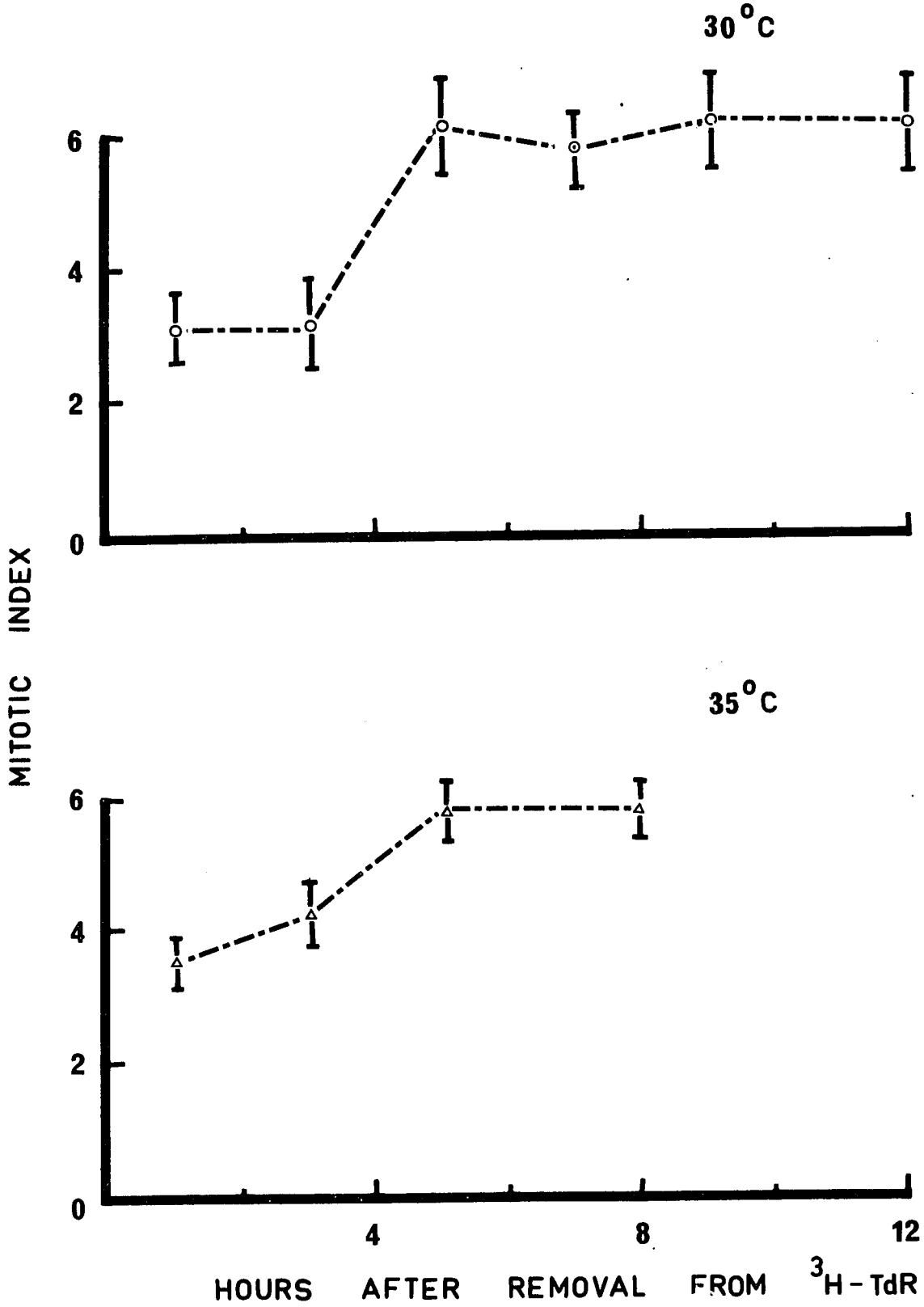

20°C
12
8
4
0
MITOTIC INDEX
25°C
12
8
4
0
8
16
24
HOURS AFTER REMOVAL FROM ^{3}H-TdR

Fig. 4.1-7 Mitotic Index, plotted against hours after removal from ^{3}H-TdR at 30°C (upper) and 35°C (lower). Standard deviations either side of the mean are indicated as a vertical bar.


30°C
35°C
MITOTIC INDEX
0
2
4
6
4
8
12
HOURS AFTER REMOVAL FROM ^{3}H-TdR

stocks are presented in Figure 3.21-1.

The changes in the proportion of labelled mitotic figures from one cycle after removal from ^{3}H-TdR treatment in diploid, autotriploid, and autotetraploid stocks of W23 are shown in Figures 4.2-1, 4.2-2 and 4.2-3. These curves all contained two peaks, an indication that at least one nuclear cycle had been studied in each stock. The labelled prophase and metaphase curves appear together, and the anaphase and telophase curves are similar and close at each level of ploidy tested. There was no pronounced difference among the diploid, autotriploid, and autotetraploid stocks as regards the apex of a 'first' and 'second' peak. In Figure 4.2-4, the percent labelled prophase cells are plotted from each of the stocks. Table B*-1 to B-6 contain the confidence limits or standard deviations.

The classification data are presented in Table B-7. A total of 100,000 nuclei were scored. At least 3000 nuclei were scored from each collection period. Estimates of the parameters of the nuclear cycle, obtained from the data in Figures 4.2-1, 4.2-2, 4.2-3, and Table B-7 are presented in Table 4.2-1.

---

*Appendix B

Fig. 4.2-1 The percent labelled mitotic cells found in root tips of W23-diploid at 0 to 18 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 25°C. For standard deviations and confidence limits see Tables B-1 and B-4 respectively.

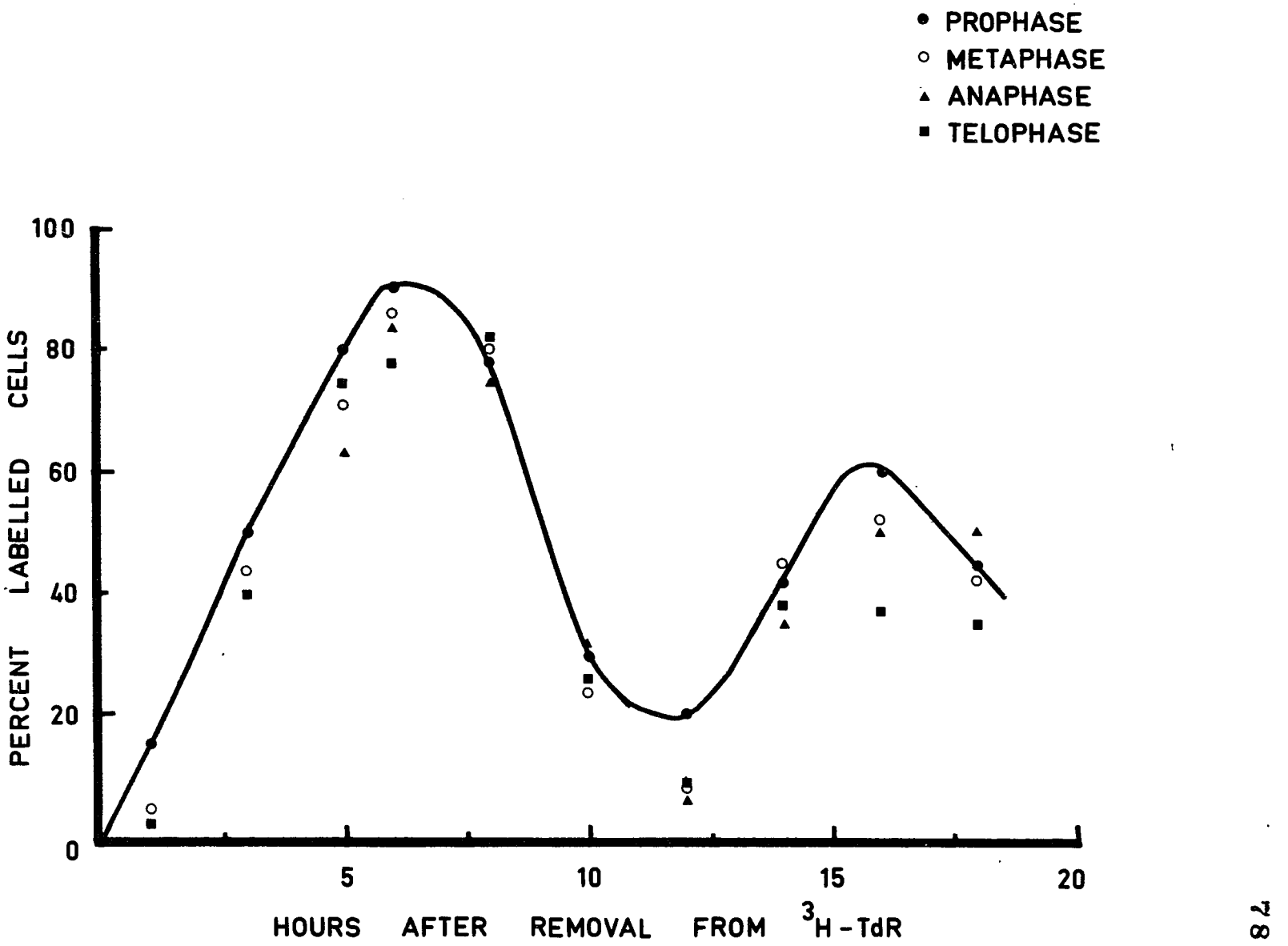

PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H - TdR

Fig. 4.2-2 The percent labelled mitotic cells found in root tips of W23-autotriploid at 0 to 18 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 25°C. For standard deviations and confidence limits see Tables B-2 and B-5.

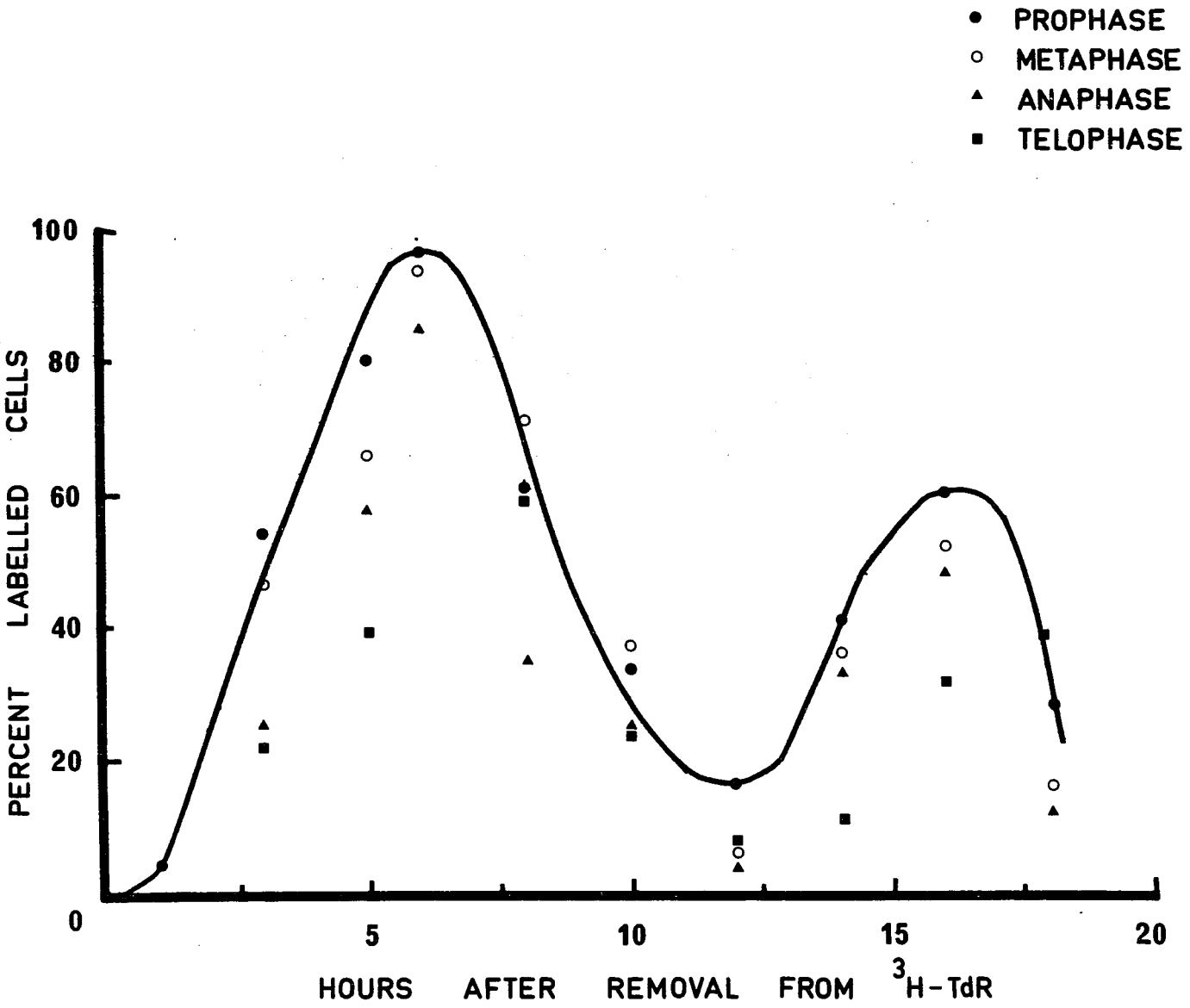

PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H-TdR

Fig. 4.2-3 The percent labelled mitotic cells found in root tips of W23-autotetraploid at 0 to 18 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 25°C. For standard deviations and confidence limits see Tables B-3 and B-6.

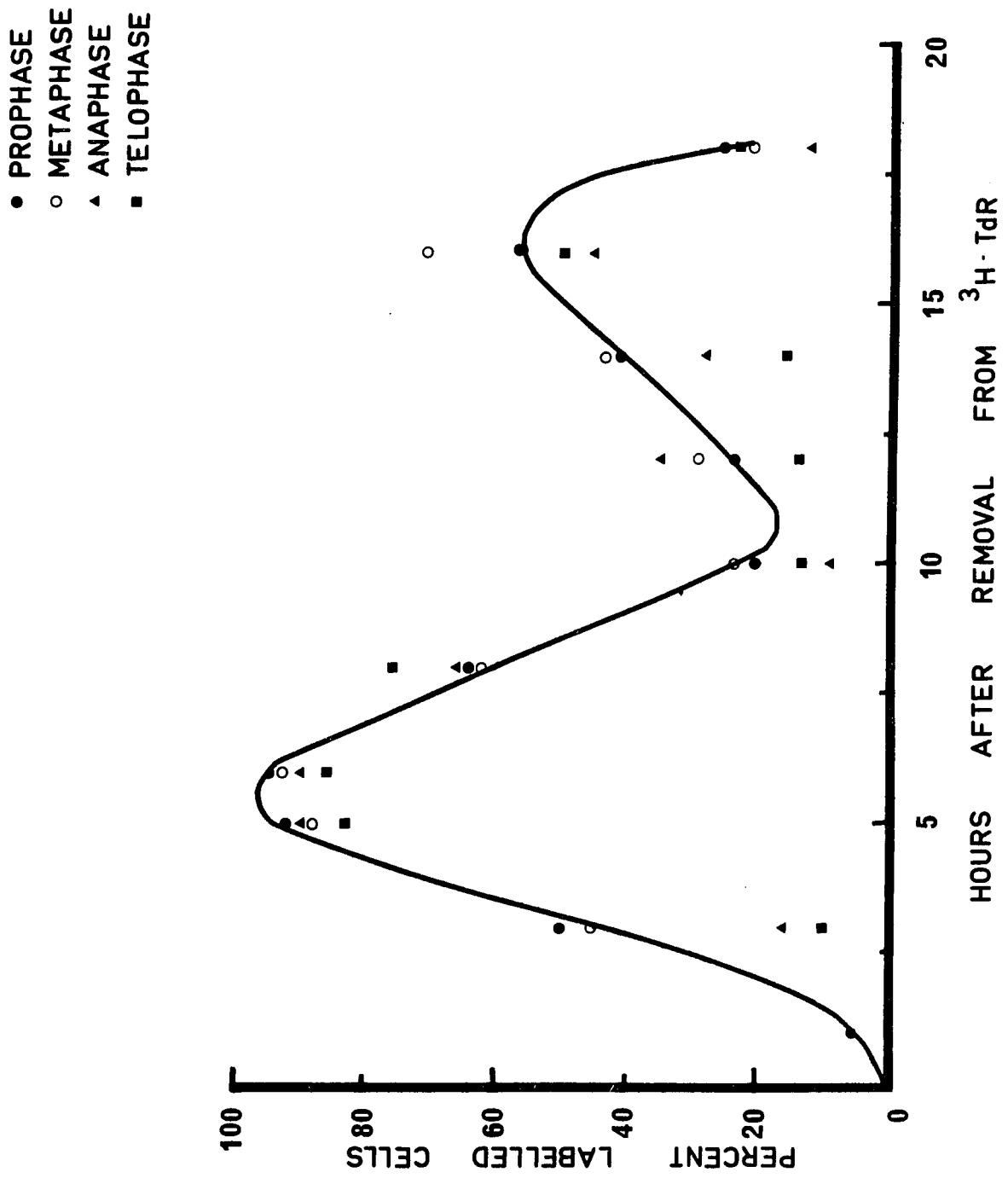

• PROPHASE
○ METAPHASE
▲ ANAPHASE
■ TELOPHASE
PERCENT LABELLED CELLS
0
20
40
60
80
100
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H-TdR

Fig. 4.2-4 Comparison of percent labelled prophase curves among diploid, autotriploid, and autotetraploid stocks of W23 at 25°C.

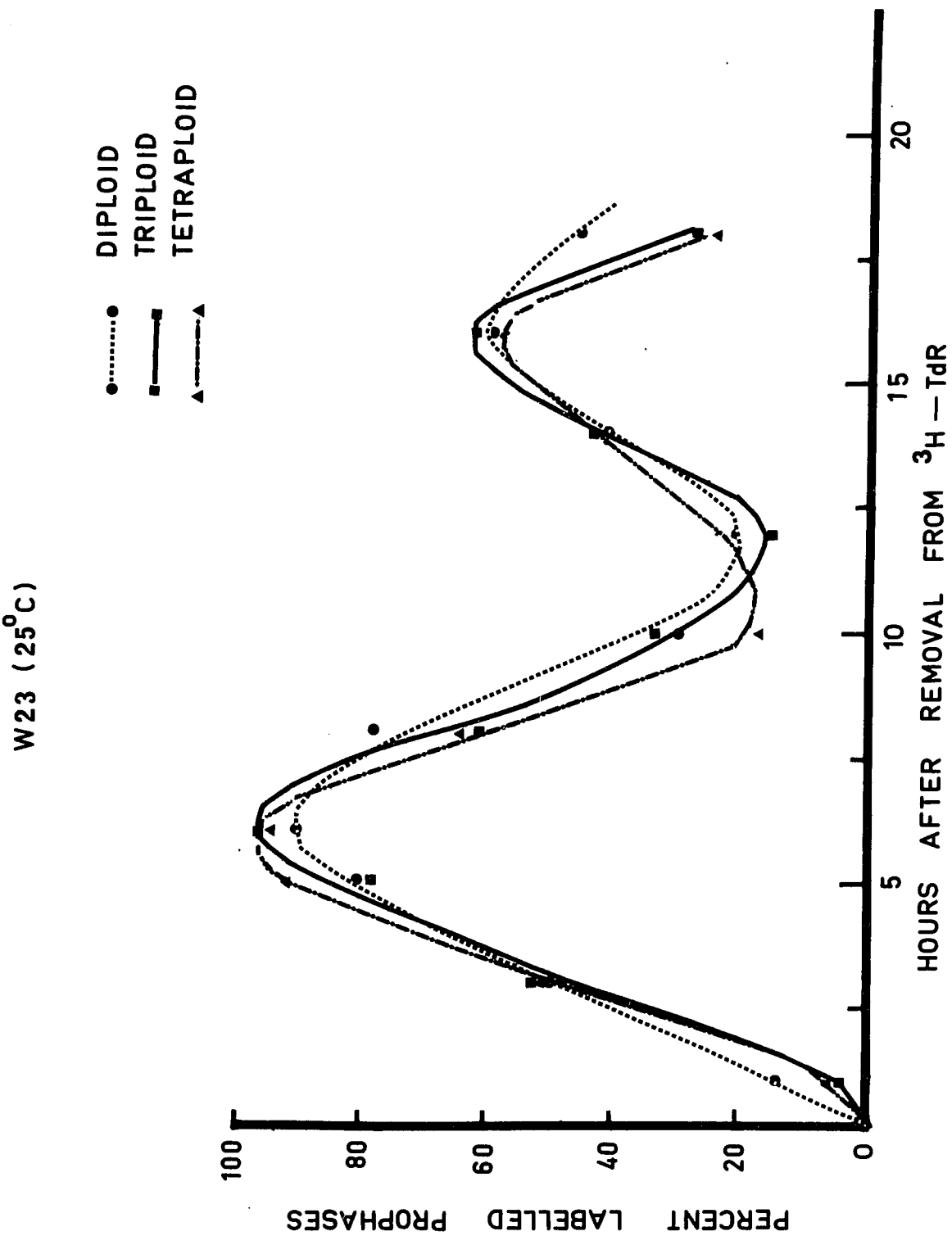

W23 (25°C)
DIPLOID
TRIPLOID
TETRAPLOID
PERCENT LABELLED PROPHASES
0
20
40
60
80
100
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H — TdR

TABLE 4.2-1

Estimates of the duration (hours) of the nuclear cycle in primary root tips of W23 (2N, 3N, and 4N) at 25°C *

Phase		Ploidy		
		Diploid	Autotriploid	Autotetraploid
Interphase				
	G_1	1.8	2.6	2.3
	S	5.5	5.0	5.2
	G_2	2.7	2.8	2.7
	Sub-total	10.0	10.4	10.2
Mitosis				
	Prophase	0.68	0.56	0.68
	Metaphase	0.23	0.28	0.28
	Anaphase	0.06	0.06	0.07
	Telophase	0.32	0.21	0.31
	Sub-total	1.29	1.11	1.34
Total		11.3	11.5	11.5

*Durations are calculated by method '2'

The results presented in Table 4.2-1 indicate that the duration of the nuclear cycle and in particular, estimates of tS in diploid, autotriploid, and autotetraploid stocks, are similar. Harvey's (1970) method was employed for testing the significance of means, in which the three ploidy levels were treated as columns. There was no significant difference among columns.

Standard deviations of the nuclear cycle and its component phases are presented in Table 4.2-2. The variances of the nuclear cycle estimates can be found in Table B-10. The variances of S and G_2 + ½ prophase are similar in diploid and autotriploid. The variances of the total nuclear cycle are also the same between diploid and autotetraploid while the variances for the phases were heterogeneous.

In Table 4.2-3 the nuclear cycle estimates are presented as percentages. In Table 4.2-4, the mitotic indices are tabulated. There is no significant difference in mitotic indices between the diploid and autotetraploid. However, in the autotriploid stock the mitotic index is significantly lower than either the diploid or autotetraploid.

From our data, it is clear that there is not an increase in the duration of either the DNA synthesis period or the entire nuclear cycle among diploid, autotriploid, and autotetraploid stocks of Z. mays.

TABLE 4.2-2

Standard deviations (hours) of the nuclear cycle components in the primary root tips of W23 (2N, 3N, and 4N) at 25°C

Phase	Ploidy		
	Diploid	Autotriploid	Autotetraploid
G_1 + Mitosis	0.20	0.14	0.24
S	0.15	0.14	0.05
G_2 + ½ Prophase	0.18	0.17	0.12
Total Nuclear Cycle	0.26	0.17	0.27

TABLE 4.2-3

Nuclear cycle duration (percent) in W23 (2N, 3N, and 4N) at 25°C*

Phase		Ploidy		
		Diploid	Autotriploid	Autotetraploid
Interphase				
	G_1	15.6	22.7	20.0
	S	49.1	43.5	45.2
	G_2	23.8	24.2	23.1
	Sub-total	88.5	90.4	88.3
Mitosis				
	Prophase	6.1	4.9	5.9
	Metaphase	2.1	2.4	2.5
	Anaphase	0.5	0.5	0.6
	Telophase	2.8	1.8	2.7
	Sub-total	11.5	9.6	11.7

*Data from Table 4.2-1

TABLE 4.2-4

Mean mitotic indices (with standard deviations) in W23 at 25°C

Hours after pulse	Ploidy		
	Diploid	Autotriploid	Autotetraploid
1	6.2 ± 0.53	4.7 ± 0.46	5.4 ± 0.40
3	7.5 ± 0.55	5.2 ± 0.46	8.8 ± 0.56
5	9.6 ± 0.48	5.9 ± 0.38	5.2 ± 0.38
6	8.7 ± 0.59	8.6 ± 0.41	8.5 ± 0.42
8	8.4 ± 0.56	6.4 ± 0.45	8.0 ± 0.47
10	7.4 ± 0.42	7.1 ± 0.57	9.6 ± 0.50
12	8.0 ± 0.39	6.9 ± 0.37	7.3 ± 0.44
14	8.1 ± 0.41	9.5 ± 0.51	8.2 ± 0.43
16	7.4 ± 0.45	7.7 ± 0.46	7.6 ± 0.42
18	7.1 ± 0.46	6.0 ± 0.34	7.7 ± 0.41
Mean	7.89 ± 0.15	6.41 ± 0.13	7.92 ± 0.41

4.3 Mitotic Poisons:

Recognizing the effect of temperature on the nuclear cycle, a second set of parameters was considered for investigation, namely chemical additives which would, perhaps, alter the nuclear cycle. Cycloheximide and chloramphenicol were selected for testing at 25 or 30°C. The concentrations chosen for each chemical resulted from survey experiments.

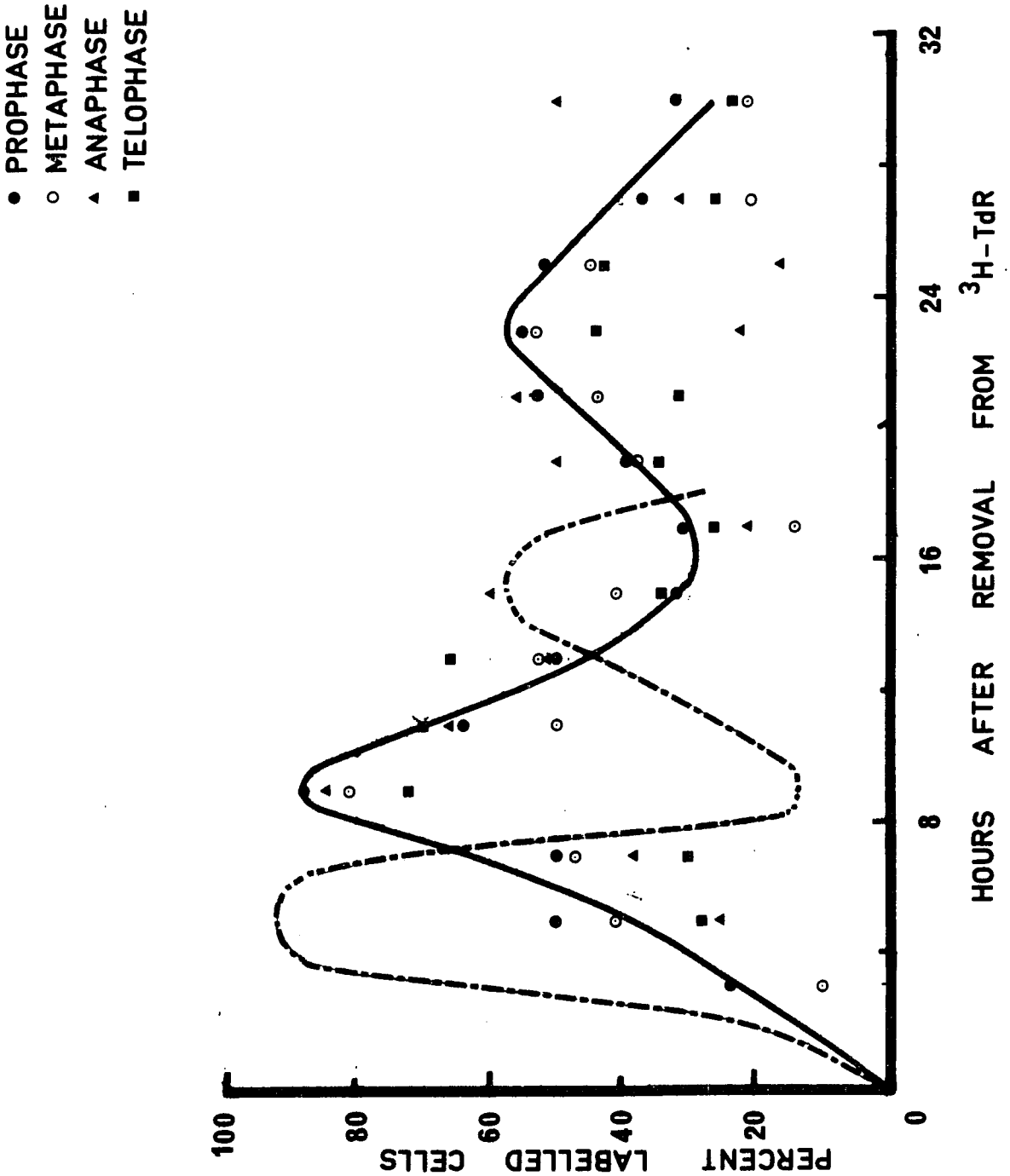
4.31 Estimation of the Nuclear Cycle:

4.311 Cycloheximide:-

Preliminary experiments were performed to ascertain the suitable concentrations for cycloheximide treatment. Roots were treated with 0.01% cycloheximide for two hours. This concentration proved lethal to some roots. It was found that root growth was visibly not altered with 0.001% cycloheximide. Roots were grown for three to four days and no death was observed. A reduction over the control value of 50% in the mitotic index occurred following a 0.001% cycloheximide treatment for two hours. Consequently, this concentration was chosen for the present study.

The results of an experiment conducted at 25°C are summarized in Figure 4.311-1. The data for the control curve were taken from Figure 3.41-2 inasmuch as the experiments were conducted under identical conditions. It should be noted that there is a delay of three hours in

Fig. 4.311-1 The percent labelled mitotic cells found in root tips after treating with cycloheximide (0.001%) for 2 hours at 25°C. The percent labelled prophase curve of the control experiment is shown as a broken line. For standard deviations and confidence limits see Tables C-1 and C-5.


PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
0
20
40
60
80
100
8
16
24
32
HOURS AFTER REMOVAL FROM ^{3}H-TdR

the appearance of the 'first' peak in the treatment curve and the 'second' peak was delayed until the 23rd hour.

A second experiment with cycloheximide (0.001%) was conducted at 30°C. The summary of this experiment can be seen in Figure 4.311-2. The data from Figure 4.1-2 have been employed as a control. There was a delay of two hours before the appearance of the 'first' peak and a delay of five hours in the appearance of the 'second' peak in comparison with the control value (Tables C*-1 and C-3).

The classification data are presented in Table C-9. A total of approximately 83,000 nuclei were scored for the two experiments. The estimates of the nuclear cycle that can be derived from Figures 4.311-1 and 4.311-2 are shown in Tables 4.311-1 and 4.311-2. Again, all methods for determining the duration of the nuclear cycle yielded similar results.

At 25° C, a delay was induced by cycloheximide in the G_2 and S periods. The percent changes from the control are presented in Table 4.311-3. The value of tS was increased by a factor of 1.4 and tG_2 was increased by a factor of 2.4 at 25° C. The total nuclear cycle was increased by a factor of 1.5. There was a slight effect

---

*Appendix C

Fig. 4.311-2 The percent labelled mitotic cells found in root tips after treating with cycloheximide (0.001%) for 2 hours at 30°C. The percent labelled prophase curve of the control experiment is shown as a broken line. For standard deviations and confidence limits see Tables C-3 and C-7.

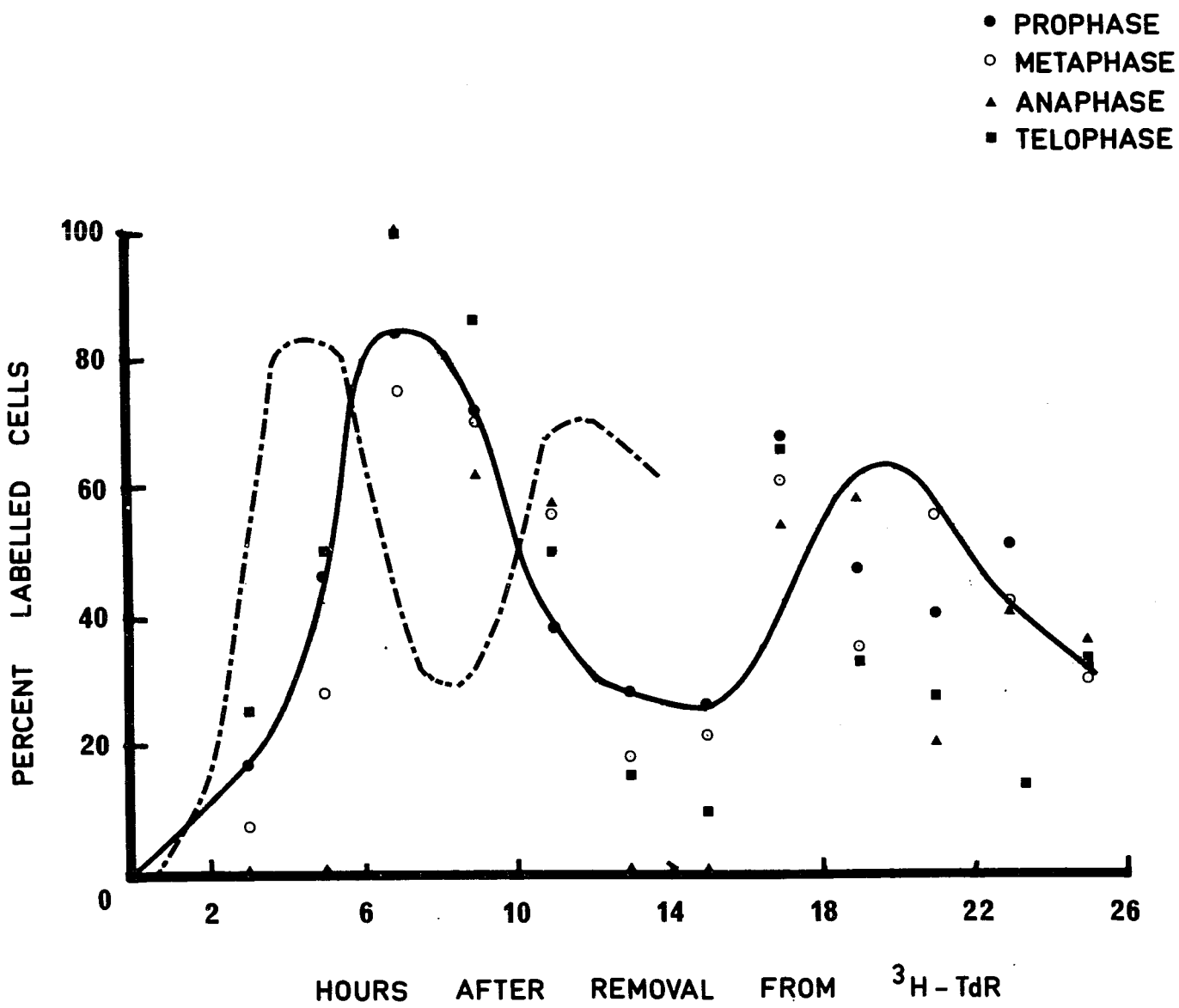

PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
2
6
10
14
18
22
26
HOURS AFTER REMOVAL FROM ^{3}H - TdR

TABLE 4.311-1

Estimates of the duration (hours) of the nuclear cycle after a 2 hour incubation in cycloheximide (0.001%) or chloramphenicol (0.03%) following 30 minutes of ^{3}H-TdR treatment at 25°C*

Phase		Treatment		
		Control**	Cyclohe-ximide	Chloram-phenicol
Interphase				
	G_1	1.7	1.4	1.6
	S	5.0	7.0	6.5
	G_2	2.1	5.2	5.7
	Sub-total	8.8	13.6	13.8
Mitosis				
	Prophase	0.57	0.71	0.60
	Metaphase	0.23	0.32	0.23
	Anaphase	0.06	0.09	0.07
	Telophase	0.24	0.31	0.30
	Sub-total	1.10	1.43	1.20
Total		9.9	15.0	15.0

*Durations are calculated by method '2'

**From Table 4.1-1

TABLE 4.311-2

Estimates of the duration (hours) of the nuclear cycle after a 2 hour incubation in cycloheximide (0.001%) or chloramphenicol (0.03%) following 30 minutes of ^{3}H-TdR treatment at 30°C.*

Phase		Treatment		
		Control**	Cyclohe-ximide	Chloram-phenicol
Interphase				
	G_1	0.4	1.6	1.80
	S	3.5	5.0	6.0
	G_2	2.3	4.9	3.8
	Sub-total	6.2	11.5	11.6
Mitosis				
	Prophase	0.40	0.30	0.49
	Metaphase	0.18	0.15	0.18
	Anaphase	0.04	0.04	0.06
	Telophase	0.21	0.11	0.21
	Sub-total	0.83	0.60	0.94
Total		7.0	12.0	12.5

*Durations are calculated by method '2'.

**From Table 4.1-1.

TABLE 4.311-3

Percent increase or decrease over control after treatment with cycloheximide (0.001%) or chloramphenicol (0.03%) for 2 hours following 30 minutes ^{3}H-TdR exposure*

Phase		25°C		30°C	
		Cyclohe-ximide	Chloram-phenicol	Cyclohe-ximide	Chloram-phenicol
Interphase					
	G_1	-16.8	-5.9	318.9	386.4
	S	40.0	30.0	42.8	71.4
	G_2	145.2	171.4	110.8	63.4
	Interphase	54.2	56.8	84.8	87.4
Mitosis					
	Prophase	24.6	5.2	-25.0	22.5
	Metaphase	39.1	0	-16.7	0
	Anaphase	50.0	16.7	0	50.0
	Telophase	29.2	25.0	-47.6	0
	Mitosis	30.0	9.1	-27.7	13.2
Total		51.5	51.5	71.4	78.6

*Calculations are based on the data from Tables 4.311-1 and 4.311-2

on mitosis which appears inflated in Table 4.311-3. Apparently cycloheximide does not effect tG_1 at 25°C.

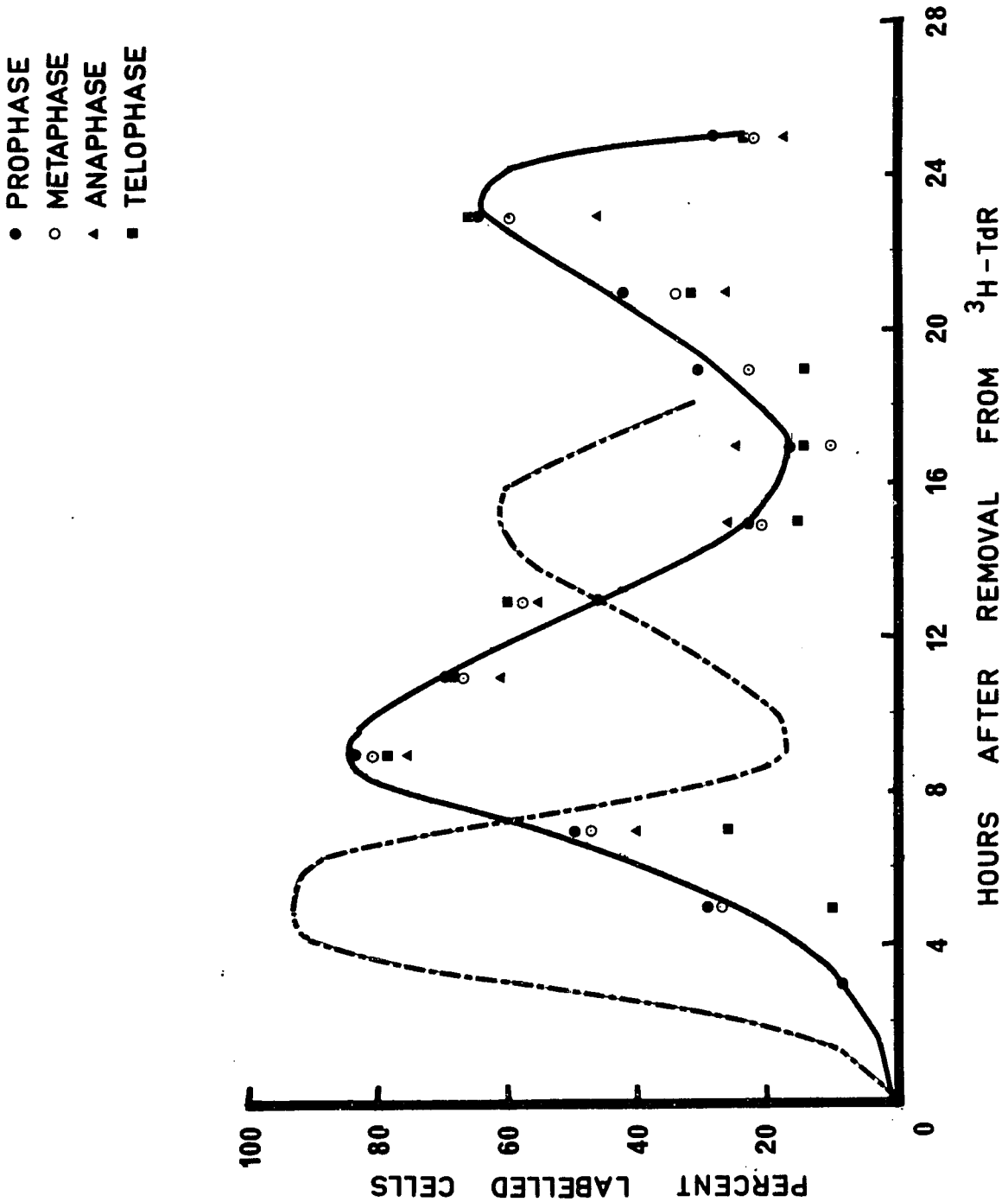
At 30°C, the G_2 and S periods also were delayed. In general, tS was increased by a factor of 1.4 while tG_2 was increased by a factor of 2.1. At 30°C, tG_1 was also affected by a factor of 4.2 while at 25°C, cycloheximide did not affect tG_1. Furthermore, this chemical does not have any effect on mitosis at higher temperature (i.e. 30°C) (Table 4.311-2).

4.312 Chloramphenicol

Preliminary experiments were conducted using 3.0%, 0.3% and 0.03% concentrations of chloramphenicol. All treatments were carried out on attached three days old primary roots of 'Seneca 60' at 25 or 30°C. Following treatment with 3% chloramphenicol for two hours, it was found that the roots did not grow. Growth was visibly not altered in a 0.03% chloramphenicol treatment. Roots were grown for three to four days and no death was observed. There was reduction in the mitotic index of 50 to 60% over the control value. Therefore, 0.03% was chosen for the present study.

The results of chloramphenicol (0.03%) experiment conducted at 25°C are summarised in Figure 4.312-1. The data for the control curve were taken from Figure 3.41-2. There was a three hour delay in the appearance of 'first' peak and a seven hour delay in the appearance of the 'second'

Fig. 4.312-1 The percent labelled mitotic cells found in root tips after treating with chloramphenicol (0.03%) for 2 hours at 25°C. The percent labelled prophase curve of the control experiment is shown as a broken line. For standard deviations and confidence limits see Tables C-2 and C-6.


• PROPHASE
○ METAPHASE
▲ ANAPHASE
■ TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
4
8
12
16
20
24
28
HOURS AFTER REMOVAL FROM ^{3}H-TdR

peak in the treatment curve.

The results of the chloramphenicol (0.03%) experiment conducted at 30°C are presented in Figure 4.312-2. The data for the control value were taken from Figure 4.1-2. There was a two hour delay in the appearance of 'first' peak and a seven hour delay in the appearance of the 'second' peak.

A total of approximately 89,000 nuclei were scored from the two experiments (Table C-9). The durations of the phases of the nuclear cycle were altered after treatment with chloramphenicol at both temperatures. There was an increase in tG_2 and tS by factors of 1.3 and 2.7 respectively at 25°C; by factors of 1.6 and 1.7 at 30°C.

The tG_1 was not affected at 25°C but at 30°C, tG_1 was increased by a factor of approximately 5.0. For percent increments, the reader is referred to Table 4.311-3 (section 4.311). In Tables 4.312-1 and 4.312-2, the time estimates are presented as percentages. Standard deviations for control and treated experiments are presented in Table 4.312-3. The variances of nuclear cycle estimates can be found in Table C-14.

4.32 Mitotic Index:

4.321 Cycloheximide:

The mitotic indices of the control and the treated

Fig. 4.312-2 The percent labelled mitotic cells found in root tips after treating with chloramphenicol (0.03%) for 2 hours at 30°C. The percent labelled prophase curve of the control experiment is shown as a broken line. For standard deviations and confidence limits see Tables C-4 and C-8.

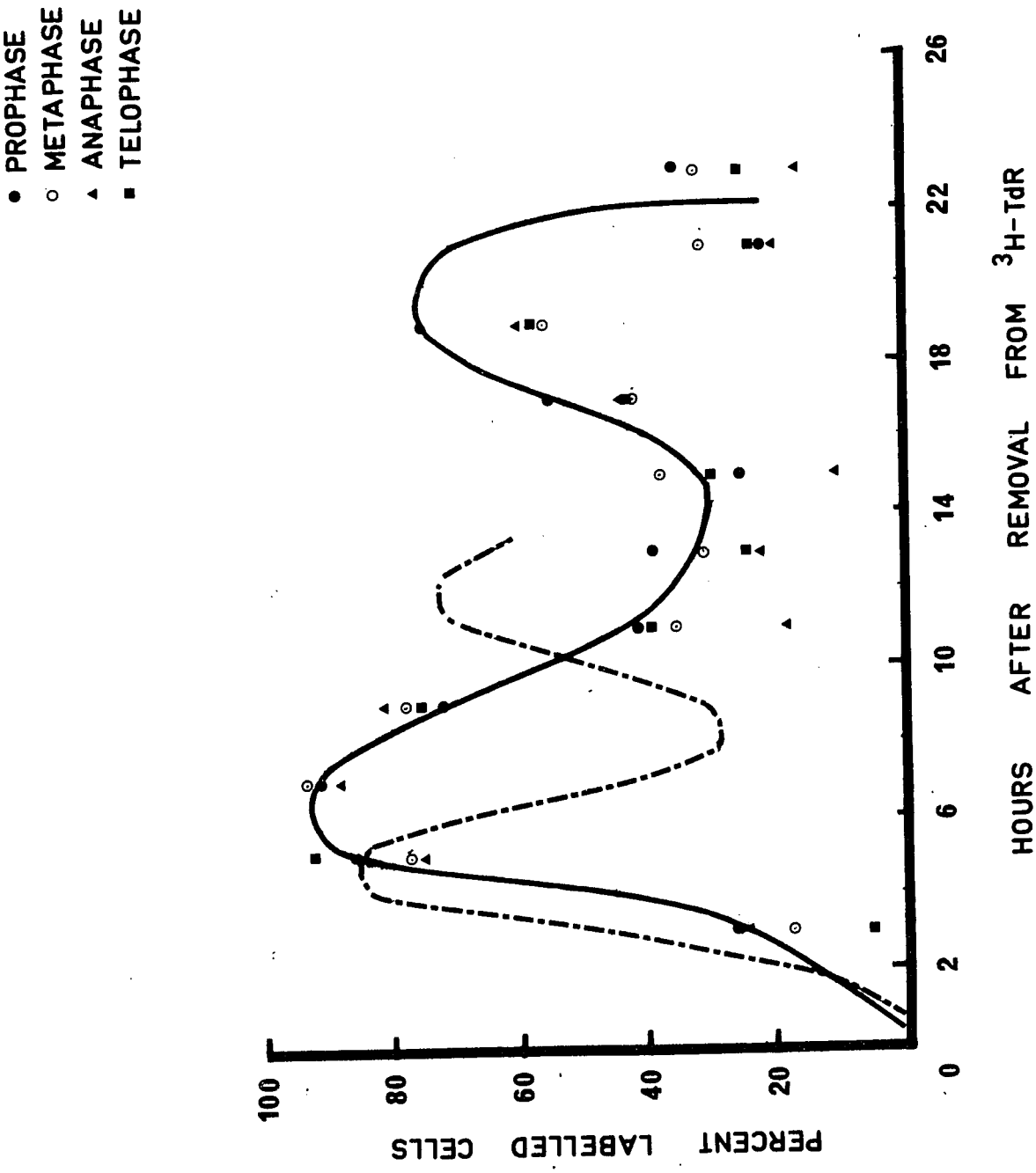

• PROPHASE
○ METAPHASE
▲ ANAPHASE
■ TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
2
6
10
14
18
22
26
HOURS AFTER REMOVAL FROM ^{3}H-TdR

TABLE 4.312-1

Nuclear cycle duration as percentage at 25°C

Phase		Treatment		
		Control*	Cyclohe-** ximide	Chloram-phenicol
Interphase				
	G_1	17.3	9.5	10.7
	S	50.5	46.7	43.3
	G_2	21.2	34.3	38.0
	Sub-total	89.0	90.5	92.0
Mitosis				
	Prophase	5.7	4.7	4.0
	Metaphase	2.3	2.1	1.5
	Anaphase	0.6	0.6	0.5
	Telophase	2.4	2.6	2.0
	Sub-total	11.0	9.5	8.0

*From Table 4.1-3

**Calculations are based on the data from Table 4.311-1

TABLE 4.312-2

Nuclear cycle duration as percentage at 30°C

Phase		Treatment		
		Control*	Cycloheximide**	Chloramphenicol
Interphase				
	G_1	5.3	12.9	14.4
	S	50.0	41.7	48.0
	G_2	32.7	40.4	30.1
	Sub-total	88.0	95.0	92.5
Mitosis				
	Prophase	5.7	2.5	3.9
	Metaphase	2.5	1.3	1.4
	Anaphase	0.6	0.3	0.5
	Telophase	3.2	0.9	1.7
	Sub-total	12.0	5.0	7.5

*From Table 4.1-3

**Calculations are based on the data from Table 4.311-2

TABLE 4.312-3

Standard deviations (hours) of the nuclear cycle components estimates

Phase	25°C			30°C		
	Control*	Cyclohe-ximide (0.001%)	Chloram-phenicol (0.03%)	Control*	Cyclohe-ximide (0.001%)	Chloram-phenicol (0.03%)
G_1 + Mitosis	0.31	0.37	0.15	0.21	0.50	0.52
S	0.22	0.14	0.07	0.33	0.51	0.13
G_2 + ½ Prophase	0.09	0.30	0.23	0.21	0.36	0.23
Total Nuclear Cycle	0.40	0.49	0.18	0.36	0.71	0.59

*From Table 4.1-2

roots have been plotted in Figure 4.321-1. At 25°C, the control values remain fairly constant over the duration of the experiment with a mean of 9.1 ± 0.29 whereas the value from the treated roots was 6.07 ± 0.11. There was a significant difference between the means of the mitotic index values (cycloheximide vs control).

At 30°C, the mean mitotic index in the control experiment was 5.3 ± 0.28 whereas the value from the treated root tips was 5.1 ± 0.11. There was not a significant difference between mean mitotic indices at 30°C.

4.322 Chloramphenicol:

At 25°C, the mean mitotic index from the treated root tips was 5.34 ± 0.45 which is roughly half the value of the control. The mitotic indices were affected immediately, apparently even during incubation period. The mitotic indices of the control and the treated roots have been plotted in Figure 4.322-1. Mitotic indices did not attain the control value after 25 hours at 25°C. There was a significant difference between the mitotic index value of the control and treated roots.

The mean mitotic index from treated root tips was 5.5 ± 0.11 at 30°C. There was no significant difference between the means of the mitotic index values (chloramphenicol vs control). Again an immediate effect on the mitotic indices after treatment was recorded at 30°C (Figure 4.322-1).

Fig. 4.321-1 Mitotic indices in control or treated (cycloheximide, 0.001%, 2 hours) at 25°C (upper) and 30°C (lower). Data for controls are taken from Figures 4.1-6 and 4.1-7. Standard deviations either side of the mean are indicated as vertical bar.

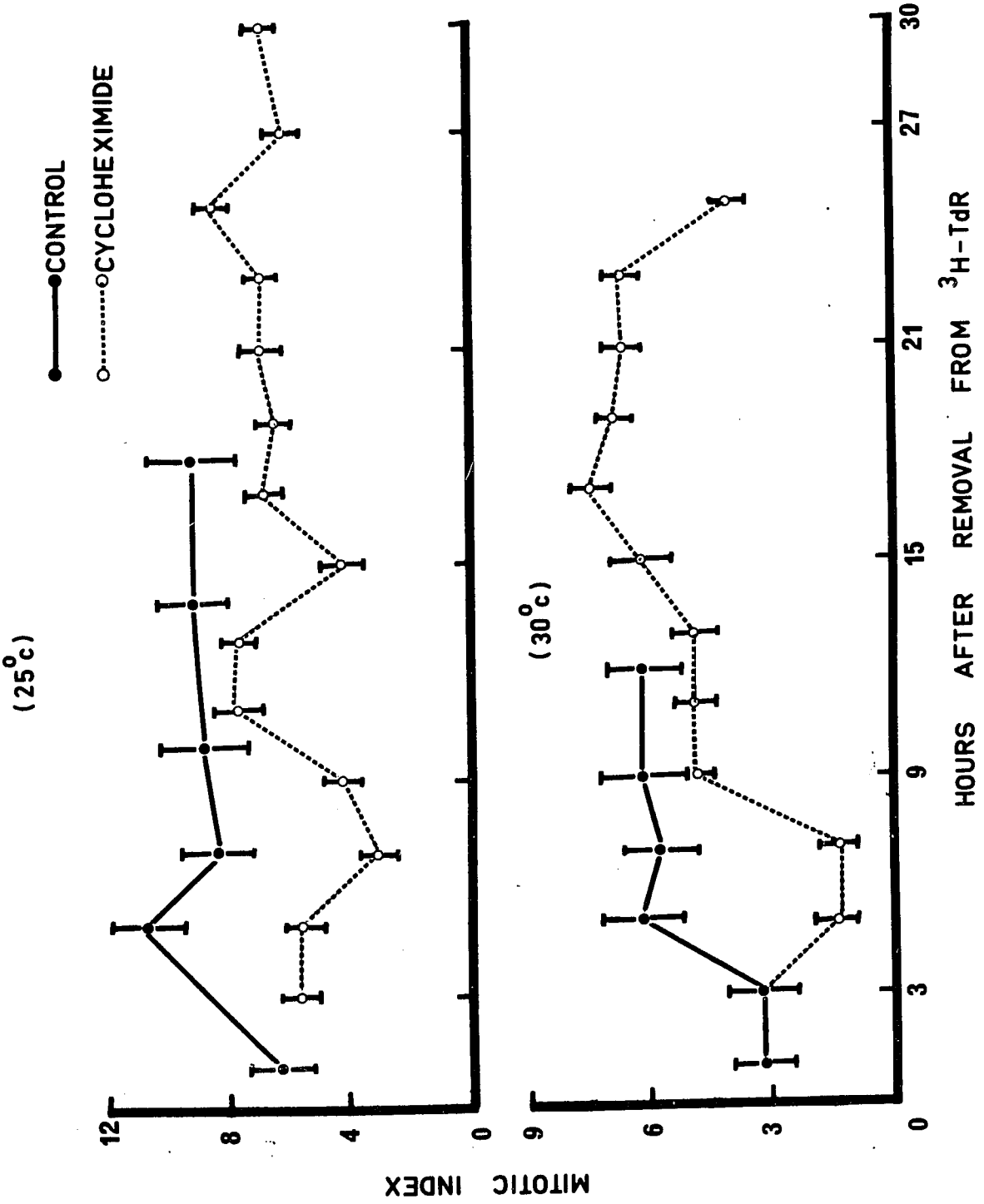
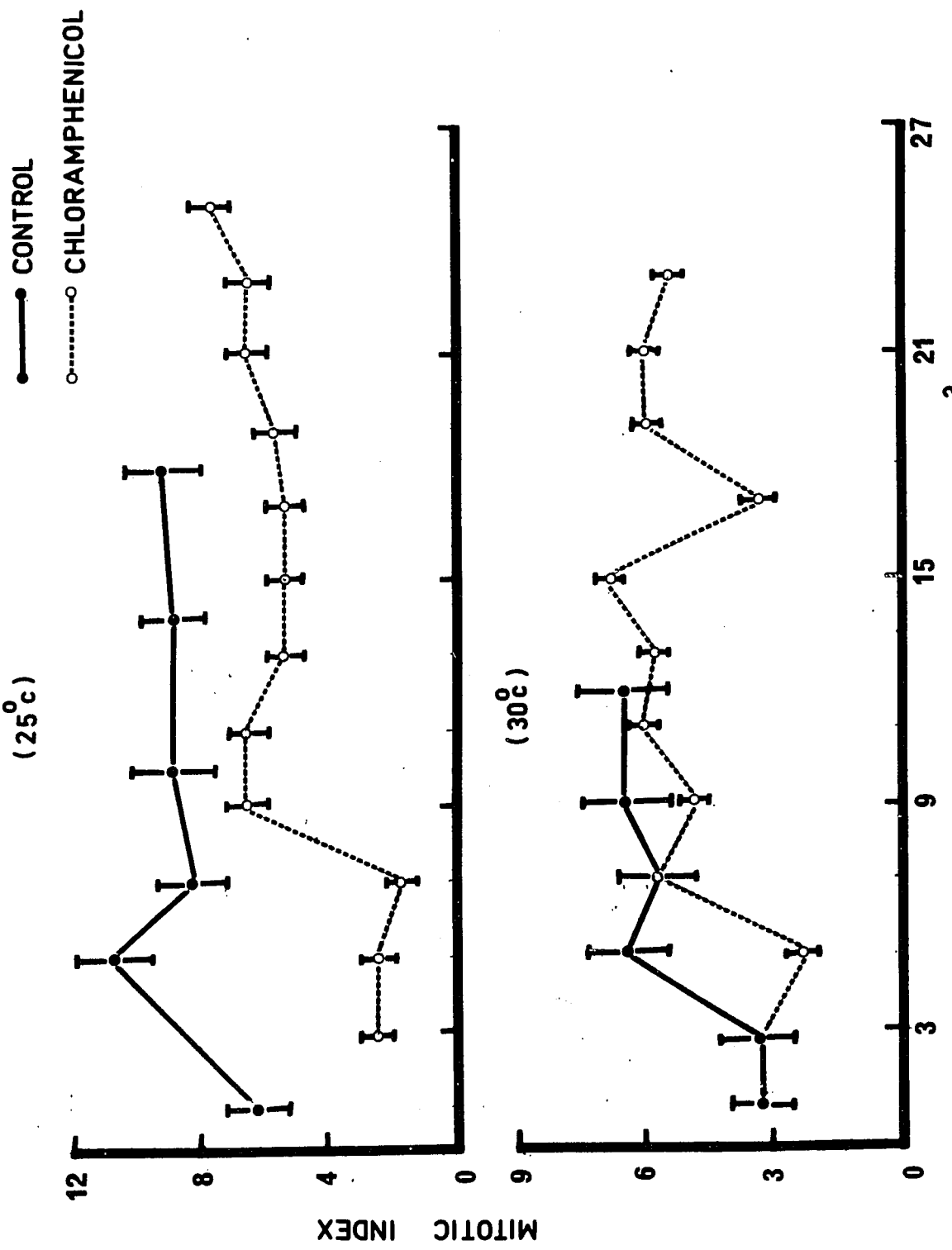

CONTROL
CYCLOHEXIMIDE
(25°c)
(30°c)
MITOTIC INDEX
HOURS AFTER REMOVAL FROM ^{3}H-TdR
0
4
8
12
0
3
6
9
0
3
9
15
21
27
30

Fig. 4.322-1 Mitotic indices in control or treated (chloramphenicol, 0.03%, 2 hours) at 25°C (upper) and 30°C (lower). Data for controls are taken from Figures 4.1-6 and 4.1-7. Standard deviations either side of the means are indicated as vertical bar.


CONTROL
CHLORAMPHENICOL
(25°c)
(30°c)
MITOTIC INDEX
12
8
4
0
9
6
3
0
3
9
15
21
27
HOURS AFTER REMOVAL FROM ^{3}H-TdR

4.4 Other Stocks:

I included in my protocol (Figure 3.1-1) an analysis of the nuclear cycle of several diverse genetic stocks in an attempt to ascertain evidence for genetic control of the cycle. The percent labelled mitotic figure curves from 'Seneca 60', W23-diploid, a chromosome 9 tester stock, and KYS are shown in Figures 3.41-2, 4.2-1, 4.4-1, and 4.4-2 respectively. Each curve contains two peaks, indicating that at least one nuclear cycle has been studied in each stock. No pronounced differences existed among stocks with regard to the appearance of the 'first' and 'second' peaks. It should be noted that the 'first' peak appeared between six and seven hours and the 'second' peak was found between 15 and 16 hours, after removal from ^{3}H-TdR. There is little difference among the slopes of the ascending and descending portions of the curves. The slight differences among the four stocks are probably due to experimental variation rather than to any real stock difference.

The various parameters of the nuclear cycle can be calculated from Figures 3.41-2, 4.2-1, 4.4-1, 4.4-2, Table D*-5 and are presented in Table 4.4-1. In Table 4.4-2, the estimates of the nuclear cycle are presented as percentages. Approximately 135,000 nuclei were scored in this study.

---

*Appendix D

Fig. 4.4-1 The percent labelled mitotic cells found in root tips of a chromosome 9 tester stock at 0 to 21 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 25°C. For standard deviations and confidence limits see Tables D-2 and D-5.

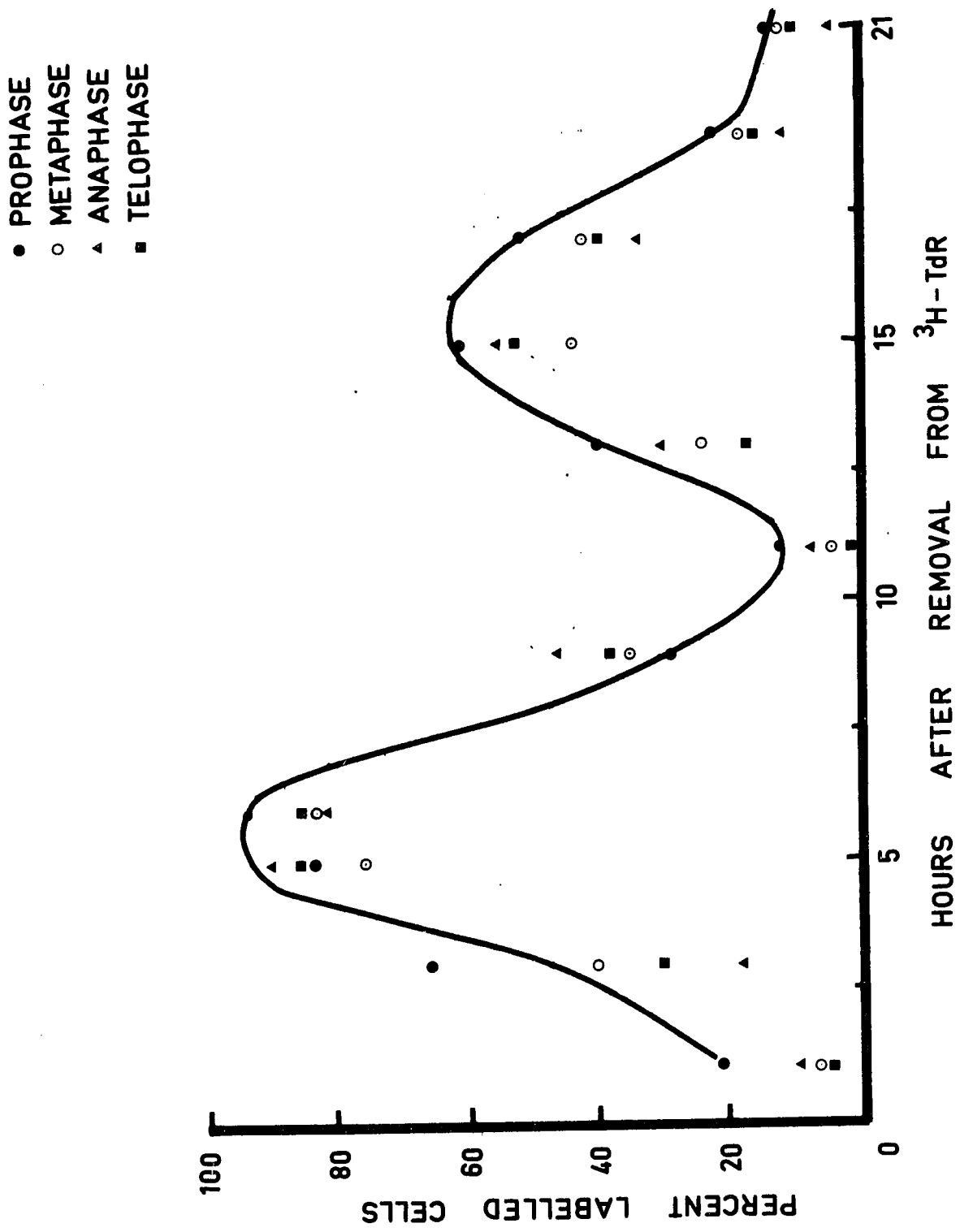

• PROPHASE
○ METAPHASE
▲ ANAPHASE
■ TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
5
10
15
21
HOURS AFTER REMOVAL FROM ^{3}H-TdR

Fig. 4.4-2 The percent labelled mitotic cells found in root tips in a KYS stock at 0 to 18 hours following pulse labelling with ^{3}H-TdR for 30 minutes at 25°C. For standard deviations and confidence limits see Tables D-3 and D-6.

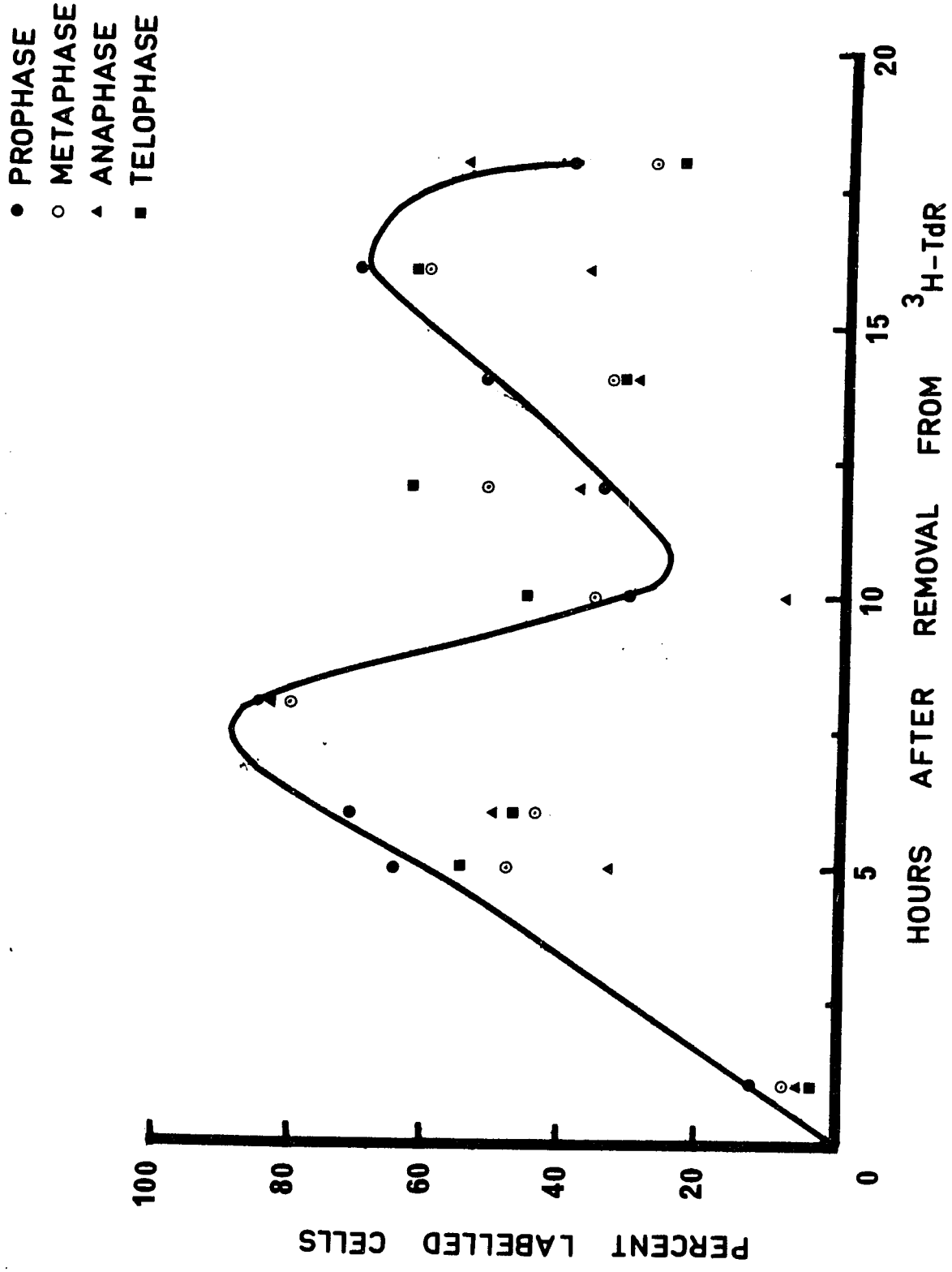

PROPHASE
METAPHASE
ANAPHASE
TELOPHASE
PERCENT LABELLED CELLS
100
80
60
40
20
0
5
10
15
20
HOURS AFTER REMOVAL FROM ^{3}H-TdR

TABLE 4.4-1

Estimates of the duration (hours) of the nuclear cycle in primary root tips of 'Seneca 60', 9-tester, W23-2N, and KYS at 25°C*

Phase		Stocks			
		Seneca 60**	W23-2N***	9-tester	KYS
Interphase					
	G_1	1.7	1.8	2.3	0.7
	S	5.0	5.5	4.5	5.5
	G_2	2.1	2.7	2.7	3.2
	Sub-total	8.8	10.0	9.5	9.4
Mitosis					
	Prophase	0.57	0.68	0.57	0.41
	Metaphase	0.23	0.23	0.24	0.18
	Anaphase	0.06	0.06	0.07	0.04
	Telophase	0.24	0.32	0.24	0.18
	Sub-total	1.10	1.29	1.12	0.81
Total		9.9	11.2	10.6	10.2

*Durations are calculated by method '2'
**From Table 4.1-1
***From Table 4.2-1

TABLE 4.4-2

Nuclear cycle durations as percentage in several stocks of Zea mays (25°C)*

Phase		Percent Stocks			
		Seneca 60	W23-2N	9-tester	KYS
Interphase					
	G_1	17.3	15.6	21.4	6.7
	S	50.5	49.1	42.5	53.9
	G_2	21.2	23.8	25.6	31.4
	Sub-total	89.0	88.5	89.5	92.0
Mitosis					
	Prophase	5.7	6.1	5.4	4.2
	Metaphase	2.3	2.1	2.2	1.7
	Anaphase	0.6	0.5	0.6	0.4
	Telophase	2.4	2.8	2.3	1.7
	Sub-total	11.0	11.5	10.5	8.0

*Calculations are based on data from Table 4.4-1

The estimates obtained via the other two methods are presented in Appendix D (Tables D-6 and D-7). As mentioned, squashes of the terminal 1.5 ± 0.02 mm of the root meristem were prepared from all except from the KYS stock (root length was 2.4 ± 0.08 mm). The root size was changed because it was ascertained that variability was higher in the longer roots (Verma, 1970) and the shorter length more nearly conformed to the major region of the dividing cells as discussed by Clowes (1965) and shown in Figure 3.25-1.

Harvey's (1970) method was employed for testing the significance of means; there was no significant difference indicating that the nuclear cycles do not differ among stocks. KYS was not included in the calculations. It is recognized that the root size is longer in KYS, a factor which may be responsible for the variability in mitosis and G_2 and, consequently, G_1 (Verma, 1970). Standard deviations of the nuclear cycle estimates are presented in Table 4.4-3. The variances of the nuclear cycle estimates can be found in Table D-8. The variances are heterogeneous.

4.5 Estimates of the S Period:

An additional method (Section 3.44) was employed to determine the mean duration of the DNA synthesis period (S). The estimates of tS calculated by equation 11 in Figure 3.44-1 are presented in Table 4.5-1. The planimeter measurements are also included in the Table 4.5-1.

TABLE 4.4-3

Standard deviations (hours) of the nuclear cycle components estimates in several stocks of Zea mays

Phase	Stocks			
	Seneca 60*	W23-2N**	9-tester	KYS
G_1 + Mitosis	0.31	0.20	0.11	0.64
S	0.22	0.15	0.12	0.21
G_2 + ½ Prophase	0.09	0.18	0.04	0.08
Total Nuclear Cycle	0.40	0.26	0.35	0.68

*From Table 4.1-2

**From Table 4.2-2

TABLE 4.5-1

The results of using different methods for estimating the DNA synthesis period (hours)

Experiments		Methods			
		Gerecke (1970a)	Planimeter	Wimber (1966)	Webster (1968)
Temperature ('Seneca 60')					
	20°C	8.2	8.3	8.5	7.7
	25°C	4.5	4.5	5.5	5.0
	30°C	3.6	3.7	4.0	4.0
	35°C	2.6	2.7	3.0	2.8
Ploidy (W23)					
	2N	5.8	5.8	6.0	6.0
	3N	5.9	5.9	5.5	5.8
	4N	5.6	5.7	5.7	5.7
Mitotic Poisons ('Seneca 60')					
	25°C CYL*	7.6	7.2	7.5	7.8
	25°C CHL**	6.5	6.4	7.0	7.0
	30°C CYL	5.2	5.3	5.5	5.5
	30°C CHL	6.5	6.2	6.5	6.3
Other Stocks					
	25°C 9-tester	5.0	5.5	5.0	6.1
	25°C KYS	5.4	5.4	6.0	5.8

*Cycloheximide (0.001%)

**Chloramphenicol (0.03%)

Furthermore, this table also contains the tS values obtained from the histogram plot (50% intercept). Harvey's (1970) method was employed to test for significant differences among methods. It was found that the four methods yielded similar results.

CHAPTER 5

DISCUSSION

The observations that are described represent an elucidation of some of the variables that control the nuclear cycle and its component phases in Zea mays root meristem cells. The main aims of this study were to investigate:

(i) the effect of temperature on the nuclear cycle;

(ii) the nuclear cycle in relation to autopolyploidy;

(iii) evidence for the genetic control of the nuclear cycle;

(iv) the effect of some mitotic poisons on the nuclear cycle; and

(v) an analytical approach to nuclear cycle data.

5.1 Total Nuclear Cycle:

Estimates of the nuclear cycle and its component phases in Z. mays root tips have been reported by several workers (Clowes, 1965; Douglas, 1968, 1971; Dougall, 1970;

Evans and Rees, 1971; Jain and Gupta, 1971; and Lin and Walden, 1971). The estimates have been based on whole meristems employing the squash technique by all workers except Clowes (1965). Clowes reported on the component phases of the nuclear for four separate regions of the root meristem (the cap initials, quiescent centre, stele just above the quiescent centre, and 200μ above the quiescent centre respectively). In the protocol for the present study, the 1.5 ± 0.02 mm terminal primary root tip has been used exclusively and no attempt has been made to distinguish among cell types. My rationale for the use of the entire root tip is as follows:

Clowes (1965) stated " ... in comparing the four regions we see that S, G_2, and Mitosis do not vary very much from one region to another. It is G_1 that is variable and accounts for the prolongation of total nuclear cycle in the quiescent centre". The quiescent centre of Z. mays contains about 600 cells and possesses a mitotic index of 1.9%, i.e. approximately 12 dividing cells would be expected per quiescent centre (Clowes, 1961b, and 1965). In addition, the two regions of the stele which have similar nuclear cycles (Table 2.1-1) are most representative of the meristem as a whole. It seemed logical, therefore, that the aims of this study were approached best from the study of a population of cells which were representative of the entire meristem region.

The effect of temperature on the nuclear cycle has

been investigated at 20, 25, 30, and 35 °C. In an early stage of this investigation, an attempt was made to measure root length at 12 hour intervals (Table A-2). It was found that root length was longer at the higher temperatures. These observations are in agreement with those of Brown and Rickless (1949), who employed Cucurbita pepo roots in their studies. Other studies have indicated that the root length was primarily dependent on the rate of cell progression, which in turn, was dependent on the duration of the nuclear cycle and its component phase (Van't Hof and Sparrow, 1963b; Van't Hof and Ying, 1964b).

Estimates of the total nuclear cycle at four different temperatures are presented in Table 4.1-1. It is obvious from these data that the total nuclear cycle is shortened with increasing temperature in the intact root of Zea mays (singlecross hybrid 'Seneca 60'). Therefore, it may be presumed that the increased root length at the higher temperatures is due to a higher rate of cell proliferation. Our findings on the effect of temperature on nuclear cycle durations are in agreement with the older literature.

The total nuclear cycle durations are the same among a diploid, an autotriploid, and an autotetraploid [(inbred W23) (Table 4.2-1)]. These results are in agreement with those of Friedberg and Davidson (1970) in the case of Vicia faba root meristem cells. Yang and Dodson (1970) also found that the total nuclear cycle duration of diploid and

tetraploid species of Avena strigosa was the same. Ayonoadu and Rees (1968) reported that the presence of two or four B chromosomes (in addition to the normal diploid genome) leads to an increase in the total nuclear cycle duration in rye root tips. Gimenez-Martin et al. (1966) found that the duration of the nuclear cycle of the diploid, caffein-induced binucleate and tetraploid cells in Allium cepa root tips were the same.

The following explanation can be suggested for the apparent constancy of the nuclear cycle among diploid, autotriploid and autotetraploid stocks: All genes of the diploid complement are present in the autopolyploid cells. As long as the genes regulating the nuclear cycle are equally 'active' in autotriploid and autotetraploid nuclei, and that precursors and enzymes are not limiting factors, we would expect the autopolyploid stocks to possess a nuclear cycle similar to that possessed by the parental diploid stock.

It is clear from the present study that the increment of the nuclear DNA from the diploid to the tetraploid level does not result in an increase in the duration of the nuclear cycle. Since induced autopolyploidy may be a more critical test than comparing among allopolyploid species, it has now been demonstrated convincingly that an increase in DNA may not necessarily alter the duration of the nuclear cycle.

Initially, consideration was given to the view that the nuclear cycle was under specific genetic (gene) control. It was proposed that maize was an excellent material in which to test the hypothesis of specific genetic control inasmuch as a wide variety of markedly different agronomic and genetic stocks was available. If the duration of the nuclear cycle was related to the growth characteristics of a stock, judicious choice of a few stocks should permit the identification of different nuclear cycles under identical controlled conditions.

A survey of the nuclear cycle of several stocks (Seneca 60, KYS, chromosome 9 tester and W23) was undertaken and concomitantly all possible F_1 and F_2 stocks were developed at the field station. Upon discovering that no differences in the nuclear cycle of the different stocks at 25°C could be described (Table 4.4-1). The analysis of F_1 and F_2 stocks was deferred. It remains to be shown whether or not differences can be described at other temperatures and under the influence of other environmental conditions as a prerequisite for heritability studies although at the moment we must conclude that these differences do not exist and that further information would not be contributed by analysing the F_1 and subsequent generations. On this basis, plus the evidence from the auto-polyploids, we concluded that there was almost universal, specific, genetic control of the nuclear cycle in maize.

In summary, it is hypothesized that the nuclear cycle is under specific genetic control and it may be concluded from our observations, that the control is highly specific, as demonstrated by the lack of varietal differences, and further, that the control was not dependent upon the amount of DNA present.

In the present study, the effect on the nuclear cycle of 0.001% cycloheximide and 0.03% chloramphenicol at 25 or 30°C is reported. Preliminary experiments were

performed to ascertain the biologically acceptable concentrations for cycloheximide or chloramphenicol (Section 4.3). At both temperatures and treatments (Tables 4.311-1 and 4.311-2), the nuclear cycles have been extended in comparison to the control values. At 25°C, the total cycle duration was increased by a factor of 1.5 for cycloheximide and chloramphenicol, whereas at 30°C the increment factor was approximately 1.7 for both. The chemical additives affected the nuclear cycle to a similar extent at each temperature. The following description can be provided for the prolongation of the nuclear cycle: At 25°C, tG_2 and tS account for the prolongation of the nuclear cycle, while at 30°C all three phases of interphase were involved in lengthening the nuclear cycle duration (Tables 4.311-1 and 4.311-2).

5.11 Interphase:

5.111 Pre DNA Synthesis Period (G_1):

The following constraint inherent in the estimation of tG_1 should be noted: tG_1 has been calculated by subtracting the sum of the estimates of tS, tG_2, and Mitosis from the total nuclear cycle time (equation 3; section 3.41). Since tG_1 was obtained as a residual value, it contains error contributions from estimates of all the other phases. Thus overestimation of any of these other phases and/or underestimation of the total nuclear cycle should misrepresent

tG_1.

It is evident from Table 4.1-3 that G_1 is very sensitive to temperatures above 25°C, i.e. it is markedly telescoped. However, between 25 and 20°C, G_1 remains proportionately similar. Therefore, G_1 apparently registers a differential response to temperature in maize. In experiments in which the cycle time of mammalian cells in culture was changed by varying the temperature, Sisken *et al.* (1965), and Watnabe and Okada (1967) found that G_1 was the most sensitive phase. It increased disproportionately in relation to the other phases and such changes were largely responsible for the alteration of the over all cycle duration. By growing *Tradescantia paludosa* roots at three different temperatures, Wimber (1966) found all phases of the nuclear cycle were temperature dependent. He also noted the lack of proportional changes in the G_1 phase. In *Tetrahymena pyriformis* cells, Mackenzie *et al.* (1966) reported that the G_1 period did not appear to be the most sensitive phase to temperature change. I can provide no explanation for the observation that G_1 in animal and plant cells is more sensitive to temperature changes than are the other nuclear cycle components.

An investigation of the molecular events occuring during the G_1 stage has not been carried out in plants. However, in animal cells, it has been suggested that the 'flow' of cells from G_1 to S seems to require the synthesis of RNA and protein as well as some special enzymes, e.g.

thymidine kinase (Baserga, 1968). However, the type of RNA synthesized during G_1 is not known. Jung and Rothstein (1967) have suggested that in animal cells at the end of the G_1 period and extending into the early part of the S phase there is a marked decrease in $[K^+]$.

G_1 was the most variable period in the nuclear cycle of maize (Tables 4.1-1, 4.2-1, and 4.4-1). This variation may or may not be real. Yang and Dodson (1970) reported that G_1 was quite variable in diploid and auto-tetraploid species of Avena strigosa. Sisken and Kinasita's (1961) experiments showed that variation in the nuclear cycle of mammalian cells in culture may be attributable to the duration of G_1 rather than the duration of S or G_2. Similarly, Kaltsikes (1971) reported that in an amphiploid (Triticale) and its parental species, the most variable phase of the nuclear cycle was G_1.

A negative estimate for tG_1 in Zea mays has been reported by several workers (Douglas, 1968, and 1971; Dougall, 1970; Jain and Gupta, 1971). According to Clowes (1965), the negative value (-1.0 hour) obtained for tG_1 indicated that this stage does not exist separately but is probably accommodated in mitosis. Douglas (1971) suggested an alternative explanation, namely that the negative tG_1 is due to an overestimation and/or underestimation of the total nuclear cycle time. Evans and Rees (1971) reported that tG_1 is 0.5 hour in Z. mays root tips (Table 2.1-1), although they did not mention the environmental conditions

of their study. Upon taking into account the extensive data in this thesis, there can be little doubt that a positive tG_1 estimate is appropriate for Z. mays.

After roots were treated with cycloheximide (0.001%) for 2 hours at 25°C, G_1 was not affected; at 30°C, this period was considerably extended (Tables 4.311-1 and 4.311-2). These data suggest that the sensitivity of the G_1 phase to cycloheximide (0.001%) is temperature dependent. The observation of temperature sensitivity is not entirely without support in the literature, as 2,4,5-trichlorophenoxyacetic acid was reported (MacLeod, 1969) to lengthen the duration of S and G_2 but not G_1 at room temperature. The questions of diffusion into the nuclei and inactivation of the chemicals cannot be approached with the methods used in this study.

Chloramphenicol (0.03%) did not affect the duration of G_1 at 25°C, although at 30°C G_1 was extended considerably (Tables 4.311-1 and 4.311-2). In inhibiting sensitive systems, cycloheximide and chloramphenicol display the following common features (Ennis and Lubin, 1964):

(i) Protein synthesis is suppressed in intact cells before any affect on RNA synthesis appears;

(ii) The transfer of amino acids from sRNA to a polypeptide is inhibited.

In conclusion, it is strongly indicated from our work that G_1 exists and to it is assignable to positive estimate of time. In addition, the G_1 phase demonstrates a

differential response to temperature in maize. The duration of the G_1 phase in each stock showed more variation than other phases. At 25°C, cycloheximide and chloramphenicol did not affect the duration of G_1 while at 30°C this phase was extended considerably by both chemicals.

5.112 DNA Synthesis Period (S):

The data in Table 4.4-1 show that the S period occupied approximately 50 percent of the total nuclear cycle time, i.e. the S phase occupied the same portion of the nuclear cycle at all temperatures tested. To quote Mackenzie et al. (1966) " ... since most enzymes have temperature coefficients in the same general range, and since progress through each sub-section of the nuclear cycle is probably dependent on numerous enzymes reactions, it is not particularly surprising to find that the subsections occupy the same proportion of the nuclear cycle at all temperatures". Our results regarding the S period are in agreement with those of Mackenzie et al. (1966), working with Tetrahymena pyriformis. An observation contrary to the constancy hypothesis was made by Wimber (1966) in Tradescantia paludosa roots; he found that the duration of the S period at 30 and 21°C was approximately the same, but at 13°C, the duration of the S phase was twice that recorded at 21°C. Therefore, it might be presumed that the proportional changes of S phase may be tissue specific.

Increased autopolyploidy in Zea mays did not alter the duration of S in the nuclear cycle. Therefore, apparently

tS is independent of DNA content in the diploid-auto-polyploid stocks tested. Yang and Dodson (1970) reported that tS was identical in a diploid stock as well as in a related tetraploid species of Avena strigosa. Similar results were reported also by Camron and Stone (1964) in Tetrahymena pyriformis and by Van't Hof (1966) in diploid and autopolyploid cells in the same roots of Pisum sativum. Friedberg and Davidson (1970) also reported that tS values were similar in diploid and autotetraploid cells in the same roots of Vicia faba and Troy and Wimber (1968) demonstrated that the S period duration was independent of the nuclear DNA content (Table 2.3-1).

Thus, the results presented here should not be compared directly with those of Van't Hof's (1965b) earlier study involving a number of unrelated higher plants, nor with those of Prasad and Godward (1965) who studied the diploid Phalaris canariensis and the tetraploid p. minor. In both reports, a direct relationship between the DNA content and the value of tS was indicated.

There are conflicting views about the DNA content and the duration of S period. However, it is not appropriate to compare cytogenetically different but related species with autopolyploids. In the former class, in addition to different amounts of DNA, one may have allele differences which compound the comparison. In the latter class, such genetic differences do not exist.

The asynchrony of chromosomal DNA replication in

both plant and animal cells has been known for many years (Taylor, 1960; Darlington and Haque, 1964; Kusanagi, 1966; and several others). Taylor (1963) postulated that a chromosome consisted of many replication units. Cairn (1966) reached a similar conclusion by studying the rate of DNA synthesis in chromosomes of cultured HeLa cells. In the polytene chromosomes of Drosophila melanogaster, the replication of DNA at homologous loci is closely synchronized (Plaut and Nash, 1964). The observation that a haploid or autopolyploid stock has a tS value similar to its diploid counterpart may be explained as follows:

Presumably the number of replication units per genome is finite and constant. Thus, increased ploidy levels would not be expected to lengthen the time required for DNA synthesis unless multiple sets of replication units caused genome asynchrony. Gupta (1969) has proposed that the DNA synthesis period of the entire genome of a species is controlled by a regulatory mechanism, and this mechanism comes under the control of chromosome replicons (unit of replication).

If homologous sites on chromosomes synthesize DNA at the same time, one would expect that with an increase in ploidy, the temporal pattern of DNA replication would remain the same. In theory, corresponding portions of homologous chromosomes should replicate simultaneously, and the pattern of replication throughout the complement would be retained at various euploidy levels. Thus

increased ploidy levels would not be expected to lengthen the time required for DNA synthesis.

Apparently the morphological characteristics usually associated with increased polyploidy (the gigas characteristics) are not accounted for by the time required for metabolic events. Rather, account can be taken in the autopolyploids by an increase in the quantity of metabolites.

A comparison of the prophase labelling curves of the control roots and roots exposed to 'cycloheximide' shows that tS is increased by a factor of 1.4 at 25 and 30°C in comparison to the control value. The results reported here are in agreement with those of Bennett et al. (1964). In mammalian cells, Ennis and Lubin (1964) have shown that the primary effect of cycloheximide was the inhibition of protein synthesis and that the inhibition of DNA synthesis was an indirect effect. Morris (1967) has demonstrated in a resting cell suspension of Chlorella pyrenoidosa that protein synthesis was inhibited (80%) by cycloheximide. This inhibition appears to be a primary effect. The controversy over whether the primary effect of cycloheximide is on protein or on DNA synthesis has not been resolved.

The duration of S was increased in the presence of 'chloramphenicol' by a factor of 1.3 and 1.7 at 25 and 30°C respectively. The specific point of action for chloramphenicol is unknown. However, Demoss and Novelli (1956) and Coutsogeorgopouplos (1966) have suggested that chloramphenicol

does not inhibit the coding step but prevents polymerization leading to the synthesis of peptide bonds during protein synthesis.

In earlier studies, estimates of tS have been extrapolated from the histogram plot (50% intercept). In Gerecke's method, the value of tS is based on the area under the curve. The advantages of this latter method has been described earlier (section 3.44). The area under the prophase curve was calculated from a determination of the perimeter by the use of a planimeter. Estimates of the duration of the S period calculated by several methods are presented in Table 4.5-1. It was concluded that all methods yielded similar results. From the experience acquired in this study, it can be concluded that the accuracy of estimating tS by any of four methods depends on the sample size and the mechanics of plotting the curve.

In summary, it was observed that the S period requires the longest portion of the interphase. S occupied the same portion of the nuclear cycle at all temperatures. In addition, the duration of the S period was independent of the amount of the nuclear DNA content. It was shown that cycloheximide and chloramphenicol extend the duration of S period. More direct evidence is needed to interpret the action of cycloheximide and chloramphenicol.

5.113 Post-DNA Synthesis Period (G_2):

The lack of proportional changes in tG_2 at four

different temperatures is noted in Table 4.1-1. Two possible explanations for the constancy of the G_2 period at 25 and 30 °C can be suggested:

(i) Since there is no change in the duration of G_2 at 25 and 30 °C, conditions other than metabolic changes may be limiting the rate of G_2 processes. Brown and Whiteman (1950) has shown that at least some of the metabolites required for division are synthesized in mature tissues and transported to the meristem cells. This observation suggests that some of the processes indirectly involved in division may not be localized in the cells of the meristem and therefore may appear as temperature independent.

(ii) The observation may indicate a rather wide optimum temperature range for this phase.

It should be noted from Figure 4.2-4 that the 'first' ascending curve is practically identical among diploid, autotriploid, and autotetraploid stocks of W23. Consequently, the duration of G_2 among the stocks was similar. Prasad and Godward (1965) reached the same conclusion in Phalaris canariensis (2N) and P. minor (4N) root tips. However, Yang and Dodson (1970) reported tG_2 was 1.8 times longer in an autotetraploid than in diploid Avena strigosa.

The duration of G_2 was considerably higher in KYS in comparison to the other stocks (Table 4.4-1). This is likely due, at least, in part, to our change in experimental

technique (Verma, 1970). Douglas (1971; Table 1) suggested that the proportion of G_2 in the cycle is tissue specific.

The most obvious effect of cycloheximide was a marked increase in tG_2 relative to the control value (Tables 4.311-1 and 4.311-2). At 25°C, tG_2 increased 3.05 hours (control/treatment); at 30°C, the increase was 2.55 hours. Therefore, the G_2 phase appears to be quite sensitive to cycloheximide at both temperatures.

It has been known for some time that cycloheximide affects RNA and protein synthesis in higher plant cells (Key, 1966, 1969; Waters and Dure, 1966), and it has been established that during G_2, cells are actively engaged in the synthesis of RNA and protein (Nygaard et al. 1960; Taylor, 1960; Prescott, 1962). Verbin and Farber (1967) suggested that cycloheximide prolonged the duration of G_2 period, presumably as the result of an interference with the formation of protein(s) required for the normal progression of cells from this phase. Studies with cell-free extracts showed that the primary effect of cycloheximide was the inhibition of protein synthesis, and that the inhibition of DNA synthesis was indirect (Siegle and Sisler, 1964a, 1964b).

In the chloramphenicol treatment at 25°C the G_2 period increased 3.6 hours and at 30°C, the increase was 2.46 hours. It has been well documented that chloramphenicol is a protein synthesis inhibitor (Lacks and Gros, 1960). The findings of Prescott (1962) and others suggested that

during the G_2 period, cells are actively engaged in the synthesis of protein.

Thus, for maize we can report a lack of proportional changes in tG_2 at four different temperatures. In addition, the duration of G_2 among the stocks studied was similar. The effect of cycloheximide and chloramphenicol on tG_2 are also similar and can be interpreted as an inhibition of protein(s) or DNA synthesis.

5.12 Mitosis:

Approximately 10 to 15 percent of the total nuclear cycle is required for mitosis at 25, 30, 35 and 20°C. Therefore, proportional changes were recorded at all temperatures. These results are in agreement with those of Lopez-Saez et al. (1966) who employed Allium cepa roots. Wimber (1966) reported the contrary observation of Tradescantia paludosa, namely that the duration of mitosis was practically identical at 30 and 21°C.

Since mitosis occupied the same proportion of the nuclear cycle at 25, 30, and 35°C, it is presumed that those enzymes which are active during mitosis, and events taking place in mitosis, have temperature coefficients in the same general range.

There is no pronounced difference among diploid, autotriploid, and autotetraploid in regard to the phase times of mitosis. It should be noted from Tables 4.311-1 and 4.311-2 that the duration of mitosis is similar in the

chemical treatment and control experiments. Therefore, it is concluded that neither cycloheximide nor chloramphenicol has any effect on mitosis, attesting to the localized effect of those two chemicals.

5.2 Mitotic Index:

Determination of mitotic indices were made and are presented graphically in Figures 4.1-6 and 4.1-7. The higher mitotic indices were recorded at the lower temperatures. These results are in agreement with those of Murin (1966) in the case of Vicia faba root tips. It can be noted from Figures 4.1-6 and 4.1-7 that during the first few hours after removal from ^{3}H-TdR, there was considerable fluctuation in the mitotic indices; this is believed to be due to a sudden change in the environmental conditions, namely, washing the roots after removal from the isotope solution, and a possible metabolic effect of ^{3}H-TdR itself.

The mitotic indices were the same in the diploid and autotetraploid (W23), but a significantly lower mitotic index was found in the autotriploid stocks. An effect of cycloheximide or chloramphenicol appeared to result in a reduction of the mitotic indices at both temperatures studied. Since the mitotic indices were affected immediately, even during the incubation period, it is suggested that the chemical is incorporated rapidly into the cells. Since the effect of cycloheximide or chloramphenicol is mainly on G_2 or S, it may be presumed that the reduction in mitotic

indices were mainly due to prolongation of G_2 or/and S.

CHAPTER 6

SUMMARY

The intent of this study was to investigate: (i) the effect of temperature on the nuclear cycle and its component phases; (ii) the nuclear cycle in relation to autopolyploidy (autotriploidy, and autotetraploidy); (iii) the effect of some chemicals, cycloheximide (0.001%) and chloramphenicol (0.03%), on nuclear cycle; (iv) the genetic control of nuclear cycle; and (v) an analytical approach to nuclear cycle data. The findings are summarized below:

6.1 Temperature:

1. The effect of different temperatures on the duration of nuclear cycle in Zea mays (Singlecross hybrid 'Seneca 60') root meristem cells, was studied with autoradiographic techniques and it was shown that all component phases of the nuclear cycle are shortened by an increase in temperature from 20 to 35°C.

2. The durations of total nuclear cycle at 20, 25, 30, and 35°C were 16.5, 9.9, 7.0, and 4.4 hours respectively while the durations of mitosis were 2.7, 1.1, 0.8, and 0.4 hours respectively.

3. 85-90 percent of the nuclear cycle is required for interphase while the remaining 10-15 percent of the cycle is occupied by mitosis.

4. It is presumed that the longer root length at the higher temperatures is due to a shorter duration of the nuclear cycle.

6.2 Autopolyploidy:

1. The durations of various component phases of derived autotriploid and autotetraploid stocks, and a diploid stock of Z. mays (W23) were estimated from autoradiographic data.

2. The results indicate that the duration of the nuclear cycle, and in particular the DNA synthesis period in diploid, autotriploid and autotetraploid stocks were similar. At 25°C, the total nuclear cycle was 11.2 to 11.5 hours and the DNA synthesis period was 5.0 to 5.5 hours for each of 2N, 3N, and 4N stocks of W23.

3. The duration of S period has been estimated by several methods and it was found that all methods yielded similar results.

4. It was concluded that the nuclear cycle was under specific genetic control and that the DNA synthesis period was not dependent upon the amount of DNA present in a cell.

5. The results support the Troy and Wimber views that the homologous chromosomes or whole genomes require

a similar duration for DNA synthesis.

6.3 Mitotic Poisons:

1. Cycloheximide (0.001%) and chloramphenicol (0.03%) appear to act mainly on G_2 and S phases, extending the duration of S and G_2 periods at 25 and 30°C.

2. Neither chemicals affected the duration of G_1 at 25°C. However, at 30°C, G_1 was extended considerably by both chemicals.

3. Mitosis was not affected at either 25°C or 30°C by either chemicals. Therefore, the prolongation of the duration of nuclear cycle was mainly because of interphase.

4. There was a significant reduction in the mitotic indices after treatment with both chemicals in comparison to the control value. These results suggest that some interphase processes have been delayed or inhibited by both chemicals and have resulted in a fall of mitotic indices.

6.4 Other Stocks:

1. The nuclear cycle among several inbred stocks of Z. mays were compared and it was found that there was no significant difference among the nuclear cycle durations.

2. The total nuclear cycle was 10 to 11 hours and mitosis was 0.9 to 1.29 hours at 25°C.

3. The S period is the longest interval (50% of

the total time) of the nuclear cycle; of the rest of the cycle, G_2 is longer than G_1 or Mitosis among all stocks.

4. The constancy of the nuclear cycle among several stocks was adduced as evidence for strict genetic control of the cycle.

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APPENDIX A

TEMPERATURE

TABLE A-1

Percent germination in Seneca 60*

Temperature (°C)	Percent Germination					
	24	36	48	60	72	84
20	0	2	42	60	80	100
25	4	94	100			
30	34	96				
35	58	100				

*Data are based on 50 seeds

TABLE A-2

Elongation of radicle of germinating maize seedlings (Seneca 60)*

Temperature (°C)	Root length in cm					
	48	60	72	84	96	108
20						
Mean	-	-	-	1.25	1.89	2.68
S.D.**				0.27	0.39	0.44
25						
Mean	1.02	1.63	2.38	3.38	4.48	5.58
S.D.	0.39	0.21	0.67	0.83	1.21	0.86
30						
Mean	2.04	2.82	3.47	4.09	4.97	5.99
S.D.	0.62	0.61	0.67	0.64	0.11	1.02
35						
Mean	3.55	4.38	5.37	6.22	7.29	-
S.D.	0.84	0.80	0.90	1.40	1.01	

*Data are based on 50 seeds

**Standard Deviation

TABLE A-3

Percentage of labelled nuclei in root tips of Seneca 60 at 20°C

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	12.24 ± 2.0	3.33 ± 1.7	10.71 ± 5.8	2.65 ± 1.4
5	46.74 ± 3.0	27.47 ± 4.5	4.34 ± 4.2	20.19 ± 3.8
7	62.06 ± 3.2	38.55 ± 5.2	53.84 ± 13.7	49.33 ± 5.4
9	90.07 ± 1.0	80.11 ± 3.0	82.00 ± 5.4	89.60 ± 2.0
11	78.18 ± 1.4	56.01 ± 2.8	56.09 ± 5.5	74.21 ± 2.2
13	59.72 ± 2.0	62.87 ± 2.8	64.70 ± 5.5	71.94 ± 2.4
15	33.44 ± 1.7	32.17 ± 2.0	42.85 ± 5.7	39.56 ± 2.2
17	16.13 ± 1.4	13.89 ± 2.0	20.54 ± 4.7	13.36 ± 2.2
19	28.45 ± 2.8	16.36 ± 2.7	40.00 ± 7.3	25.27 ± 3.0
21	43.65 ± 1.7	51.94 ± 2.5	42.00 ± 5.0	50.74 ± 2.3

TABLE A-4

Percentage of labelled nuclei in root tips of Seneca 60 at 25°C

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	6.01 ± 1.0	2.50 ± 1.4	0	0.93 ± 0
3	63.44 ± 3.4	39.68 ± 6.0	19.04 ± 8.5	32.20 ± 6.0
5	92.56 ± 1.0	74.84 ± 3.2	80.43 ± 5.8	74.70 ± 3.3
6	92.18 ± 2.1	95.58 ± 2.3	90.00 ± 6.7	91.02 ± 3.2
7	66.22 ± 2.3	52.35 ± 3.3	60.97 ± 6.5	52.20 ± 4.3
8	16.93 ± 2.6	19.56 ± 4.0	11.42 ± 5.2	23.36 ± 4.0
10	17.41 ± 1.4	12.28 ± 2.3	16.66 ± 7.4	13.01 ± 2.4
12	35.90 ± 2.2	14.28 ± 2.5	18.51 ± 7.4	9.92 ± 1.7
14	55.95 ± 2.0	45.07 ± 3.4	48.48 ± 6.0	40.67 ± 3.2
16	58.55 ± 2.5	51.23 ± 4.6	42.30 ± 9.6	55.03 ± 4.0
18	29.92 ± 2.5	35.64 ± 4.3	34.48 ± 8.8	37.93 ± 4.5

TABLE A-5

Percentage of labelled nuclei in root tips of Seneca 60 at 30°C

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	23.21 ± 5.4	16.12 ± 6.6	0	15.21 ± 0.0
3	50.00 ± 6.8	46.87 ± 6.2	16.66 ± 10.7	6.66 ± 5.3
4	75.80 ± 3.5	63.33 ± 4.6	23.52 ± 10.2	47.16 ± 4.5
5	83.33 ± 2.0	69.04 ± 4.0	76.66 ± 7.7	78.28 ± 6.7
6	68.08 ± 3.0	75.00 ± 4.3	67.64 ± 8.1	77.56 ± 3.3
7	40.19 ± 3.3	36.36 ± 4.2	41.66 ± 8.3	49.59 ± 4.5
8	24.40 ± 2.8	18.55 ± 3.9	15.78 ± 8.3	16.49 ± 3.7
9	38.36 ± 3.4	19.17 ± 4.6	20.00 ± 12.6	12.82 ± 3.7
10	52.96 ± 2.4	29.89 ± 4.6	30.00 ± 10.2	40.00 ± 4.7
11	58.00 ± 2.6	45.63 ± 4.6	42.22 ± 10.2	33.55 ± 4.7
12	79.17 ± 2.0	13.25 ± 2.5	76.78 ± 5.5	68.69 ± 3.0

TABLE A-6

Percentage of labelled nuclei in root tips of Seneca 60 at 35°C

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	12.26 ± 2.5	1.19 ± 1.0	0	0
2	64.61 ± 6.0	44.34 ± 8.3	0	11.53 ± 6.3
3	88.05 ± 2.3	57.77 ± 7.4	66.66 ± 10.3	63.82 ± 7.0
4	68.63 ± 3.0	83.00 ± 5.2	71.42 ± 12.0	85.18 ± 3.6
5	28.75 ± 2.5	41.66 ± 4.5	38.00 ± 6.4	47.77 ± 3.9
6	56.44 ± 3.2	34.30 ± 3.6	24.39 ± 6.6	41.71 ± 3.6
7	40.05 ± 2.2	60.30 ± 5.6	58.82 ± 12.0	66.31 ± 4.8
8	12.60 ± 2.0	6.79 ± 1.7	12.00 ± 6.6	12.14 ± 2.6

TABLE A-7

95% Confidence limits on labelled mitoses in 'Seneca 60' at 20°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	16.16 - 8.32	7.03 - 0	22.06 - 0	9.44 - 0
5	52.08 - 40.68	36.64 - 18.30	12.56 - 0	27.90 - 12.37
7	69.27 - 54.86	49.02 - 28.00	80.94 - 26.84	59.95 - 38.70
9	92.50 - 87.63	86.09 - 74.12	92.64 - 71.36	93.80 - 85.19
11	81.11 - 75.28	61.71 - 51.00	66.83 - 45.35	79.01 - 69.41
13	63.45 - 55.82	68.69 - 57.04	75.30 - 53.25	76.99 - 66.88
15	37.19 - 29.79	37.87 - 26.47	54.34 - 31.26	45.36 - 33.56
17	19.25 - 13.00	18.10 - 9.70	29.80 - 11.27	17.88 - 8.82
19	34.00 - 22.79	22.00 - 10.71	54.31 - 25.69	31.58 - 18.95
21	47.18 - 40.11	58.72 - 45.14	51.67 - 32.22	55.67 - 45.80

TABLE A-8

95% Confidence limits on labelled mitoses in 'Seneca 60' at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	8.86 - 3.15	5.29 - 0	-	2.64 - 0
3	70.36 - 56.51	51.76 - 27.60	35.83 - 2.15	44.12 - 20.28
5	95.06 - 90.15	81.58 - 68.10	91.89 - 68.97	81.23 - 68.17
6	97.06 - 87.53	100.4 - 90.69	103.14 - 76.96	97.36 - 84.68
7	70.87 - 62.06	59.85 - 44.55	75.70 - 46.04	60.59 - 43.71
8	22.06 - 11.49	27.66 - 11.46	21.95 - 0.89	31.37 - 15.25
10	21.01 - 13.50	17.19 - 7.67	29.99 - 3.43	18.08 - 7.94
12	40.29 - 31.52	19.80 - 8.76	33.05 - 3.97	13.59 - 6.15
14	60.32 - 51.58	52.09 - 38.06	60.35 - 36.61	46.93 - 34.41
16	63.74 - 53.26	60.12 - 42.23	61.29 - 23.11	63.01 - 47.05
18	35.14 - 24.50	44.98 - 26.30	51.77 - 17.19	46.75 - 29.11

TABLE A-9

95% Confidence limits on labelled mitoses in 'Seneca 60' at 30°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	33.49 - 12.43	29.06 - 3.28	-	25.58 - 4.84
3	63.23 - 36.77	59.08 - 34.56	37.66 - 0	15.58 - 0
4	81.69 - 65.	72.22 - 54.23	43.38 - 3.36	60.59 - 33.73
5	87.46 - 79.19	77.11 - 60.97	91.79 - 61.63	84.73 - 71.73
6	73.66 - 62.10	83.22 - 66.68	83.16 - 51.92	84.10 - 71.02
7	46.91 - 33.37	44.26 - 28.16	57.76 - 25.26	58.42 - 40.76
8	30.22 - 18.88	26.28 - 10.82	32.07 - 0	23.57 - 9.11
9	45.91 - 30.81	28.20 - 10.14	44.79 - 0	20.23 - 5.41
10	58.73 - 47.19	39.00 - 20.78	50.00 - 10.00	49.37 - 30.63
11	63.24 - 52.56	55.14 - 36.02	56.71 - 27.85	41.05 - 26.05
12	83.10 - 75.14	18.16 - 8.34	87.83 - 65.73	74.48 - 63.00

TABLE A-10

95% Confidence limits on labelled mitoses in 'Seneca 60' at 35°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	17.16 - 7.36	3.15 - 0	-	-
2	76.37 - 52.85	60.54 - 28.14	-	23.87 - 0
3	92.55 - 83.55	72.27 - 43.27	86.84 - 46.48	77.54 - 50.10
4	74.51 - 62.75	93.19 - 72.81	94.94 - 47.90	92.23 - 78.13
5	33.65 - 23.85	50.48 - 32.84	50.54 - 25.46	55.41 - 40.12
6	62.71 - 50.17	41.35 - 27.24	37.32 - 11.46	48.76 - 34.66
7	44.36 - 35.74	71.24 - 49.30	82.23 - 35.30	75.71 - 56.91
8	16.52 - 86.60	10.12 - 3.46	24.93 - 0	17.23 - 7.05

TABLE A-11

Classification and frequency of nuclei scored from root tips (Zea mays, 'Seneca 60') following pulse labelling at 4 temperatures

Phase	Temperature (°C)			
	20	25	30	35
Interphase				
Labelled	25334	30828	21181	22198
Mitosis				
Prophase				
Labelled	2380	1639	1450	746
Unlabelled	2335	1818	978	998
Metaphase				
Labelled	832	547	537	238
Unlabelled	1148	865	583	494
Anaphase				
Labelled	247	151	140	66
Unlabelled	305	218	147	118
Telophase				
Labelled	1080	572	612	330
Unlabelled	1110	896	610	459
Total	34771	37534	26238	25647

TABLE A-12

Estimates of the duration (hours) of the nuclear cycle in primary root tips of hybrid 'Seneca 60' at four temperatures*.

Phase		Temperature			
		20°C	25°C	30°C	35°C
Interphase					
	G_1	2.16	1.98	0.57	0.19
	S	8.0	5.0	3.5	2.5
	G_2	3.65	1.83	2.09	1.28
	Sub-total	13.82	8.81	6.16	3.97
Mitosis					
	Prophase	1.34	0.56	0.41	0.22
	Metaphase	0.56	0.23	0.18	0.09
	Anaphase	0.16	0.06	0.05	0.02
	Telophase	0.62	0.24	0.20	0.10
	Sub-total	2.68	1.09	0.84	0.43
Total		16.5	9.9	7.0	4.4

*Durations are calculated by method '1'

TABLE A-13

Estimates of the duration (hours) of the nuclear cycle in primary root tips of hybrid 'Seneca 60' at four temperatures*

Phase		Temperature			
		20°C	25°C	30°C	35°C
Interphase					
	G_1	0.72	2.58	0.60	0.15
	S	7.15	4.5	3.5	2.3
	G_2	4.86	2.21	2.29	1.35
	sub-total	12.73	9.29	6.39	3.80
Mitosis					
	Prophase	1.27	0.57	0.42	0.29
	Metaphase	0.53	0.23	0.18	0.11
	Ana+Telophase	0.72	0.31	0.26	0.15
	sub-total	2.52	1.11	0.86	0.55
Total		15.25	10.40	7.25	4.35

*Durations are calculated by method '3'

TABLE A-14

Variances (hours) of the nuclear cycle components estimates in the primary root tips of 'Seneca 60' at four temperatures

Phase	Temperature			
	20°C	25°C	30°C	35°C
G_1 + Mitotis	0.15	0.10	0.05	0.23
S	0.02	0.05	0.11	0.03
G_2 + ½ Prophase	0.002	0.01	0.05	0.01
Total Nuclear Cycle	0.18	0.16	0.13	0.26

APPENDIX B

AUTOPOLYPLOIDY

TABLE B-1

Percentage of labelled nuclei in root tips of W23-2N at 25°C

Hours after pulse	Percent Labelled Mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	14.86 ± 4.2	5.26 ± 5.1	0	3.12 ± 3.1
3	50.00 ± 5.3	44.44 ± 9.5	50.00 ± 20.4	40.00 ± 7.3
5	80.41 ± 2.8	70.76 ± 5.6	63.16 ± 11.1	75.00 ± 4.7
6	89.56 ± 2.8	86.66 ± 6.2	83.34 ± 10.7	78.04 ± 6.5
8	78.21 ± 4.1	79.16 ± 5.9	75.00 ± 15.3	81.25 ± 5.6
10	29.72 ± 3.7	24.39 ± 6.7	31.25 ± 11.5	26.38 ± 5.2
12	21.24 ± 2.5	8.06 ± 3.6	6.25 ± 6.1	7.92 ± 2.7
14	41.46 ± 3.8	45.56 ± 5.6	35.00 ± 10.7	38.29 ± 5.0
16	60.00 ± 4.2	52.63 ± 8.1	50.00 ± 13.4	37.70 ± 6.2
18	46.53 ± 4.7	43.24 ± 8.1	50.00 ± 15.8	35.59 ± 6.2

TABLE B-2

Percentage of labelled nuclei in root tips of W23-3N at 25°C

Hours after pulse	Percent labelled nuclei			
	Prophase	Metaphase	Anaphase	Telophase
1	3.84 ± 3.8	0	0	0
3	53.84 ± 8.0	47.50 ± 7.9	25.00 ± 21.6	22.22 ± 8.1
5	80.83 ± 3.6	66.00 ± 6.7	58.33 ± 14.2	39.53 ± 7.4
6	96.96 ± 1.3	94.50 ± 2.3	85.00 ± 8.0	98.48 ± 1.1
8	61.36 ± 5.2	71.11 ± 6.8	35.71 ± 12.8	60.46 ± 7.4
10	34.72 ± 5.6	37.14 ± 8.2	25.00 ± 21.7	25.00 ± 7.2
12	16.12 ± 3.1	6.00 ± 2.9	4.54 ± 4.4	7.35 ± 3.1
14	40.96 ± 3.8	36.61 ± 5.7	33.33 ± 15.7	11.66 ± 4.1
16	60.62 ± 4.3	52.92 ± 6.1	50.00 ± 10.7	32.20 ± 6.1
18	28.45 ± 4.1	16.41 ± 4.6	12.50 ± 8.3	39.00 ± 5.4

TABLE B-3

Percentage of labelled nuclei in root tips of W23-4N at 25°C

Hours after pulse	Percent labelled nuclei			
	Prophase	Metaphase	Anaphase	Telophase
1	4.93 ± 0.1	0	0	0
3	50.00 ± 4.4	43.5 ± 7.3	16.66 ± 15.2	10.81 ± 5.0
5	92.24 ± 2.3	87.27 ± 4.5	89.47 ± 7.0	83.33 ± 4.4
6	95.65 ± 1.5	93.54 ± 6.3	89.65 ± 5.6	86.59 ± 3.5
8	64.60 ± 4.5	61.72 ± 5.4	66.66 ± 12.2	76.78 ± 5.6
10	20.49 ± 3.2	24.24 ± 5.3	9.09 ± 8.7	13.09 ± 3.7
12	24.62 ± 3.7	29.16 ± 6.6	35.71 ± 12.8	14.54 ± 4.7
14	41.61 ± 3.9	43.05 ± 5.8	28.57 ± 12.0	16.88 ± 4.3
16	58.06 ± 4.0	71.21 ± 5.6	45.45 ± 15.0	50.72 ± 6.0
18	24.87 ± 3.1	21.73 ± 6.1	12.50 ± 8.3	23.52 ± 5.1

TABLE B-4

95% Confidence limits on the labelled mitoses in W23-2N at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	23.01 - 6.71	15.29 - 0	-	9.13 - 0
3	60.32 - 39.68	63.07 - 25.71	90.00 - 10.00	54.20 - 25.50
5	85.97 - 74.84	81.81 - 59.71	84.82 - 41.48	84.15 - 65.65
6	95.14 - 83.97	98.81 - 74.21	104.41 - 62.17	90.70 - 65.48
8	86.24 - 70.17	90.64 - 67.68	104.98 - 45.02	92.18 - 70.21
10	37.07 - 22.27	37.52 - 11.26	53.54 - 8.66	36.55 - 16.21
12	26.21 - 16.26	15.11 - 1.06	18.10 - 0	13.17 - 2.67
14	48.98 - 33.84	56.53 - 34.49	55.89 - 14.11	48.10 - 28.47
16	68.25 - 51.75	68.48 - 36.78	76.18 - 23.52	49.85 - 25.44
18	55.74 - 37.32	59.19 - 27.29	80.98 - 19.02	47.80 - 23.28

TABLE B-5

95% Confidence limits on the labelled mitoses in W23-3N at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	11.20 - 0	-	-	-
3	69.48 - 38.20	62.86 - 32.04	67.43 - 0	38.19 - 6.55
5	87.86 - 73.49	79.13 - 52.66	86.22 - 30.45	54.13 - 25.23
6	99.58 - 94.43	98.92 - 90.08	100.64 - 69.36	100.50 - 94.40
8	71.53 - 51.19	84.34 - 57.98	50.79 - 10.62	75.06 - 45.56
10	45.71 - 23.43	53.13 - 21.15	67.43 - 0	39.13 - 10.87
12	22.09 - 10.35	11.72 - 1.00	13.12 - 0	13.34 - 1.16
14	48.40 - 33.42	47.80 - 25.42	64.02 - 2.44	19.75 - 3.47
16	69.04 - 52.10	64.36 - 41.07	70.89 - 29.11	44.11 - 20.29
18	36.40 - 20.49	25.00 - 7.50	28.68 - 0	49.54 - 28.46

TABLE B-6

95% Confidence limits on the labelled mitoses in W23-4N at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	4.96 - 4.89	-	-	-
3	58.58 - 41.41	57.77 - 29.27	46.47 - 0	20.66 - 0.96
5	96.84 - 87.63	96.10 - 78.46	103.26 - 75.68	91.93 - 74.33
6	98.59 - 92.31	105.88 - 81.30	100.72 - 78.58	93.25 - 79.83
8	73.32 - 55.78	72.20 - 51.14	90.51 - 43.11	87.75 - 65.81
10	26.72 - 14.25	34.09 - 13.82	26.06 - 0	20.30 - 5.58
12	31.91 - 17.33	42.01 - 16.41	60.79 - 10.62	23.65 - 5.12
14	49.25 - 33.17	54.47 - 31.63	52.12 - 5.32	25.12 - 8.54
16	65.90 - 50.22	82.02 - 60.30	74.86 - 16.04	62.40 - 39.25
18	30.90 - 18.83	33.34 - 9.82	28.68 - 0	33.49 - 13.35

TABLE B-7

Classification and frequency of nuclei scored from root tips (Zea mays W23) following pulse labelling at 25°C

Phase		Ploidy level		
		2N	3N	4N
Interphase				
	Labelled	10409	9641	10639
	Unlabelled	19209	21747	22150
Mitosis				
Prophase				
	Labelled	679	563	709
	Unlabelled	647	518	736
Metaphase				
	Labelled	210	242	294
	Unlabelled	236	306	287
Anaphase				
	Labelled	56	48	71
	Unlabelled	71	84	75
Telophase				
	Labelled	260	153	274
	Unlabelled	377	236	377
Total		32154	33538	35612

TABLE B-8

Estimes of the duration (hours) of the nuclear cycle in primary roots tips of W23 (2N, 3N, and 4N) at 25°C*

Phase		Ploidy		
		Diploid	Autotriploid	Autotetraploid
Interphase				
	G_1	2.1	2.89	2.65
	S	5.5	5.0	5.2
	G_2	2.31	2.5	2.31
	Sub-total	9.91	10.39	10.16
Mitosis				
	Prophase	0.68	0.56	0.68
	Metaphase	0.23	0.28	0.28
	Anaphase	0.06	0.06	0.07
	Telophase	0.32	0.21	0.31
	Sub-total	1.29	1.11	1.34
Total		11.2	11.5	11.5

*Durations are calculated by method '1'

TABLE B-9

Estimates of the duration (hours) of the nuclear cycle in primary root tips of W23 (2N, 3N, and 4N) at 25°C

Phase		Ploidy		
		Diploid	Autotriploid	Autotetraploid
Interphase				
	G_1	1.25	2.16	2.26
	S	5.5	5.3	5.2
	G_2	2.66	2.73	2.64
	sub-total	9.31	10.19	10.10
Mitosis				
	Prophase	0.67	0.54	0.72
	Metaphase	0.23	0.26	0.28
	Anaphase+ Telophase	0.39	0.26	0.40
	sub-total	1.29	1.06	1.40
Total		10.7	11.25	11.50

*Durations are calculated by method '3'

TABLE B-10

Variances (hours) of the nuclear cycle components estimates in the primary root tips of W23 (2N, 3N, and 4N) at 25°C

Phase	Ploidy		
	Diploid	Autotriploid	Autotetraploid
G_1 + Mitosis	0.04	0.02	0.06
S	0.02	0.02	0.003
G_2 + ½ Prophase	0.03	0.03	0.01
Total Nuclear Cycle	0.07	0.03	0.07

APPENDIX C

MITOTIC POISONS

TABLE C-1

Percentage of labelled nuclei in root tips of Seneca 60 at 25°C treated with cycloheximide (0.001%)

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
3	24.63 ± 5.3	10.00 ± 3.9	0	0
5	50.00 ± 7.7	40.90 ± 7.3	25.00 ± 21.6	28.57 ± 2.0
7	50.00 ± 6.1	47.45 ± 6.5	38.46 ± 13.5	30.00 ± 10.2
9	87.61 ± 3.2	81.48 ± 7.4	85.71 ± 13.1	72.41 ± 8.2
11	64.28 ± 7.3	50.00 ± 13.3	66.66 ± 19.2	70.00 ± 14.5
13	50.45 ± 4.7	53.33 ± 7.4	50.00 ± 13.3	66.10 ± 6.2
15	32.35 ± 8.0	41.55 ± 10.0	60.00 ± 21.9	33.33 ± 9.6
17	31.66 ± 4.2	14.89 ± 5.1	21.05 ± 9.3	25.89 ± 5.7
19	39.53 ± 4.2	39.53 ± 7.4	50.00 ± 13.3	34.54 ± 6.4
21	54.31 ± 4.6	42.85 ± 7.6	56.25 ± 12.4	31.14 ± 5.9
23	56.48 ± 4.7	53.65 ± 8.1	22.22 ± 13.8	44.44 ± 6.2
25	52.81 ± 4.1	45.16 ± 6.2	16.66 ± 8.8	43.40 ± 6.8
27	37.19 ± 4.3	20.75 ± 5.5	31.25 ± 11.6	26.56 ± 5.5
30	32.45 ± 3.7	20.68 ± 5.2	50.00 ± 10.6	22.98 ± 4.4

TABLE C-2

Percentage of labelled nuclei in root tips of Seneca 60 at 25°C, treated with chloramphenicol (0.03%)

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
3	8.33 ± 4.6	0	0	0
5	29.03 ± 8.1	26.66 ± 11.4	0	10.52 ± 7.0
7	50.00 ± 10.6	37.50 ± 17.1	40.00 ± 21.9	26.31 ± 10.1
9	83.33 ± 2.6	82.97 ± 5.5	75.00 ± 10.8	79.54 ± 4.2
11	69.51 ± 5.0	67.30 ± 6.5	61.53 ± 13.5	68.51 ± 6.2
13	46.51 ± 5.3	57.78 ± 7.3	55.55 ± 11.7	60.30 ± 5.9
15	23.00 ± 3.8	20.83 ± 5.8	26.66 ± 11.4	15.68 ± 5.0
17	16.80 ± 3.3	10.00 ± 4.2	25.00 ± 10.8	15.38 ± 4.5
19	33.65 ± 4.6	22.72 ± 6.2	14.28 ± 13.1	17.39 ± 5.6
21	43.67 ± 3.7	34.09 ± 7.1	26.31 ± 10.1	32.85 ± 5.6
23	66.11 ± 2.2	60.52 ± 7.9	46.15 ± 13.8	66.66 ± 6.5
25	27.97 ± 3.4	20.89 ± 4.9	17.39 ± 7.8	23.52 ± 4.6

TABLE C-3

Percentage of labelled nuclei in root tips of Seneca 60 at 30°C, treated with cycloheximide (0.001%)

hours after pulse	Percentage labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
3	17.24 ± 7.0	7.5 ± 2.8	0	25.00 ± 15.3
5	46.15 ± 13.8	28.57 ± 17.0	0	50.00 ± 35.3
7	83.87 ± 6.5	75.0 ± 15.3	100.00 ± 30.0	100.00 ± 30.0
9	72.00 ± 6.3	69.23 ± 12.8	62.50 ± 17.1	86.66 ± 8.7
11	38.88 ± 4.7	56.75 ± 8.1	57.14 ± 18.7	50.00 ± 9.4
13	28.76 ± 5.3	18.51 ± 7.4	0	15.15 ± 6.2
15	26.82 ± 6.8	21.42 ± 10.9	0	9.09 ± 8.7
17	68.50 ± 3.7	61.40 ± 6.4	54.54 ± 10.7	67.18 ± 7.1
19	46.95 ± 4.5	35.84 ± 6.5	58.82 ± 9.1	35.84 ± 6.6
21	39.28 ± 4.6	55.55 ± 7.3	20.00 ± 8.9	27.08 ± 6.5
23	51.61 ± 5.1	41.93 ± 8.8	41.66 ± 14.2	13.04 ± 5.9
25	32.78 ± 6.0	30.76 ± 6.3	36.36 ± 10.2	31.24 ± 8.3

TABLE C-4

Percentage of labelled nuclei in root tips of Seneca 60 at 30°C, treated with chloramphenicol (0.03%)

Hours after pulse	Percent labelled nuclei			
	Prophase	Metaphase	Anaphase	Telophase
3	26.31 ± 5.8	17.39 ± 7.9	25.00 ± 15.3	5.00 ± 4.8
5	86.11 ± 6.0	76.92 ± 11.7	75.00 ± 21.6	92.30 ± 7.3
7	91.40 ± 2.4	93.54 ± 4.3	88.23 ± 7.8	93.93 ± 2.8
9	72.54 ± 4.3	78.57 ± 6.3	81.81 ± 11.6	76.92 ± 5.8
11	40.96 ± 3.7	35.93 ± 5.9	18.18 ± 8.2	39.13 ± 5.8
13	39.13 ± 4.1	31.48 ± 6.3	22.22 ± 9.8	23.19 ± 5.0
15	25.71 ± 4.2	38.23 ± 8.3	10.00 ± 9.5	29.19 ± 9.3
17	55.55 ± 3.6	42.46 ± 5.7	43.47 ± 10.3	42.25 ± 5.8
19	75.45 ± 4.0	56.81 ± 7.4	60.00 ± 12.64	58.82 ± 8.4
21	22.00 ± 3.3	31.25 ± 6.6	21.73 ± 8.5	23.28 ± 4.9
23	35.21 ± 5.6	32.14 ± 9.3	16.66 ± 10.7	25.80 ± 6.8

TABLE C-5

95% Confidence limits on the labelled mitoses treated with cycloheximide (0.001%) at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
3	34.69 - 14.17	17.58 - 2.32	0	0
5	65.05 - 34.95	55.58 - 26.52	67.39 - 0	32.46 - 24.28
7	61.91 - 38.09	60.15 - 34.35	64.90 - 12.02	50.07 - 9.93
9	93.80 - 81.42	96.00 - 67.48	111.56 - 60.16	88.56 - 56.26
11	78.88 - 50.10	76.14 - 23.46	104.35 - 28.97	98.40 - 41.60
13	59.64 - 41.26	67.85 - 39.11	76.14 - 23.76	78.17 - 54.03
15	48.03 - 16.67	61.26 - 22.06	102.92 - 17.18	52.02 - 14.54
17	39.97 - 23.25	24.86 - 4.02	39.31 - 2.79	37.14 - 14.24
19	47.84 - 31.22	54.05 - 25.01	76.06 - 23.74	47.08 - 22.00
21	63.18 - 45.34	57.76 - 28.15	80.47 - 32.03	42.62 - 19.56
23	65.69 - 47.29	69.59 - 38.04	49.36 - 0	56.57 - 32.11
25	60.88 - 44.64	57.39 - 33.03	33.68 - 0	56.67 - 30.21
27	45.00 - 28.66	53.38 - 9.86	53.42 - 8.68	37.28 - 15.84
30	39.78 - 25.12	31.04 - 10.32	70.83 - 29.17	31.17 - 14.11

TABLE C-6

95% Confidence limits on the labelled mitoses treated with chloramphenicol (0.03%) at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
3	17.30 - 0	-	-	-
5	44.94 - 13.12	49.00 - 4.21	-	24.14 - 0
7	70.83 - 29.17	70.97 - 4.23	82.52 - 0	46.08 - 6.54
9	88.50 - 78.16	93.69 - 72.12	96.18 - 53.72	87.85 - 71.23
11	79.31 - 59.71	80.00 - 54.70	87.97 - 35.09	80.74 - 56.38
13	56.87 - 36.15	72.06 - 43.40	78.48 - 32.62	71.87 - 48.71
15	30.58 - 15.42	32.15 - 9.01	49.00 - 4.42	25.28 - 5.48
17	23.28 - 10.31	18.31 - 1.69	46.18 - 3.72	24.04 - 6.62
19	42.42 - 24.67	34.95 - 10.59	39.83 - 0	28.28 - 6.50
21	51.00 - 36.34	48.08 - 20.71	46.08 - 6.54	43.74 - 21.96
23	70.50 - 62.04	75.94 - 45.10	73.13 - 19.07	79.40 - 54.02
25	34.55 - 21.19	30.47 - 11.31	32.71 - 1.97	32.39 - 14.55

TABLE C-7

95% Confidence limits on the labelled mitoses treated with cycloheximide (0.001%) at 30°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
3	30.96 - 3.62	13.02 - 1.98	-	54.66 - 0
5	73.23 - 19.07	61.98 - 0	-	119.27 - 0
7	96.70 - 71.14	104.96 - 45.04	158.00 - 41.20	158.80 - 41.20
9	84.38 - 59.72	94.23 - 44.23	95.97 - 29.13	103.71 - 69.61
11	48.07 - 29.69	72.56 - 40.84	93.65 - 20.53	68.42 - 31.58
13	39.12 - 18.40	33.03 - 3.89	-	27.22 - 3.08
15	40.24 - 13.40	42.78 - 0	-	26.06 - 0
17	75.72 - 61.16	73.64 - 48.86	75.42 - 33.16	81.25 - 53.01
19	55.77 - 38.13	48.67 - 23.11	76.65 - 40.99	48.67 - 23.01
21	48.25 - 30.31	69.93 - 41.47	37.52 - 2.48	39.80 - 14.46
23	61.58 - 41.64	58.79 - 24.19	69.51 - 13.41	24.62 - 1.46
25	44.24 - 21.00	43.14 - 18.48	56.43 - 16.29	47.51 - 14.79

TABLE C-8

95% Confidence limits on the labelled mitoses treated with chloramphenicol (0.03%) at 30°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
3	37.73 - 14.59	32.71 - 1.97	54.45 - 0	14.28 - 0
5	97.83 - 74.29	99.77 - 54.17	117.29 - 32.67	106.60 - 78.10
7	96.18 - 86.62	102.07 - 85.02	103.53 - 73.03	99.41 - 88.44
9	81.07 - 64.02	90.85 - 66.29	104.56 - 59.16	88.32 - 65.52
11	48.29 - 33.53	47.54 - 24.54	34.11 - 2.05	50.55 - 27.71
13	47.20 - 31.04	43.34 - 19.32	41.40 - 3.04	32.39 - 13.29
15	34.02 - 17.40	54.29 - 21.97	28.58 - 0	47.35 - 11.03
17	62.20 - 48.50	53.51 - 31.21	63.53 - 23.21	53.25 - 30.83
19	83.15 - 67.61	71.33 - 42.18	84.77 - 35.63	75.32 - 42.22
21	28.48 - 15.52	44.24 - 18.36	38.46 - 5.00	32.76 - 13.30
23	46.28 - 24.14	50.40 - 13.78	37.67 - 0	37.25 - 14.35

TABLE C-9

Classification and frequency of nuclei scored from root tips (Zea mays Seneca 60) following pulse labelling at 25° and 30°C

Phase	Treatments			
	Cycloheximide (0.001%)		Chloramphenicol (0.03%)	
	25°C	30°C	25°C	30°C
Interphase				
Labelled	13139	14896	13317	16533
Unlabelled	29200	20985	30785	23891
Mitosis				
Prophase				
Labelled	639	413	550	627
Unlabelled	717	459	686	616
Metaphase				
Labelled	230	160	184	209
Unlabelled	389	264	291	245
Anaphase				
Labelled	67	49	56	64
Unlabelled	98	73	95	99
Telophase				
Labelled	226	135	258	240
Unlabelled	372	194	369	292
Total	45077	37628	46591	42816

TABLE C-10

Estimates of the duration (hours) of the nuclear cycle with a 2 hour incubation in cycloheximide (0.001%) or chloramphenicol (0.03%) following 30 minutes of ^{3}H-TdR treatment at 25°C

Phase		Treatment		
		Control**	Cyclohe-ximide	Chloram-phenicol
Interphase				
	G_1	1.98	1.77	1.89
	S	5.0	7.0	6.5
	G_2	1.83	4.8	5.4
	Sub-total	8.81	13.57	13.79
Mitosis				
	Prophase	0.56	0.71	0.60
	Metaphase	0.23	0.32	0.23
	Anaphase	0.06	0.09	0.07
	Telophase	0.24	0.31	0.30
	Sub-total	1.09	1.43	1.2
Total		9.9	15.0	15.0

*Durations are calculated by method '1'

**From Table A-12

TABLE C-11

Estimates of the duration (hours) of the nuclear cycle with a 2 hour incubation in cycloheximide (0.001%) or chloramphenicol (0.03%) following 30 minutes of ^{3}H-TdR treatment at 25°C*

Phase		Treatment		
		Control**	Cyclohe-ximide	Chloram-phenicol
Interphase				
	G_1	2.58	1.01	1.97
	S	4.5	7.25	6.5
	G_2	2.21	5.08	5.41
	Sub-total	9.29	13.34	13.88
Mitosis				
	Prophase	0.57	0.83	0.58
	Metaphase	0.23	0.37	0.22
	Anaphase + Telophase	0.31	0.46	0.32
	Sub-total	1.11	1.66	1.12
Total		10.4	15.0	15.0

*Durations are calculated by Method '3'

**From Table A-13

TABLE C-12

Estimates of the duration (hours) of the nuclear cycle with a 2 hour incubation in cycloheximide (0.001%) or chloramphenicol (0.03%) following 30 minutes of ^{3}H-TdR treatment at 30°C

Phase		Treatment		
		Control**	Cyclohe-ximide	Chloram-phenicol
Interphase				
	G_1	0.57	1.7	2.04
	S	3.5	5.0	6.0
	G_2	2.09	4.7	3.52
	Sub-total	6.16	11.4	11.56
Mitotis				
	Prophase	0.41	0.30	0.49
	Metaphase	0.18	0.15	0.18
	Anaphase	0.05	0.04	0.06
	Telophase	0.20	0.11	0.21
	Sub-total	0.84	0.60	0.93
Total		7.0	12.0	12.5

*Durations are calculated by method '1'

**From Table A-12

TABLE C-13

Estimates of the duration (hours) of the nuclear cycle with a 2 hour incubation in cycloheximide (0.001%) or chloramphenicol (0.03%) following 30 minutes of ^{3}H-TdR treatment at 30°C

Phase		Treatment		
		Control**	Cyclohe-ximide	Chloram-phenicol
Interphase				
	G_1	0.60	2.32	2.01
	S	3.5	5.0	5.75
	G_2	2.29	4.5	3.75
	Sub-total	6.39	11.82	11.51
Mitosis				
	Prophase	0.42	0.33	0.51
	Metaphase	0.18	0.17	0.19
	Anaphase + Telophase	0.26	0.18	0.29
	Sub-total	0.86	0.68	0.99
Total		7.25	12.5	12.5

*Durations are calculated by method '3'

**From Table A-13

TABLE C-14

Variances of the nuclear cycle component estimates (hrs).

Phase	25°C			25°C		
	Control*	Cyclohe ximide	Chloram phenicol	Control*	Cyclohe ximide	Chloram phenicol
G_1 + Mitosis	0.10	0.14	0.02	0.05	0.25	0.27
S	0.05	0.02	0.004	0.11	0.26	0.02
G_2 + ½ Prophase	0.01	0.09	0.05	0.05	0.13	0.05
Total Nuclear Cycle	0.16	0.25	0.03	0.13	0.50	0.35

*From Table A-14

APPENDIX D

OTHER STOCKS

TABLE D-1

Percentage of labelled nuclei in root tips of chromosome 9-tester at 25°C

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	21.09 ± 2.5	5.78 ± 2.0	8.69 ± 5.8	6.52 ± 2.3
3	66.25 ± 5.2	40.42 ± 7.1	18.18 ± 11.6	29.54 ± 6.8
5	82.97 ± 3.1	76.56 ± 5.2	90.00 ± 9.5	84.48 ± 4.7
6	94.28 ± 1.0	83.72 ± 3.1	82.85 ± 6.3	84.35 ± 2.8
9	29.35 ± 2.5	35.48 ± 4.2	46.66 ± 8.8	38.23 ± 4.1
11	12.19 ± 2.1	4.85 ± 2.0	7.69 ± 7.3	1.04 ± 1.0
13	39.64 ± 3.7	24.61 ± 5.3	30.00 ± 10.2	17.18 ± 4.7
15	61.14 ± 3.5	44.18 ± 7.5	55.55 ± 11.7	53.02 ± 6.0
17	52.19 ± 2.6	42.74 ± 4.2	83.33 ± 6.7	39.86 ± 4.0
19	22.36 ± 2.3	18.11 ± 3.2	11.74 ± 7.8	16.92 ± 3.1
21	14.35 ± 2.2	13.48 ± 3.5	4.76 ± 4.5	10.97 ± 3.3

TABLE D-2

Percentage of labelled nuclei in root tips of KYS at 25°C

Hours after pulse	Percent labelled mitoses			
	Prophase	Metaphase	Anaphase	Telophase
1	12.04 ± 2.5	7.57 ± 3.2	6.25 ± 6.0	5.00 ± 2.4
5	65.21 ± 5.7	48.57 ± 8.5	33.33 ± 19.2	55.55 ± 11.7
6	72.89 ± 3.3	44.77 ± 6.0	50.00 ± 14.4	47.94 ± 5.9
8	85.89 ± 2.0	80.23 ± 4.3	84.61 ± 7.0	84.82 ± 3.2
10	31.91 ± 3.6	36.53 ± 6.7	8.33 ± 8.0	46.66 ± 6.4
12	35.10 ± 3.5	52.17 ± 7.3	38.46 ± 13.2	63.79 ± 5.2
14	52.77 ± 3.0	34.71 ± 4.3	29.41 ± 7.8	33.83 ± 4.0
16	71.36 ± 2.8	61.53 ± 4.3	37.50 ± 8.5	62.40 ± 4.2
18	40.00 ± 4.5	28.57 ± 5.1	55.55 ± 11.7	24.32 ± 5.0

TABLE D-3

95% Confidence limits on the labelled mitoses in 9-tester at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	25.45 - 16.19	9.70 - 1.96	20.05 - 0	11.02 - 2.02
3	76.44 - 56.06	54.13 - 26.81	40.93 - 0	42.76 - 16.32
5	89.04 - 76.90	86.75 - 66.37	108.62 - 71.38	93.69 - 75.27
6	96.18 - 92.22	89.79 - 77.65	95.19 - 70.61	89.83 - 78.86
9	34.15 - 24.25	43.61 - 27.25	63.90 - 29.42	46.26 - 30.19
11	16.30 - 8.08	8.77 - 1.03	21.99 - 0	3.00 - 0
13	46.89 - 32.29	34.69 - 14.23	49.99 - 10.01	26.39 - 7.97
15	68.00 - 54.25	58.88 - 29.74	78.48 - 32.62	64.48 - 41.26
17	57.28 - 47.10	50.97 - 34.21	46.46 - 20.31	47.70 - 32.02
19	26.86 - 18.06	24.38 - 11.87	27.02 - 0	22.79 - 10.85
21	18.66 - 10.24	20.34 - 6.62	13.48 - 0	17.43 - 4.30

TABLE D-4

95% Confidence limits on the labelled mitoses in KYS at 25°C

Hours after pulse	Prophase	Metaphase	Anaphase	Telophase
1	16.94 - 7.24	13.74 - 1.30	18.01 - 0	9.70 - 1.00
5	76.38 - 54.04	65.23 - 31.01	70.96 - 0	78.38 - 32.62
6	79.35 - 66.42	56.53 - 33.01	78.22 - 21.78	59.50 - 36.38
8	89.81 - 82.07	88.65 - 71.41	98.33 - 70.99	91.09 - 78.55
10	38.96 - 24.66	49.66 - 23.40	24.01 - 0	59.10 - 34.12
12	41.96 - 28.24	66.47 - 37.87	64.33 - 12.46	73.58 - 53.40
14	58.65 - 46.89	43.03 - 26.29	44.39 - 14.13	41.67 - 25.99
16	76.84 - 66.08	69.95 - 53.00	54.04 - 20.84	70.63 - 54.07
18	48.82 - 31.18	38.56 - 18.58	78.48 - 32.62	34.12 - 14.52

TABLE D-5

Classification and frequency of nuclei scored from root tips of several stocks following pulse labelling at 25°C

Phase	Stocks			
	9-tester	W23-2N*	S-60**	KYS
Interphase				
Labelled	19529	10409	30828	21740
Mitosis				
Prophase				
Labelled	1086	679	1639	851
Unlabelled	1340	647	1818	753
Metaphase				
Labelled	355	210	547	308
Unlabelled	681	236	865	372
Anaphase				
Labelled	87	56	151	69
Unlabelled	144	71	218	100
Telophase				
Labelled	381	260	572	349
Unlabelled	682	377	896	364
Total	24285	11791	37534	24906

*From Table B-7

**From Table A-11

TABLE D-6

Estimates of the duration (hours) of the nuclear cycle in primary root tips of 9-tester, W23-2N, Seneca 60 and KYS at 25°C.*

Phase		Stocks			
		9-tester	W23-2N**	Seneca 60***	KYS
Interphase					
	G_1	2.57	2.10	1.98	0.79
	S	4.5	5.5	5.0	5.5
	G_2	2.43	2.31	1.83	3.09
	Sub-total	9.5	9.91	8.81	9.38
Mitosis					
	Prophase	0.56	0.68	0.56	0.41
	Metaphase	0.24	0.23	0.23	0.17
	Anaphase	0.05	0.06	0.06	0.04
	Telophase	0.25	0.32	0.24	0.18
	Sub-total	1.1	1.29	1.09	0.82
Total		10.6	11.2	9.9	10.2

*Durations are calculated by method '1'

**From Table B-8

***From Table A-12

TABLE D-7

Estimates of the duration (hours) of the nuclear cycle in primary root tips of 9-tester, W23-2N, Seneca 60, and KYS at 25°C.*

Phase		Stocks			
		9-tester	W23-2N**	Seneca 60***	KYS
Interphase					
	G_1	2.62	1.25	2.58	0.30
	S	4.6	5.5	4.5	5.25
	G_2	2.79	2.66	2.21	3.1
	Sub-total	9.0	9.31	9.29	8.65
Mitosis					
	Prophase	0.42	0.67	0.57	0.99
	Metaphase	0.18	0.23	0.23	0.41
	Anaphase + Telophase	0.44	0.39	0.31	0.53
	Sub-total	1.04	1.29	1.11	1.93
Total		11.05	10.7	10.4	10.5

*Durations are calculated by method '3'

**From Table B-9

***From Table A-13

TABLE D-8

Variances (hours) of the nuclear cycle component estimates in 9-tester, W23-2N, Seneca 60, and KYS at 25°C

Phase	Stocks			
	9-tester	Seneca 60*	W23-2N**	KYS
G_1 + Mitosis	0.01	0.10	0.04	0.41
S	0.01	0.05	0.02	0.04
G_2 + ½ Prophase	0.002	0.01	0.03	0.006
Total Nuclear Cycle	0.12	0.16	0.07	0.47

*From Table A-14

**From Table B-10

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