Growth of Binucleate Cells in *Vicia faba*

GROWTH OF SISTER NUCLEI IN BINUCLEATE CELLS INDUCED BY TREATMENT WITH METHYLXANTHINES

By

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A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

July 1976

MASTER OF SCIENCE (1976) McMASTER UNIVERSITY

Biology Hamilton, Ontario

TITLE: Growth of Sister Nuclei in Binucleate Cells Induced by Treatment with Methylxanthines.

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SUPERVISOR: Dr. D. Davidson

NUMBER OF PAGES: xii, 65

ABSTRACT

The responses of primary root cells of Vicia faba to two methylxanthines, 8-ethoxycaffeine and 3-isobutyl-1 methylxanthine were compared. The object was to determine whether or not the binucleate condition, induced by treatment with methylxanthines, could be used to estimate cell cycle duration in a marked subpopulation of cells. Binucleate cells induced by EOC did not divide; about 75% of those induced by IBMX had a cell cycle duration of 15-16 hours, i.e. close to the duration estimated for fast cycling cells.

Methylxanthines may also induce other effects, e.g. (1) chromatid stickiness and tetraploid nucleus formation, (2) depression of mitotic index EOC and IBMX differed in their ability to induce these changes.

Using binucleate cells, nuclear growth was followed throughout interphase. Nuclei did not grow continuously throughout interphase; there was a period of little or no growth initially and then a period in which the nuclei contracted. In IBMX induced binucleate cells, nuclear growth occurred and was rapid in the 2 hours prior to the entry of nuclei into mitosis. The other aspect of nuclear growth that was studied concerned the relative growth of the two sister nuclei of a binucleate cell. Sister nuclei were rarely of identical volumes.

In most cases, large differences were seen between sister nuclei; these differences were maintained throughout interphase and

into prophase. Nuclear shape changed during interphase but sister nuclei in a binucleate cell tended to have the same shape. The change in nuclear shape and the volume differences between sister nuclei are discussed in terms of nucleo-cytoplasmic interactions and the origin of the differential behaviour of sister nuclei present in a common cytoplasm.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks and appreciation to Dr. D. Davidson for his supervision and assistance during the course of this study. I also wish to thank Mr. J. Thomas and Mr. T. Y. Wong for their help in computer programming and in statistical analyses.

Thanks are also due to Dr. J. Bansal, Mrs. M. Eastman, Mr. K. C. Lee, Mr. J. Thomas and Mr. T. Y. Wong for their helpful suggestions and moral support during the preparation of this thesis. My sincere gratitude is extended to Mrs. J. Grenville for typing the thesis.

Financial aid received in the form of a McMaster Graduate Fellowship was greatly appreciated.

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ABBREVIATIONS

t	time from the beginning of treatment, in hours
p	the number of prophase cells
M	the number of metaphase cells
A	the number of anaphase cells
T	the number of telophase cells
I	the number of interphase cells
SA	the number of sticky anaphase cells
ST	the number of sticky telophase cells
SI	the total number of sticky interphase and tetraploid cells
M.I.	mitotic index
S.D.	standard deviation of the mitotic index
%BN(I)	the percent of binucleate cells in interphase
%BN(M)	the percent of binucleate cells in mitosis

INTRODUCTION

The regulation of growth of proliferating cells continues to be a topic of major interest in cell biology. Some systems are simple; all cells have about the same generation time. Other systems are complex and consist of two or more subpopulations that proliferate at different rates. Meristems of Vicia faba, for example, consist of fast, slow and non cycling cells. The composition of the primary root meristem has been determined: 1) fast cycling cells, which constitute either 68% (Murin, 1966) or 41% (MacLeod, 1971) of all cells present. MacLeod (1971) suggests the values obtained depend upon the length of apical meristem studied (1.5 mm and 2.0 mm, in the two studies cited). It is estimated that the fast cycling cells comprise 68% of all cells in mitosis at any time (MacLeod, 1971). Mean cell cycle durations have been estimated to be 22.2 hours (Murin, 1966) and 18.0 hours (Van't Hof, 1967; MacLeod, 1971), 2) slow cycling cells, which constitute either 22% (Murin, 1966) or 19% (MacLeod, 1971) of the total cell population, 3) non cycling cells, which constitute either 10% (Murin, 1966) or 40% (MacLeod, 1971) of the total cells in primary root meristems. To maintain a stable composition of the cell population in such a complex or heterogeneous system will clearly require accurate control of cell cycle durations.

In order to determine cell cycle durations in vivo, cells must be marked; and preferably they should be marked during a specific stage in the growth cycle. One marking technique is to induce binucleate cells by treatments with methylxanthines. Kihlman (1955) reported that the methylxanthines, 8-ethoxycaffeine (EOC) and theophylline inhibit cell

wall formation; cells that passed through telophase during treatment did not produce a new cell plate, and, at the end of mitosis, a binucleate cell was formed. Paul and Goff (1973) carried out an electron microscope study of the response to methylxanthines and showed that vesicle formation was abnormal; the result was the suppression of cross-wall formation. In addition, they showed that caffeic acid, caffeine and aminophylline, which are also methylxanthines, inhibited wall formation and induced the formation of binucleate cells. The induced binucleate condition served as a morphological marker and provided a labelled population of cells which could be followed through a cell cycle (Howard and Dewey, 1960; Giménez-Martín et al., 1965, 1966, 1968; González-Fernández et al., 1966). However, methylxanthine induced binucleate cells do not provide a useful marking technique in every species. In lateral roots of V. faba, for example, binucleate cells induced by EOC do not come back into division and could not be used to determine cell cycle durations (Stallwood, 1974).

Methylxanthines inhibit 3',5'-cyclic monophosphate phosphodiesterase; this enzyme hydrolyzes cyclic-AMP to 5'-AMP. Thus, treatment with methylxanthines may result in an increase in intracellular levels of cyclic-AMP due to the inhibition of phosphodiesterase (Abell and Monahan, 1973). Elevated levels of cyclic-AMP within cells may arrest cells in G_1 (Froehlich and Rachmeler, 1974; Coffino et al., 1975) or G_2 (Millis, 1973; Stambrook et al., 1976), inhibit cell growth or induce cell death (Bourne et al., 1975). In addition, methylxanthines have effects that are independent of their action on phosphodiesterase and intracellular levels of c-AMP; they disturb cell proliferation (Bourne

et al., 1975) and they induce chromatid stickiness and the formation of tetraploid nuclei (Stallwood, 1974).

Primary root meristems were treated with two methylxanthines, EOC and 3-isobutyl-1 methylxanthine (IBMX), in order to determine whether or not binucleate cells in these meristems would complete a cell cycle (Fig. 1). It was found that EOC induced binucleate cells did not come back into division while IMBX induced binucleate cells did divide. The proportion of binucleate cells that completed a cell cycle and its duration were determined. This labelled subpopulation of cycling cells was used to study nuclear growth. Furthermore, since a binucleate cell contains two sister nuclei resulting from the same mitosis, the relative rates of growth of sister nuclei in a common cytoplasm could be determined. In the present study, nuclear volumes were determined as binucleate cells proceeded through the cell cycle.

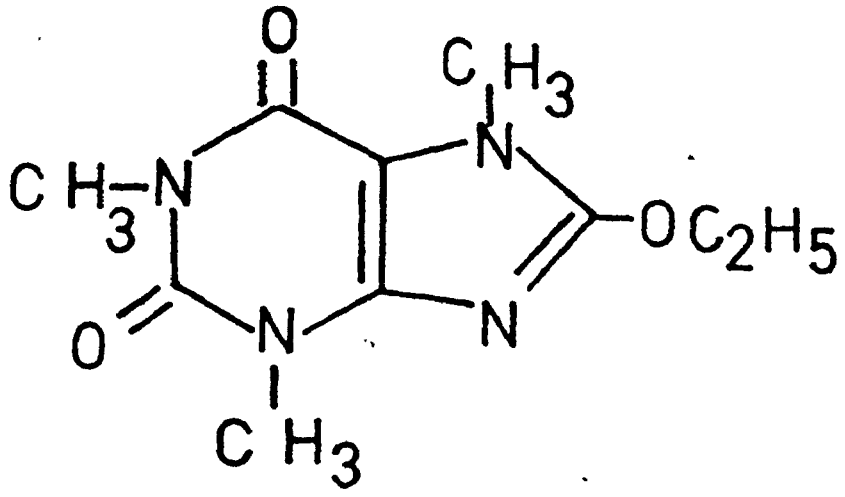
Heterogeneity in nuclear morphology has been described in root meristems; nuclei can be classified into four shapes: spheroids, nearly spheroids, oval and elongate (Bansal, 1975). The frequencies of nuclei of different shapes were also determined in the binucleate cell population as they proceeded through the cycle.

Figure 1. Structure of Methylxanthines:

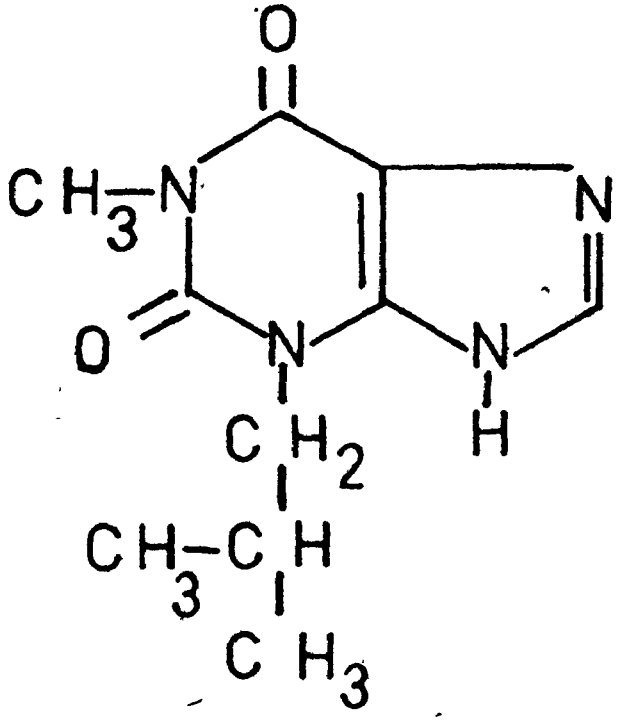
(a) 8-ethoxycaffeine

(b) 3-isobutyl-1-methylxanthine

(a)



(b)



MATERIALS AND METHODS

After soaking Vicia faba L. seeds in distilled water for 24 hours, the testas were removed, the beans were soaked in distilled water and then suspended in plexiglass tanks containing distilled water (pH 7.0 $\pm$ 0.1), which was continuously aerated and changed every 12 hours. The growth chamber was maintained at 21°C $\pm$ 1°C and kept dark. Primary roots were obtained 64 to 90 hours after the initial suspension of beans into the growth tanks.

1. Treatments

Beans were treated with 5×10^{-3} M 8-ethoxycaffeine (EOC) or 3-isobutyl-1-methylxanthine (IBMX) solutions for 1 hour, then washed carefully with distilled water, transferred to tanks of distilled water. Whole primary roots were fixed at the end of treatment and one hour from the end of treatment. Whole primary roots were also fixed every hour from 13 to 21 hours from the end of treatment. All treatments were carried out in the growth chamber; both the treatment and solutions and the distilled water used was maintained at 21°C $\pm$ 1°C.

2. Fixation, staining and preparation of primary root meristems

Whole primary roots were fixed in 20 ml. of a chilled mixture of absolute alcohol and glacial acetic acid in a ratio of 3:1 (v/v) containing 2-3 drops of formaldehyde. Roots were washed for 1 hour in 3 or 4 changes of distilled water and hydrolyzed in 1NHCl at 60°C for $\pm$ 1°C for 10 minutes. They were stained for 1 hour in Feulgen's reagent.

The root cap was removed from each primary root. The apical 1 mm. of the meristem was removed and cut into thirds or quarters depending upon the size of the root; each segment was made into a permanent preparation. The meristem was dissected into columns of cells using needles with fine points. Malachite green, which was added as a counterstain, helped define cell wall boundaries.

3. Scoring of slides

At each fixation time, 5,000 cells were scored per root meristem. Samples were taken equally from each of the 3 or 4 slides to minimize any heterogeneity within the different sections of the root meristem. Mitotic indices and the frequencies of binucleate cells, tetraploid cells and abnormal cells were determined for the same 5,000 cells scored per root at each hour.

4. Nuclear volumes and shapes

Both nuclear volume and nuclear morphology were determined in the binucleate cell population induced by either EOC or IBMX treatment in primary root meristems. Using phase-contrast microscopy, major (long) and minor (narrow) axes were measured using a Zeiss ocular micrometer calibrated against a stage micrometer. The measurement of nuclear dimensions was performed on 150 binucleate interphase cells at the end of treatment and 1, 13 and 15 hours from the end of treatment. Prophase nuclei were measured in 150 binucleate cells and in 300 uninucleate cells 15 hours from the end of IBMX treatment.

5. Calculations of nuclear volumes

Nuclear volume (V) was determined as follows:

(i) Spherical nuclei

$$V = 4/3\ \pi\ r^3$$

r = 1/2 diameter

(ii) Nearly-spherical nuclei

$$V = 4/3\ \pi\ ab^2$$

(iii) Oval nuclei

$$V = 4/3\ \pi\ ab\left(\frac{a+b}{2}\right)$$

(iv) Elongated nuclei

$$V = (4/3\ \pi\ b^3) + (4/3\ \pi\ ab^2)$$

a = 1/2 major axis

b = 1/2 minor axis

6. Nuclear morphology

Nuclei were classified into four different morphological classes, according to their shape.

class I Spherical

class II Nearly-spherical

class III Oval

class IV Elongate

7. Calculation cumulative percentage frequency of nuclear volumes

The percentage cumulative frequency of nuclear volumes were computed. The volumes were ranked into an ascending order and the

cumulative percentage frequency associated with a particular volume (P_{xi}) was then computed as follows:

$$P_{xi} = \frac{2i-1}{2N} \times 100$$

i = rank

xi = nuclear volume at rank i

P_{xi} = cumulative percentage frequency associated with xi

N = number of observations

8. Probit analysis of nuclear volumes

Cumulative percentage frequencies of nuclear volumes were plotted as a function of linear and log values of nuclear volumes. Where a plot of nuclear volumes on probability scale generates a straight line, it indicates that the sample plotted represents a normally distributed population (Sokal and Rohlf, 1969).

9. Statistical analyses

9.1 Empirical Distribution Function Test

A test of goodness of fit of values of nuclear volumes to normal distribution was made using statistics based on the empirical distribution function, EDF (Stephens, 1974). The statistics used in this test are modified statistics, D, W^2, U^2 and A^2. They were modified in order to ignore sample sizes.

9.2 The G test was performed on 150 pairs of binucleate nuclei obtained either 1 hour or 13 hours after a 1 hour treatment of EOC or IBMX, respectively. Where the frequency of one class was < 5 it

was combined with a class of nuclei which most resembled it in shape, since the test does not work well with classes having < 5 values (Sokal and Rohlf, 1969). In addition, the test of proportions was performed on the same samples of nuclei. Both the frequencies of class I nuclei, during interphase, and class IV nuclei, during late interphase, were considered too low to be used for statistical purposes (Table 12). Both these tests were used to determine if the frequency of pairs of sister nuclei with identical shapes were significantly different from that expected on the basis of random assortment.

RESULTS

1. Methylxanthines: 1 Hour Treatment and 1 Hour Recovery

Treatment with EOC or IBMX for 1 hour resulted in the formation of binucleate cells in young primary roots of Vicia faba. EOC treatment yielded 52 binucleate cells per 5,000 cells scored, a frequency of 1.0%. With IBMX, 2.4% of the cells were binucleate (Table 1). IBMX, is the more effective drug; it produces 2.3 times more binucleate cells than EOC. After recovery for 1 hour, the frequency of binucleate cells was slightly reduced to 0.7% in EOC treated roots but was still 2.6% after IBMX treatment (Table 1).

EOC also induced chromatid stickiness; this prevented chromatid segregation, led to the appearance of sticky anaphases, telophases and interphase figures and gave rise to some tetraploid nuclei. The frequency of cells affected in this way, at the end of treatment, was 0.7% (Table 1); IBMX did not induce chromatid stickiness.

Both EOC and IBMX treatments reduced the mitotic index (M.I.) to the same extent. Following EOC treatment, mean M.I. $\pm$ S.D. was 3.7 $\pm$ 0.9; after IBMX treatment, mean M.I. $\pm$ S.D. was 3.9 $\pm$ 0.1. At the end of a one hour recovery from either treatment, M.I. had increased. However, after recovery from EOC, mean M.I. $\pm$ S.D. was 4.6 $\pm$ 1.0, i.e. lower than that, 6.5 $\pm$ 0.3, of control roots, while roots treated with IBMX recovered more rapidly and showed a mean M.I. $\pm$ S.D., of 6.9 $\pm$ 0.7 (Table 2).

TABLE 1

Number of affected (A.C.) and binucleate cells (BN) per 5,000 cells scored per root at each fixation time after 5×10^{-3} M treatment (one hour). This data is from the same roots as those scored for Table 2. EOC affected cells include sticky anaphases, sticky telophases, sticky interphases and tetraploid nuclei.

t - time from the beginning of treatment, in hours.

TABLE 1

		EOC			IBMX		CONTROL	
t	A.C.	%A.C.	BN	%BN	BN	%BN	BN	%BN
1	26	0.5	52	1.0	139	2.8	0	
	42	0.8	54	1.1	122	2.5	1	0.02
	40	0.8	49	1.0	105	2.1		
Mean	36	0.7	52	1.0	122	2.4		
1 + 1	58	1.2	34	0.7	130	2.6	1	0.02
	38	0.8	38	0.8	117	2.3	1	0.02
	70	1.4	36	0.7	137	2.7		
Mean	55	1.1	36	0.7	128	2.6		

TABLE 2

Primary roots of *V. faba* treated with 5×10^{-3} M EOC or IBMX for 1 hour followed by a 1 hour recovery.

TABLE 2

Normal Primary Roots

t	P	M	A	T	M.I. ± S.D.
1	230	58	21	18	
	215	54	21	23	
1 + 1	241	47	23	28	
	226	50	28	22	
Mean	228	52	22	23	6.5 ± 0.3

IBMX

t	P	M	A	T	M.I. ± S.D.
1	158	11	8	16	
	167	8	6	21	
	139	16	15	33	
Mean	155	12	10	20	3.9 ± 0.1
1 + 1	239	47	16	11	
	236	61	32	17	
	320	16	15	33	
Mean	265	41	21	20	6.9 ± 0.7

EOC

t	P	M	A	SA	T	ST	SI	M.I. ± S.D
1	87	16	6	8	19	6	13	
	156	19	3	16	27	10	16	
	115	17	4	12	23	7	13	
Mean	119	17	4	12	23	8	14	3.7 ± 0.9
1 + 1	128	28	6	10	5	15	33	
	152	23	2	10	16	12	16	
	170	45	5	16	27	23	31	
Mean	150	32	4	12	16	17	27	4.6 ± 1.0

Within one hour, prophases showed the greatest absolute drop in number; they decreased by 109 in EOC treated roots and 73 after IBMX treatment, as compared to the mean values found in control roots (Table 2). These values are 48% and 32% respectively, of control levels. Metaphases also dropped in number, 35 after EOC treatment and 40 in IBMX treated roots; these values represent decreases of 67% and 77% respectively, of the mean values found in control roots (Table 2). EOC continued to depress the mitotic and in particular, the prophase indices for a further hour with additional accumulations of sticky telophases, whereas, IBMX treated roots showed a rapid recovery of mitotic activity with both the mitotic and phase indices similar to those of control roots (Tables 2 and 3).

The drop in M.I. within 1 hour, therefore mainly results from a fall in the prophase index. This shows that both methylxanthines have an immediate effect on cells in G_2 and delay their entry into prophase. With EOC, this effect continues for a further hour while with IBMX, cells show a rapid recovery.

2. Nuclear Growth in Binucleate Cells

Volumes of nuclei of binucleate cells were determined at the end of a 1 hour treatment and after 1, 13 and 15 hours recovery. Binucleate cells induced by IBMX completed interphase and underwent mitosis; measurements of their nuclear volumes, therefore, provide an estimate of their growth as they proceed through interphase. EOC induced binucleate cells, however, did not divide. They provide evidence on the growth of nuclei that will not go on to divide.

TABLE 3

Mitotic and phase indices at the end of a 1 hour treatment of 5×10^{-3} M EOC or IBMX followed by a 1 hour recovery. This data is from the same roots as those scored for Table 2. Mean values per root were used.

t - time from the beginning of treatment, in hours.

TABLE 3

	IBMX		EOC		CONTROL
t	1	1 + 1	1	1 + 1	
Mitotic Index	3.9	6.9	3.7	4.6	6.5
Prophase Index	3.1	5.3	2.4	3.0	4.6
Metaphase Index	0.2	0.8	0.3	0.6	1.0
Anaphase Index	0.2	0.4	0.3	0.3	0.4
Telophase Index	0.4	0.4	0.6	0.7	0.5

2.1 IBMX: One Hour Treatment

At the end of treatment, nuclei showed a 7.3 - fold range in volume, from 249 to 1836 μm^3; the mean volume was 707 $\pm$ 284 μm^3 and the median volume was 641 μm^3. The range, $\pm$ 1 S.D. on probit plot was 452 - 966 μm^3, i.e. a 2.1 - fold range in volume (Table 4). The nuclei in binucleate cells must be just entering interphase, i.e. early G_1. Even in early G_1, a large range of volumes occurs in nuclei; the lower 50% of the values extend over a range of only 392 μm^3, i.e. 249 - 641 μm^3; while the upper 50% cover a range of 1195 μm^3, i.e. 641 - 1836 μm^3. The spread of these values show that some nuclei grow disportionately large, relative to others in the population. This further suggests that either large differences in nuclear volumes occur when nuclei first enter G_1 or that large differences in rates of increase in nuclear volumes occur when nuclei enter G_1. Since fast, slow and non cycling cells may be represented in this binucleate cell population, such volume differences could result from the heterogeneity in cell cycle duration.

Both linear (Fig. 2) and log (Fig. 3) transformed values of nuclear volumes fail, on probit plots, to yield one straight line. These graphs reveal that nuclear volumes are neither linear nor log normally distributed. Tests using EDF statistics show that nuclear volumes do not follow a linear (Tables 5.1 and 5.2) or a log (Tables 5.1 and 5.3) normal distribution. At the beginning of interphase, it appears that nuclei from binucleate cells do not constitute a single, normally distributed population.

TABLE 4

Mean, median and range of volumes of interphase and prophase nuclei from IBMX induced binucleate cells; 300 nuclei were measured for each fixation time. The median value is taken from the 50 percent point on a probit plot of log values of nuclear volumes. The range $\pm$ 1 S.D. covers the values between the 15.9 and 84.1 percent points; $\pm$ 2 S.D. covers the values between 2.3 and 97.7 percent points. Primary roots of V. faba were treated with 5×10^{-3} M IBMX for 1 hour followed by up to 15 hours recovery.

TABLE 4

Nuclear Volumes (μm^3)

Treatment (hours)	Type of Nucleus	Range	Mean ± S.D.	Median	Range between ± 1 S.D.	Range between ± 2 S.D.	Difference between Intervals: -2 + -1 S.D.	-1 S.D. & Median	Median & + 1 S.D.	+1 & +2 S.D.
1	interphase	249-1836	707±284	641	452-966	324-1479	128	189	325	513
1 + 1	interphase	249-2100	575±284	468	351-861	299-1288	52	117	393	427
1 + 13	interphase	279-1031	545±131	531	427-676	355-861	72	104	145	185
1 + 15	interphase	427-1211	827±151	822	676-989	501-1138	175	146	166	149
1 + 15	binucleate prophase	742-2470	1320±297	1267	1023-1640	851-1995	172	244	373	356
1 + 15	uninucleate prophase	713-2591	1343±238	1318	1122-1569	933-1862	189	196	250	294
Control All Stages	interphase	230-2251	827±343	770	500-1157	280-1528	220	270	387	371
Control	prophase	780-2830	1592±388	1565	1200-1960	885-2430	315	365	395	470

Figure 2. Cumulative percent frequency on a probability scale of nuclear volumes from IBMX induced binucleate cells.

● 1 At the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

■ 1 + 1 1 hour from the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

▲ 1 + 13 13 hours from the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

▲ 1 + 15 15 hours from the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

● 1 + 15 15 hours from the end of a 1 hour treatment with IBMX; based on 300 prophase nuclei of binucleate cells.

■ 1 + 15 15 hours from the end of a 1 hour treatment with IBMX; based on 300 prophase nuclei of mononucleate cells.

○ Control untreated roots, same age as treated roots, 1 + 15; based on 300 interphase nuclei of mononucleate cells. This data is from the same control roots as those scored for Figure 4.

□ Control untreated roots, same age as treated roots, 1 + 15; based on 300 prophase nuclei of mononucleate cells.

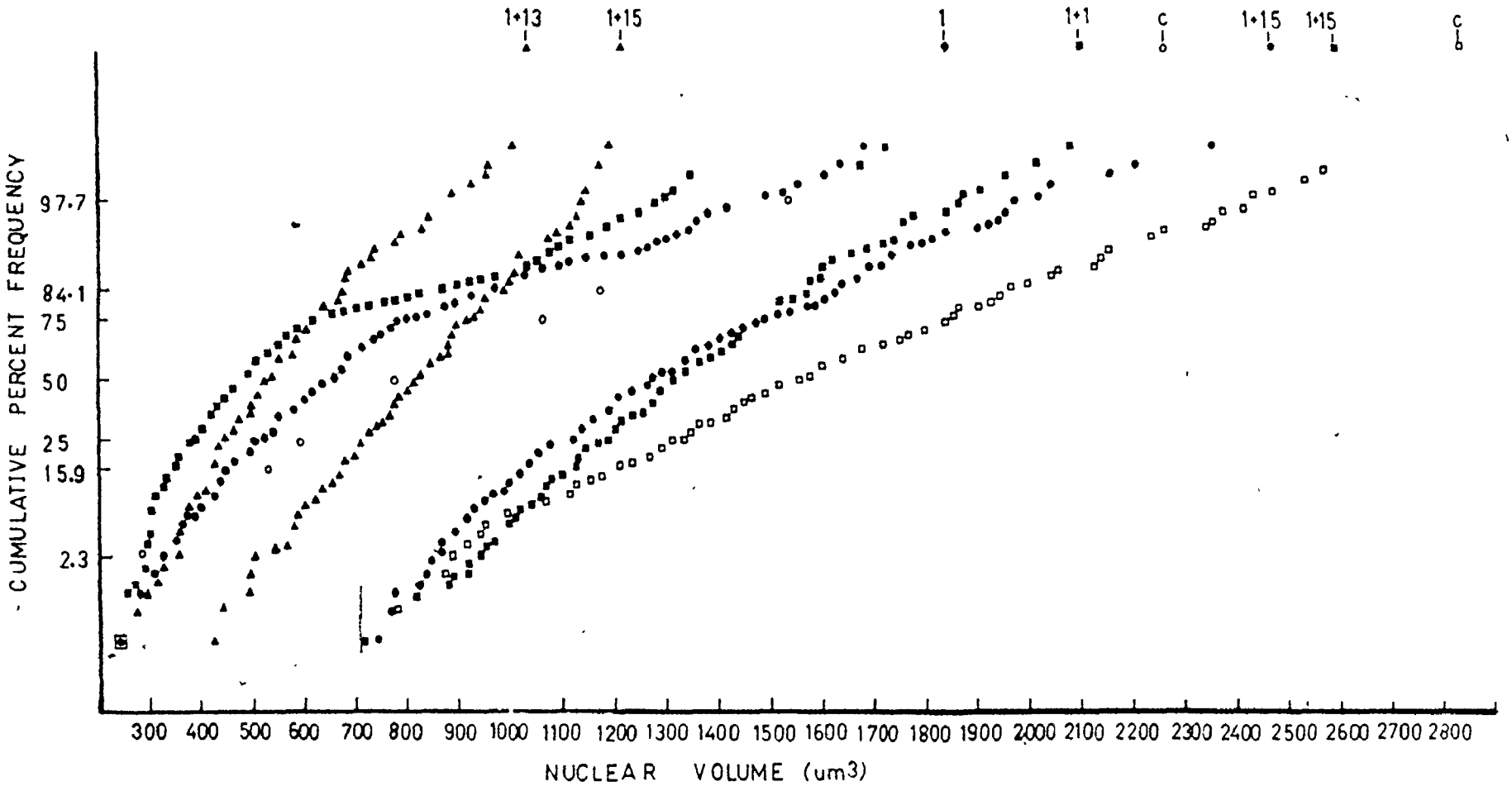

1·13
1·15
1
1·1
C
1·15
1·15
C
CUMULATIVE PERCENT FREQUENCY
97·7
84·1
75
50
25
15·9
2·3
300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200 2300 2400 2500 2600 2700 2800
NUCLEAR VOLUME (um3)

Figure 3. Cumulative percent frequency on a probability scale of log values of nuclear volumes from IBMX induced binucleate cells.

● 1 At the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

■ 1 + 1 1 hour from the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

▲ 1 + 13 13 hours from the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

▲ 1 + 15 15 hours from the end of a 1 hour treatment with IBMX; based on 300 interphase nuclei.

● 1 + 15 15 hours from the end of a 1 hour treatment with IBMX; based on 300 prophase nuclei of binucleate cells.

■ 1 + 15 15 hours from the end of a 1 hour treatment with IBMX; based on 300 prophase nuclei of mononucleate cells.

○ Control untreated roots, same age as treated roots, 1 + 15; based on 300 interphase nuclei of mononucleate cells. This data is from the same control roots as those scored for Figure 5.

□ Control untreated roots, same age as treated roots, 1 + 15; based on 300 prophase nuclei of mononucleate cells.

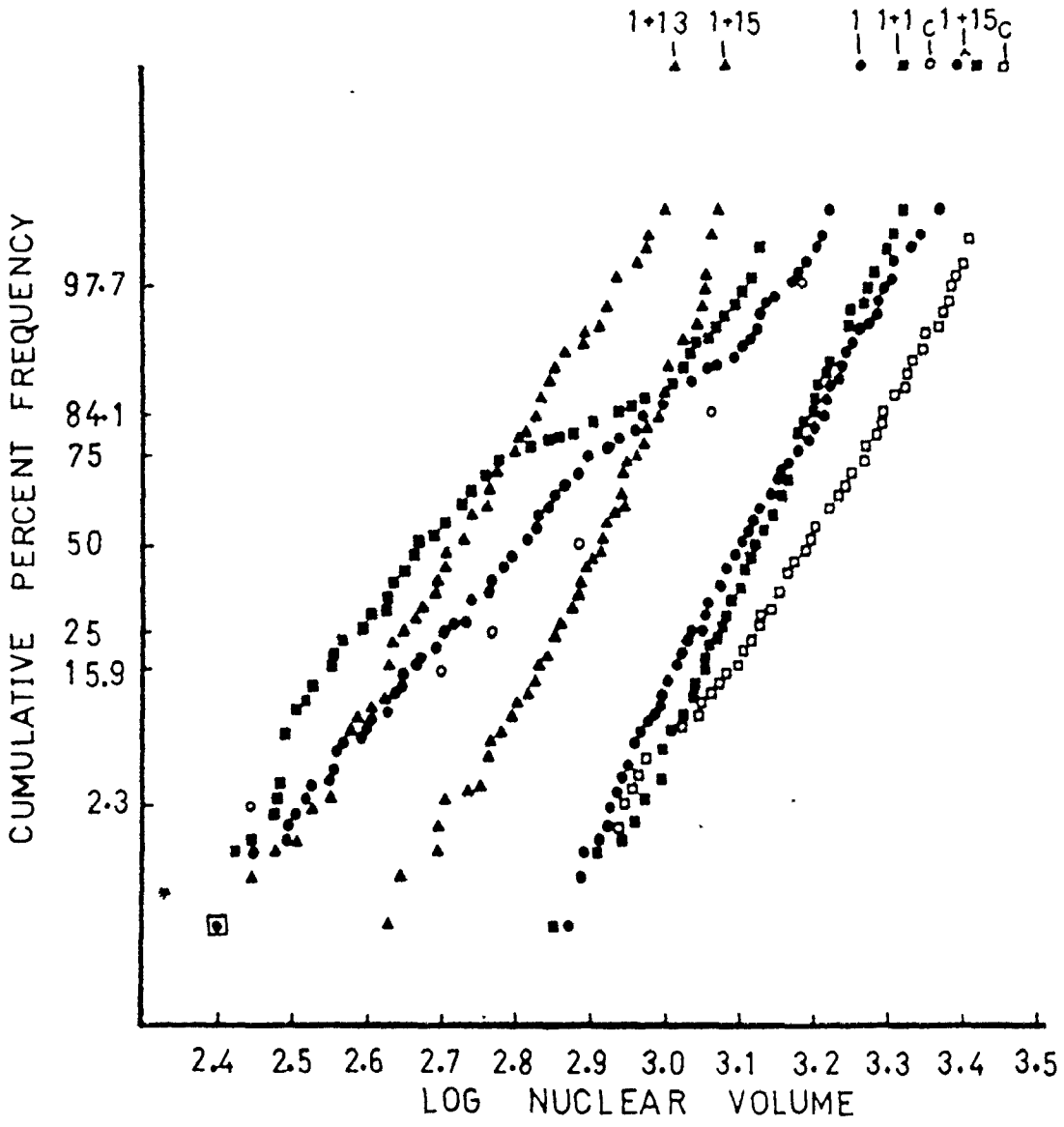

1+13
1+15
1
1+1c
1+15c
CUMULATIVE PERCENT FREQUENCY
97.7
84.1
75
50
25
15.9
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
3.5
LOG NUCLEAR VOLUME

TABLE 5.1

Percentage points for a test of normality (μ and σ^2 unknown) based on the empirical distribution function. The statistics used in the test are D, W^2, U^2 and A^2. (5% level is commonly accepted level of significance)

TABLE 5.1

Test Statistic	Critical Percentage Points				
	15.0	10.0	5.0	2.5	1.0
D	0.775	0.819	0.895	0.955	1.035
W^2	0.091	0.104	0.126	0.148	0.178
U^2	0.085	0.096	0.116	0.136	0.163
A^2	0.576	0.656	0.787	0.918	1.092

TABLE 5.2

Results of the empirical distribution function (EDF) test of goodness of fit to one linear normal distribution for the interphase and prophase nuclear volumes of primary meristems of V. faba treated with 5×10^{-3} M IBMX or EOC for 1 hour followed by up to 15 hours recovery.

TABLE 5.2

IBMX Treatment	Type of Nuclei	D	W^2	U^2	A^2
1	interphase	2.471	1.605	1.276	9.431
1 + 1	interphase	3.616	3.767	3.181	20.675
1 + 13	interphase	1.632	0.486	0.370	3.180
1 + 15	interphase	0.939	0.075	0.073	0.491
1 + 15	binucleate prophase	1.483	0.452	0.350	2.631
1 + 15	mononucleate prophase	0.925	0.144	0.113	1.015
EOC Treatment					
1	interphase	2.016	0.853	0.663	5.338
1 + 1	interphase	1.409	0.489	0.366	3.201
1 + 13	interphase	1.759	0.617	0.510	3.512
1 + 15	interphase	1.631	0.639	0.514	4.139

TABLE 5.3

Results of the empirical distribution function (EDF) test of goodness of fit to one log normal distribution for the interphase and prophase nuclear volumes of primary meristems of V. faba treated with 5×10^{-3} M IBMX or EOC for 1 hour followed by up to 15 hours recovery.

TABLE 5.3

IBMX Treatment	Type of Nuclei	D	W^2	U^2	A^2
1	interphase	1.181	0.222	0.197	1.322
1 + 1	interphase	1.962	1.023	0.815	6.416
1 + 13	interphase	0.987	0.115	0.111	0.726
1 + 15	interphase	1.168	0.174	0.139	1.095
1 + 15	binucleate prophase	0.725	0.079	0.073	0.461
1 + 15	mononucleate prophase	0.819	0.071	0.069	0.477
EOC Treatment					
1	interphase	0.926	0.133	0.127	0.814
1 + 1	interphase	0.696	0.0785	0.0785	0.534
1 + 13	interphase	1.199	0.216	0.204	1.225
1 + 15	interphase	1.416	0.279	0.259	1.690

2.2 IBMX: One Hour Treatment + One Hour Recovery

There was a 8.4 - fold range of nuclear volumes, from 249 - 2100 μm^3. Though the range of volumes is slightly greater than that found 1 hour after treatment, relatively few nuclei, the upper 2.3% of the population, have values that cover the range 1289 - 2100 μm^3. Futhermore, mean nuclear volume has fallen from 707 $\pm$ 284 μm^3 to 574 $\pm$ 284 μm^3 (Table 4). It appears that most of the nuclei are contracting. From a probit plot of log transformed nuclear volumes we see that all nuclei had reduced volumes; the greatest reductions occurred in the lower 80% of the values, when compared to those values found in corresponding percentiles, after a 1 hour treatment (Fig. 3). Graphic and statistical analyses indicate that values for nuclear volumes do not form a linear or log normal distribution, which confirms that these nuclei do not form a single uniform population.

2.3 IBMX: One Hour Treatment + 13 Hours Recovery

Nuclei showed a 3.7 - fold range in volume i.e. 279 - 1031 μm^3 and the mean volume was 545 + 131 μm^3. The spread of values at 1 + 13 hours is considerably smaller than that at 1 hour, i.e. 752 cf. 1587 μm^3 or at 1 + 1 hours, i.e. 752 cf. 1851 μm^3. This decrease, therefore, is due largely to the absence of the nuclei whose volumes ranged from 1031 to 1836 or 2100 μm^3. It is suggested that these nuclei underwent contraction. However, most nuclei, i.e. those lying below the 78th percentile on probit plots (Figs. 2 and 3), have increased in volume. Thus unequal growth rates are still present in the binucleate cells.

On a probit plot, linear values of nuclear volumes (Fig. 2) did not fall into one straight line, and appear not to follow a linear normal distribution. Tests using EDF statistics showed that the values were significantly different ($0.01 < p < 0.025$) from a linear normal population. A probit plot of log values of nuclear volumes (Fig. 3) revealed that the distribution formed a straight line for 92% of the values, i.e. 1^{st} - 93^{rd} percentiles; this suggested that these values represented a one log normally distributed population. All EDF statistics, except D, agreed that the distribution of log values did not deviate significantly from a one log normal distribution, at the 5% level, the level of significance for these tests. The D statistic indicated that these values did significantly differ from a log normal population, at the 1% level. Therefore, on the whole, statistical analyses indicated that these nuclear volumes seemed to form a log normally distributed population.

In the 12 hour recovery period, from 1 to 13 hours, the larger nuclei have contracted but many of the smaller nuclei have undergone some growth. This is accompanied by a change in the form of the distribution of nuclear volumes; they take on a log normal distribution. Overall, there is not a great deal of nuclear growth, since mean nuclear volume decreases slightly, 575 to 545 μm^3; nevertheless some fundamental change occurs that converts the population into one whose values are now log normally distributed.

2.4 IBMX: One Hour Treatment + 15 Hours Recovery

Nuclei showed a 2.8 - fold range of values, 427 to 1211 μm^3, and the mean volume was 827 $\pm$ 151 μm^3. The important difference between nuclei at the 15 hours and 13 hours recovery was the presence at 15 hours of binucleate cells in mitosis (Table 6.1). Thus, the interphase nuclei at 15 hours are only part of the total population of binucleate cells, i.e. 25%, they represent the fraction that had not entered prophase or, perhaps would never divide. Their volumes, therefore, are from a residual group of cells whose nuclei may have been smaller than those that entered prophase.

Both graphic and statistical analyses agreed that nuclear volumes were not log normally distributed. However, a probit plot of linear values of nuclear volumes suggested that values for about 83% of the nuclei, from the 5^{th} to 88^{th} percentiles, fitted a linear normal distribution. The W^2, U^2 and A^2 statistical tests agreed, at a 15% level, that nuclear volumes were linear normally distributed, whereas the D statistic rejected ($0.05 < p < 0.025$) that values for nuclear volumes followed a linear normal distribution (Tables 5.1 and 5.2).

2.5 IBMX: One Hour + 15 Hours Recovery: Prophase Nuclei

Prophase nuclei in binucleate cells had a mean volume of 1320 $\pm$ 297 μm^3 and they showed a 3.3 - fold range in values, i.e. 742 to 2470 μm^3. Probit plots of linear values did not give a straight line and three of the EDF statistics confirmed that prophase nuclear

TABLE 6.1

Primary roots of *V. faba* treated with 5×10^{-3} M IBMX for 1 hour followed by up to 21 hours recovery.

TABLE 6.1

IBMX Treatment	Diploid Cells P M A T	Binucleate Cells I P M A T	Tetraploid Cells I P M A T	M.I. + S.D.	%BN(I)	%BN(M)
1 + 13	362 92 40 63	77 - - - -	- - - - -	9.8 ± 2.3	1.54	
1 + 15	285 79 29 52	41 67 6 2 1	4 - 4 3 -	12.3 ± 1.0	0.82	1.52
1 + 17	193 52 23 50	85 10 1 - -	2 - - - -	6.8 + 1.5	1.70	0.22
1 + 19	216 61 24 51	83 - - - -	- - - - -	7.0 ± 1.1	1.66	
1 + 21	147 37 19 35	79 - - - -	- - - - -	4.8 ± 0.5	1.58	

TABLE 6.2

Primary roots of V. faba treated with 5×10^{-3} M EOC for 1 hour followed by up to 21 hours recovery.

TABLE 6.2

EOC Treatment	Diploid Cells P	M	A	T	Binucleate Cells I	Tetraploid Cells I	M.I. ± S.D.	%BN(I)
1 + 13	274	69	30	60	33	-	8.7 ± 2.0	0.66
1 + 15	279	73	34	72	23	2	9.2 ± 1.4	0.46
1 + 17	221	50	20	48	17	-	6.8 ± 1.0	0.34
1 + 19	158	38	28	19	8	-	4.9 ± 0.8	0.16
1 + 21	169	46	22	43	5	-	5.6 + 1.9	0.10
Control	209	76	39	60			7.7 ± 0.6	

volumes are not normally distributed; only the U^2 statistic did not reject, at the 5% level, the hypothesis that the volumes were normally distributed. Log transformed values, however, gave a straight line for most of the volumes, i.e. 5^{th} to 70^{th} percentiles, and tests with EDF statistics indicate that the values form a log normal distribution (acceptable at the 15% level of significance).

Prophase nuclei volumes were also determined for normal, i.e. mononucleate cells. Mean nuclear volume was $1343 \pm 238\ \mu m^3$ and the values were not significantly different, at the 5% level, from a log normal distribution (EDF statistics; Fig. 3).

The binucleate cells seen in mitosis 15 hours after the end of treatment are the first marked cells to complete interphase. They do so in 14-15 hours (i.e. 15 hours minus 1 hour for prophase from the end or 16 hours from the beginning of treatment).

This is close to the durations reported for fast cycling cells of primary roots, i.e. 15 hour (MacLeod, 1971) and 16.5 hours (Van't Hof, 1967). Several conclusions can be reached concerning this marked population of fast cycling cells.

1. From 13 to 15 hours, these cells must have been in G_2. In this period, mean volume of interphase nuclei increases from 545 to 827 μm^3. This is a 52% increase in volume. Also, the nuclei of these treated cells undergo almost all their growth in the last two hours of interphase (Table 4).

2. The 52% increase in nuclear volume from 13 to 15 hours is probably an underestimate of their growth since the volumes of the prophase

nuclei are even larger than those of interphase nuclei. Their mean volume is 1320 ± 297 μm^3; 51% of these nuclei are larger than the largest interphase nucleus of a binucleate cell at 15 hours and 77% are larger than the largest interphase nucleus at 13 hours.

3. Prophase nuclei, like interphase nuclei, also show a wide spread of values, i.e. a 3.3 - fold range (742 to 2470 μm^3). They are as variable, in volume, as interphase nuclei.

4. No binucleate cells were seen in division at 13 hours while at 15 hours, 65.0% were dividing and at 17 hours, 11.3% were dividing (Table 6.1). Therefore, if we allow a period of 2 hours for mitosis, it appears that about 75% of the cells that were in mitosis at the time of treatment and that were induced to become binucleate have completed a cell cycle in the time taken by normal fast cycling cells.

If there were no other effects induced by IBMX, this result indicates that about 75% of the mitotic cells affected by IBMX have remained as fast cycling cells over the cell cycle following treatment.

2.6 EOC: One Hour Treatment

Nuclear volumes in binucleate cells showed a 7 - fold spread of values, 140 - 978 μm^3, and the mean volume was 357 ± 127μ m^3 (Table 7). Neither linear nor log transformed values of nuclear volume (Figs. 4 & 5), yielded a straight line on a probit plot and tests using EDF statistics show that volumes were significantly different from a linear or a log normal distribution ($0.025 < p < 0.05$).

TABLE 7

Mean, median and range of volumes of interphase nuclei from EOC induced binucleate cells; 300 nuclei were measured for each fixation time. The median value is taken from the 50 percent point on a probit plot of log values of nuclear volumes. The range $\pm$ 1 S.D. covers the values between 15.9 and 84.1 percent points; $\pm$ 2 S.D. covers the values between 2.3 and 97.7 percent points. Primary roots of *V. faba* were treated with 5×10^{-3} M EOC for 1 hour followed by up to 15 hours recovery.

TABLE 7

Nuclear Volumes (μm^3)

Treatment (hours)	Type of Nucleus	Range	Mean ± S.D.	Median	Range between ± 1 S.D.	Range between ± 2 S.D.	Difference between Intervals: -2 + -1 S.D.	-1 S.D. & Median	Median & + 1 S.D.	+1 & +2 S.D.
1	interphase	140-978	357±127	330	246-462	176-638	70	84	133	176
1 + 1	interphase	265-1763	649±237	608	426-879	309-1230	118	182	271	351
1 + 13	interphase	302-1038	576±134	543	466-710	366-916	100	78	166	207
1 + 15	interphase	319-1575	726±221	675	507-938	429-1279	79	168	263	342
Control All Stages	interphase	230-2251	827±343	770	500-1157	280-1528	220	270	387	371
Control	prophase	780-2830	1592±388	1565	1200-1960	885-2430	315	365	395	470

Figure 4: Cumulative percent frequency on a probability scale of nuclear volumes from EOC induced binucleate cells.

● 1 At the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

◕ 1 + 1 1 hour from the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

▲ 1 + 13 13 hours from the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

■ 1 + 15 15 hours from the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

○ Control untreated roots, same age as treated roots, 1 + 15; based on 300 interphase nuclei of mononucleate cells.

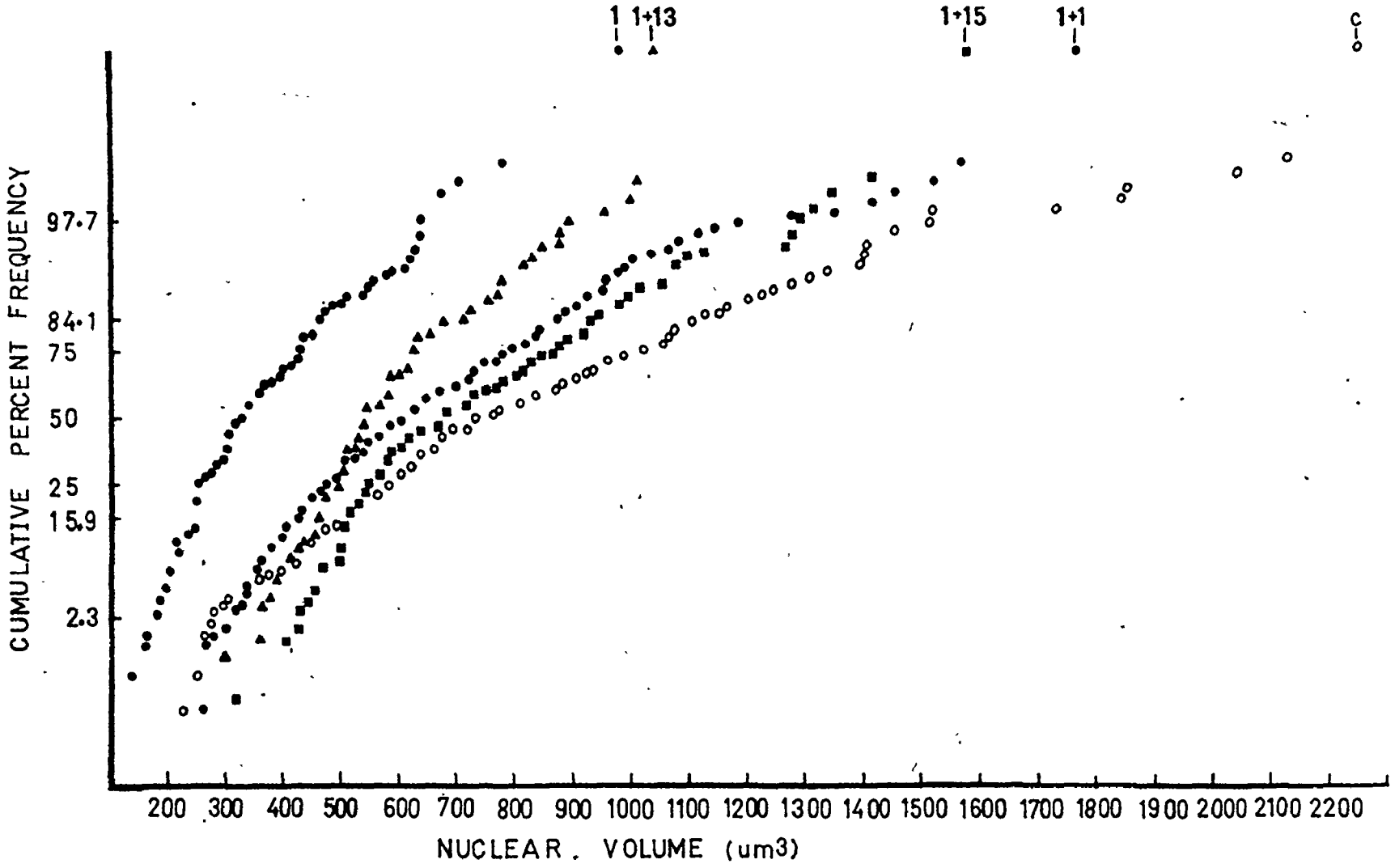

1
1+13
1+15
1+1
C
CUMULATIVE PERCENT FREQUENCY
97.7
84.1
75
50
25
15.9
2.3
200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200
NUCLEAR . VOLUME (um3)

Figure 5. Cumulative percent frequency on a probability scale of log values of nuclear volumes from EOC induced binucleate cells.

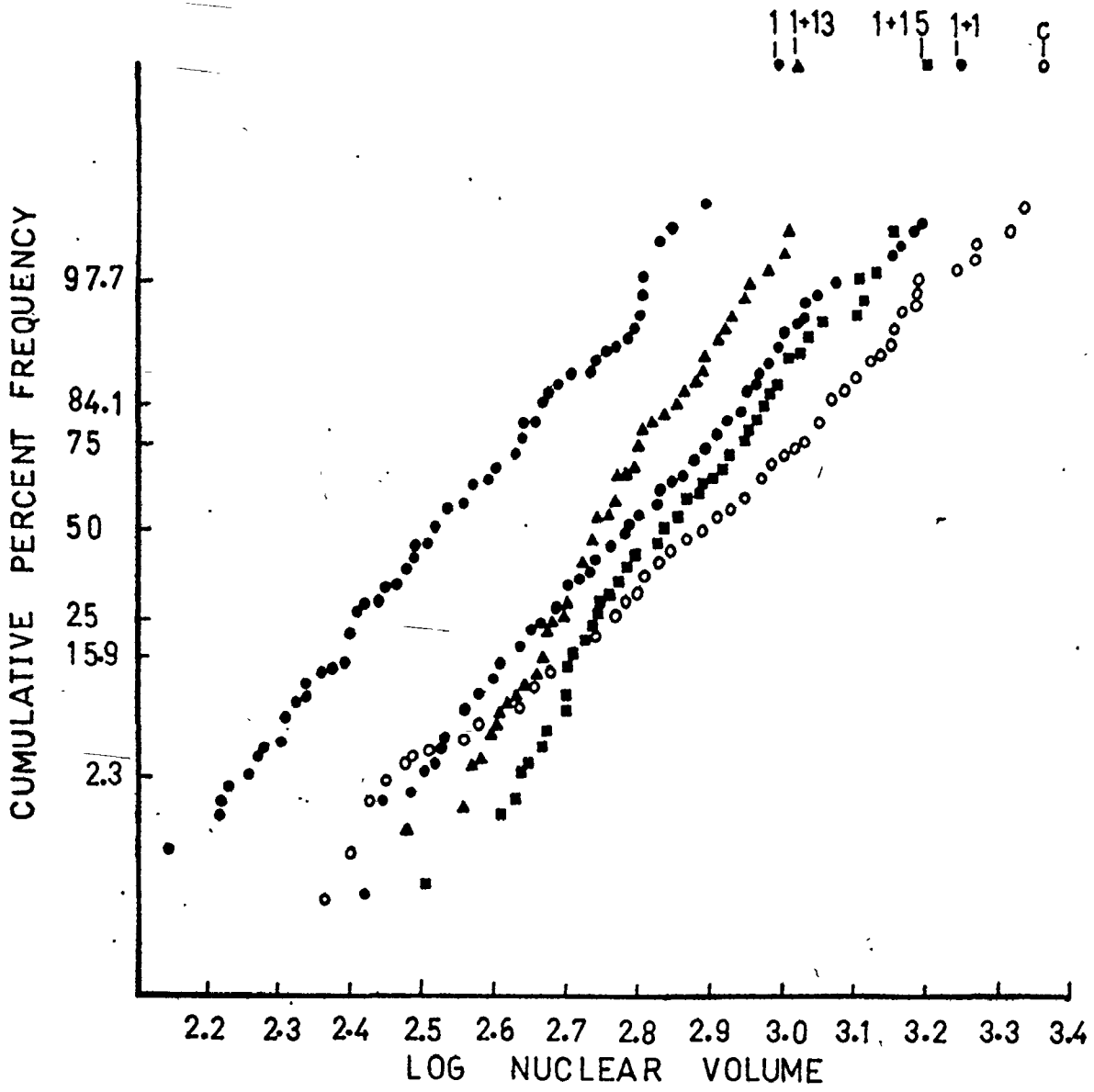
● 1 At the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

● 1 + 1 1 hour from the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

▲ 1 + 13 13 hours from the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

■ 1 + 15 15 hours from the end of a 1 hour treatment with EOC; based on 300 interphase nuclei.

○ Control untreated roots, same age as treated roots, 1 + 15; based on 300 interphase nuclei of mononucleate cells.


1
1+13
1+15
1+1
C
CUMULATIVE PERCENT FREQUENCY
97.7
84.1
75
50
25
15.9
2.3
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
3.1
3.2
3.3
3.4
LOG NUCLEAR VOLUME

Though the spread of nuclear volumes is almost as great after EOC as after IBMX treatment, 7 - fold cf. 7.3 - fold, mean nuclear volume in binucleate cells induced by EOC is only half that in IBMX induced binucleate cells (Tables 4 and 7). EOC, it appears, inhibits nuclear growth in very early G_1.

In the first hour of recovery, the nuclei grow; mean nuclear volume increases to 649 $\pm$ 237 μm^3 and the spread of values increased from 838 μm^3 to 1498 μm^3. The nuclei now approximate a log normal distribution (using EDF statistics the values were not significant at the 15% level).

In the following 12 hours of recovery (Table 7) mean nuclear volumes decreased slightly to 576 $\pm$ 134 μm^3 and the spread of values, 302 to 1038 μm^3, also decreased, to 736 μm^3. These values are almost identical with those in roots treated with IBMX (Table 4). However, nuclear volumes 13 hours after a 1 hour treatment with EOC were neither linear nor log normally distributed.

From 13 to 15 hours, mean nuclear volume increased, from 576 $\pm$ 134 μm^3 to 726 $\pm$ 221 μm^3, and the spread of values increased from 736 to 1256 μm^3. These values are similar to those found in interphase nuclei at 15 hours after IBMX. Thus, in the 2 hours from 13 to 15 hours, interphase nuclei in IBMX treated roots showed a 59.6% increase in volume and many of them went into mitosis, while in EOC treated roots there was only a 26% increase in nuclear volume and none of the binucleate cells entered mitosis (Table 6.2).

3. Nuclear Growth of Sister Nuclei

Sister nuclei in a binucleate cell often differed in volume. The extent to which they differ in size provides some indication of the extent to which differences in growth occur between sister nuclei.

At the end of treatment with IBMX only 3% of the pairs of sister nuclei had identical volumes and 25% differed by > 200.5 μm^3. The range of volume differences was 0 - 1134 μm^3 (Table 8). The mean volume of the smaller nuclei was 618 $\pm$ 226 μm^3, cf. 796 $\pm$ 309 μm^3 for the larger nuclei; the difference between mean volumes was 178 μm^3. This difference is 0.250 of the mean volume of all nuclei measured (Table 9).

After a one hour recovery period, the mean volumes of the smaller and larger nuclei were 505 $\pm$ 223 μm^3 and 645 $\pm$ 319 μm^3. The difference between the means was 140 μm^3. Though the nuclei have undergone a reduction in volume in the 1 hour period of recovery (Tables 4 and 8), the ratio between the means is 1.277 cf. 1.288 at the end of treatment. It appears that the reduction in nuclear volume was relatively the same in the larger and the smaller nucleus each pair.

In the following 12 hour period of recovery, nuclei continued to contract. Accompanying this contraction, there was a reduction in the range of differences, it was 0 - 493 μm^3, and the difference between the means of smaller and larger nuclei fell to 74 μm^3. These changes are interesting since they result, almost entirely, from changes in volume of the larger nucleus of each pair. Thus the mean volume of

TABLE 8

Range and difference between mean volumes of larger and smaller sister nuclei within binucleate cells. Primary roots of V. faba were treated with 5×10^{-3} M IBMX or EOC for 1 hour followed by up to 15 hours recovery. Number of cells scored for each time was 150. This data is from the same roots as those scored from Tables 4 and 6.

TABLE 8

IBMX Treatment	Type of Nucleus	Mean Volume (μm^3) ± S.D. Larger Nucleus (L)	Smaller Nucleus (S)	Difference (L-S)	Volume Difference (μm^3) Range	% Frequency 0	0.5-200.4	>200.5
1	Interphase	796 ± 309	618 ± 226	178	0-1134	3	72	25
1 + 1	Interphase	645 ± 319	505 ± 223	140	0-1066	3	79	18
1 + 13	Interphase	587 ± 136	513 ± 115	74	0-493	12	81	7
1 + 15	Interphase	872 ± 152	782 ± 135	90	0-474	10	78	12
1 + 15	Prophase	1415 ± 306	1225 ± 256	190	0-1020	4	62	34
EOC Treatment								
1	Interphase	396 ± 136	318 ± 104	78	0-534	4	88	8
1 + 1	Interphase	723 ± 255	575 ± 191	148	0-897	8	68	24
1 + 13	Interphase	612 ± 133	540 ± 125	72	0-294	12	83	5
1 + 15	Interphase	775 ± 225	677 ± 207	98	0-376	6	82	12

TABLE 9

Mean volumes of sister nuclei of binucleate cells induced by 5×10^{-3} M IBMX or EOC for 1 hour followed by up to 15 hours recovery.

Mean L = mean volume of larger sister nucleus

Mean S = mean volume of smaller sister nucleus

D = difference, i.e. L-S, between mean volumes

This data is obtained from Table 8.

Mean V = mean volume for total sister nuclei

This data is obtained from Tables 4 and 6.

TABLE 9

IBMX Treatment	Type of Nucleus	$\frac{D}{\text{Mean V}}$	$\frac{D}{\text{Mean L}}$	$\frac{D}{\text{Mean S}}$	$\frac{\text{Mean L}}{\text{Mean S}}$
1	Interphase	0.250	0.223	0.287	1.288
1 + 1	Interphase	0.250	0.218	0.278	1.277
1 + 13	Interphase	0.136	0.127	0.145	1.144
1 + 15	Interphase	0.109	0.103	0.115	1.115
1 + 15	Prophase	0.143	0.134	0.155	1.155
EOC Treatment					
1	Interphase	0.218	0.197	0.245	1.245
1 + 1	Interphase	0.230	0.206	0.259	1.257
1 + 13	Interphase	0.127	0.119	0.135	1.133
1 + 15	Interphase	0.136	0.128	0.146	1.145

the smaller nuclei changes very little from 1 + 1 to 1 + 13 hours, i.e. 505 $\pm$ 223 μm^3 to 513 $\pm$ 115 μm^3, while the larger nuclei were reduced from 645 $\pm$ 319 μm^3 to 587 $\pm$ 136 μm^3. These results suggest a differential behaviour of pairs of sister nuclei: the smaller nuclei show no change in volume over 12 hours, while the larger nuclei show a 9% reduction in volume. As a consequence, the mean volume ratio, larger to smaller nuclei, falls to 1.144 at 1 + 13 hours (Table 9).

From 13 to 15 hours recovery all nuclei grew. Mean volume of the larger interphase nuclei increased to 872 $\pm$ 152 μm^3, and the smaller nuclei 782 $\pm$ 135 μm^3; the volume ratio was 1.115 (Table 9). Prophase nuclei were also present at 15 hours. In prophase nuclei, the differences between sisters were even greater than those between sisters in interphase. The range of differences was 0 - 1020 μm^3 and the difference between the mean volume of smaller and larger nuclei was 190 μm^3 cf. 0 - 474 μm^3 and 90 μm^3 in interphase nuclei at 1 + 15 hours (Table 8).

In the two hour period from 1 + 13 to 1 + 15 hours, the population of binucleate cells split into two distinct sub-populations:

1. This group of binucleate cells goes on to enter mitosis. Its nuclei undergo a large increase in volume; a 2.41 - fold increase in the larger nuclei and a 2.39 - fold increase in the smaller nuclei. There is a large increase in the range of volume differences between sisters.

2. This sub population shows no change in the range of volume differences between sisters, i.e. 0 - 474 μm^3 cf. 0 - 493 μm^3 at 1 +

13 hours. However, the nuclei increase in volume. There is a 1.49 - fold increase in the smaller nuclei and a 1.52 - fold increase in the larger nuclei.

The behaviour of pairs of sister nuclei induced by EOC was, for the most part, similar to that of IBMX induced sister nuclei, i.e.

1. the range of volume differences between sister nuclei decreased from 0 - 534 μm^3 at the end of treatment to 0 -376 μm^3 at 1 + 15 hours.

2. The frequency of sister nuclei with identical volumes was always low; it never exceeded 12% and that value occurred when the difference between the mean volumes of sister nuclei was at its lowest, at 1 + 13 hours (Table 8).

3. Between 1 + 1 and 1 + 13 hours, the larger nucleus of each pair underwent a 15.4% reduction in volume cf. a 6.1% reduction in the smaller nucleus. The result was a change, in the mean volume ratio of larger to smaller nuclei, from 1.257 to 1.133 (Table 9).

4. The final point of similarity in the behaviour of sister nuclei in binucleate cells induced by EOC or IBMX is that changes in volume are rarely equal in the two nuclei, i.e. they showed differential behaviour. Whether it is the contraction of nuclei that occurs, for example, from 1 + 1 to 1 + 13 hours after IBMX or EOC (Table 8) or the increase in volume that occurs, for example, between 1 + 13 and 1 + 15 hours after IBMX, it is clear that the two sister nuclei present in the binucleate cell, do not behave in an identical manner. It appears, therefore, that even within a common cytoplasm, two sister nuclei regulate this own growth independently to some extent, of each other.

4. Nuclear Morphology

Four types of nuclear shape were found in primary root meristems: spherical, nearly-spherical, oval and elongate. Nuclear shape was not fixed since the frequencies of nuclei of different shapes changed during interphase and also differed in late interphase and prophase nuclei. The pattern of change in the frequencies of nuclei of different shapes was similar with IBMX and EOC (Table 10).

1. Spherical nuclei were the least frequent type in interphase and in prophase; they were most frequent in early G_1, when their maximum frequency, 5.3%, occurred.

2. Elongate nuclei were the next most infrequent type found; they were most frequent soon after the induction of binucleate cells, i.e. at 1 and 1 + 1 hours.

3. Oval nuclei were the most frequent shape. Furthermore, their frequency increased as the cells grew older, i.e. they made up 50.7 - 61.3% of the total sample at 1 and 1 + 1 hours and this increased to 75.0 or 77.0% by 1 + 15 hours. Of the prophase nuclei, 88.3% were oval in shape in binucleate cells and 76.3% in uninucleate cells.

The similarity in the pattern of change in the frequencies of different nuclear shapes in both EOC and IBMX induced binucleate cells suggest that nuclear shape is regulated; it may be related, in some way, to nuclear function. Further evidence that nuclear shape

TABLE 10

Percent frequencies of different morphological classes in primary root meristems of V. faba treated with 5×10^{-3} M IBMX or EOC for 1 hour followed by up to 15 hours recovery. Number of nuclei scored at each time was 300. This data is from the same roots as those scored from Tables 4 and 6.

TABLE 10

IBMX Treatment	Type of Nucleus	Class I Spherical	Class II Nearly-Spherical	Class III Oval	Class IV Elongate
1	Interphase	0.7	23.0	51.0	25.3
1 + 1	Interphase	1.3	21.0	58.3	19.3
1 + 13	Interphase	2.3	31.3	63.3	3.0
1 + 15	Interphase	0.3	22.7	77.0	
1 + 15	Binucleate Prophase		7.3	88.3	4.3
1 + 15	Uninucleate Prophase	0.6	19.6	76.3	3.5
EOC Treatment					
1	Interphase	3.3	28.0	61.3	7.3
1 + 1	Interphase	5.3	32.3	50.7	11.7
1 + 13	Interphase		20.6	79.4	
1 + 15	Interphase	1.0	23.0	75.0	1.0
Control all stages	Interphase	6.8	39.8	43.8	9.6
Control	Prophase		18.3	80.0	1.7

is regulated comes from an analysis of the frequency of sister nuclei having identical shapes (Table 11). Soon after binucleate cells are formed 56.0 - 66.0% of pairs of sister nuclei have identical shapes. By 1 + 13 and 1 + 15 hours, this frequency has risen to 74.7 - 79.0% and in binucleate cells in prophase it was 83.3%.

It may be that sister nuclei influence each other, or that both are influenced by the cytoplasm. Whatever the primary cause, tests of association have shown that the frequency of pairs of sister nuclei having identical shapes is significantly different from that expected on the basis of random association. If we assume that assortment of nuclei of different shape occurs at random, i.e. any kind of matching is equally possible, the expected frequencies of all possible matchings can be computed. The expected frequencies were compared with the observed frequencies for EOC, 1 + 1 hour, and IBMX, 1 + 13 hours (Table 12). The results of the G test for the overall binucleate cell population gave strong evidence that matching, of some sort, was more likely than random assortment of shape between sister nuclei. The test of proportions established that the proportions of III + III, II + II and IV + IV matchings were significantly greater than those expected for random assortment for the fixation time 1 + 1 hour after EOC treatment. At 1 + 13 hours after IBMX treatment, both III + III and II + II matchings were significantly greater than those expected for random matchings.

TABLE 11

Percent frequencies of pairs of sister nuclei with identical shapes. Primary roots of V. faba treated with 5×10^{-3} M IBMX or EOC for 1 hour followed by up to 15 hours recovery. Number of cells scored at each time was 150. This data is from the same roots as those scored for Tables 4 and 6.

TABLE 11

IBMX Treatment	Type of Nucleus	Identical Shape	Different Shape
1	Interphase	56.0	44.0
1 + 1	Interphase	66.0	33.0
1 + 13	Interphase	74.7	25.3
1 + 15	Interphase	78.7	21.3
1 + 15	Prophase	83.3	16.7
EOC Treatment			
1	Interphase	59.3	40.7
1 + 1	Interphase	56.0	44.0
1 + 13	Interphase	79.0	21.0
1 + 15	Interphase	76.0	24.0

TABLE 12

The G test was performed on 150 pairs of binucleate cells obtained either 1 hour or 13 hours after a 1 hour treatment of EOC or IBMX, respectively. The G values for the comparison between the observed frequencies of different nuclear class matchings and the expected frequencies, calculated on the basis of random assortment of shape, are shown.

TABLE 12

Nuclear Class Matchings	EOC (1 + 1 Hour) Observed Frequency	EOC (1 + 1 Hour) Expected Frequency	IBMX (1 + 13 Hours) Observed Frequency	IBMX (1 + 13 Hours) Expected Frequency
I + II	5	5	3	2
I + III	6	8		5
I + IV	1	2		
II + III	38	49	30	59
II + IV	6	11	1	3
III + IV	10	18	4	6
I + I	2		2	
II + II	24	16	30	15
III + III	49	39	78	60
IV + IV	9	2	2	
	150	150	150	150

$G = 38.37 > \chi^2_{0.005} 18.5 (df=6)$ $G = 37.8 > \chi^2_{0.005} 14.9 (df=4)$

DISCUSSION

1.1 Methylxanthines: Short Term Recovery

This study confirms that different methylxanthines are not equally effective in inducing changes in proliferating cells (Stallwood, 1974). In primary root meristems, a differential response to EOC and IBMX by mitotic cells present during the time of treatment was apparent:

(i) IBMX was more effective in inhibiting cytokinesis and inducing binucleate cell formation than EOC.

(ii) EOC induced chromatid stickiness at anaphase and produced tetraploid nuclei while IBMX, at the same concentration, did not induce these effects.

(iii) EOC was more efficient in inhibiting cytokinesis than at inducing chromatid stickiness since more binucleate cells than tetraploid nuclei were produced.

IBMX was a more potent drug than EOC since a 1.4-fold increase in the proportion of induced binucleate cells per root resulted from the IBMX treatment as compared to the total proportion of both induced binucleate cells and affected cells per root formed after EOC treatment (Table 1).

EOC induced the same changes in lateral roots of *V. faba* (Stallwood, 1974). Treatment with dibutyryl cyclic AMP (dbcAMP), however, did not induce chromatid stickiness or tetraploid cell formation and it did not give rise to binucleate cells. It appears that when these

changes occur after treatment with methylxanthines, the underlying mechanism is not an inhibition of phosphodiesterase and a resulting rise in intracellular levels of cAMP. Methylxanthines must act on cells in ways that are independent of their effects on levels of cAMP.

Both drugs tested were equally effective in reducing the mitotic index. Since this drop results mainly from a fall in the number of cells in prophase, it appears that methylxanthines delay cells in G_2 and prevent them from entering prophase. Within a one hour recovery period cells treated with EOC continued to show this delay, while cells treated with IBMX recovered and the mitotic index returned to normal levels. These results suggest that EOC and IBMX do not affect cells by means of a single mechanism since EOC is more effective at reducing the mitotic index while IBMX is more effective in inducing the formation of binucleate cells. These results suggest that different methylxanthines affect cells by two or more mechanisms and at two or more points in the cell cycle.

One mechanism by which methylxanthines may affect cells involves their inhibition of phosphodiesterase; this inhibition can result in a decrease in the amount of cAMP bydrolyzed by the enzyme and a consequent rise in intracellular levels of cAMP. Increased intracellular levels of cAMP, resulting from phosphodiesterase inhibition, may block cells in G_2 and thus lower the mitotic index. It has been observed (Millis, 1973) using lymphoid cells, that in late G_2, phosphodiesterase levels were twice those in late S, and by mitosis cAMP was approximately 25% of the level found in G_2. Futhermore, if levels of cAMP in G_2 are increased or prevented from decreasing by

additions of dbcAMP, the cells are blocked in G_2 and prevented from entering mitosis, hence the mitotic index drops. Recently, a reversible block of Chinese hamster V79 cells has been reported in the presence of dbcAMP (Stambrook et al., 1976). In lateral roots of V. faba treatment with dbcAMP reduced the mitotic index and it took cells up to 6 hours to recover from the delay in G_2 (Stallwood, 1974). Cells in primary roots of V. faba show a similar response to EOC and IBMX as lateral roots do to dbcAMP. Thus, it may be that at least the reduction in mitotic index by methylxanthines results from their effect on intracellular levels of cAMP.

1.2 Methylxanthines: Long Term Recovery

Not all binucleate cells induced by methylxanthines are capable of proliferation. In both primary (Table 6.2) and lateral roots (Stallwood, 1974), EOC induced binucleate cells did not divide. Since the meristematic cells not in mitosis at the time of treatment with EOC continue to proliferate, it appears that EOC has a specific effect on mitotic cells. The effect is expressed when the cells enter interphase; it converts proliferating cells into a non proliferating condition and, it inhibits growth of the nuclei of the binucleate cells. Both changes suggest that a very fundamental effect is exerted by EOC on mitotic cells.

Binucleate cells induced by IBMX on the other hand do divide. This shows that IBMX and EOC differ in their effects on proliferating cells; it also shows that the binucleate condition does not, itself, prevent a cell from completing a cell cycle. It is estimated that

about 75% of the IBMX induced binucleate cells underwent mitosis (Table 6.1). The duration of their cell cycle is approximately that of the fast cycling cells (MacLeod, 1971; Van't Hof, 1967). It appears, therefore, that of the cells in mitosis at any time, about 75% give rise to progeny that are fast cycling cells. The proportion of binucleate cells induced by EOC or IBMX decreased with time, i.e. up to 1 + 21 hours and 1 + 13 hours, respectively; this finding agrees with that reported by González-Fernández et al. (1966) in which it was shown that induced binucleate cells were being diluted out by the rest of the proliferative uninucleate cell population. Furthermore, when divisions occur in the binucleate cells in IBMX treated roots, the frequency of binucleate cells is less than would be expected if each binucleate cell gave rise to two daughter binucleates; i.e. there are only 1.66% binucleate cells at 1 + 19 hours, cf. 1.542 at 1 + 13 hours. González-Fernández et al. (1966) found, in Allium cepa, a close correlation between the frequency of parent binucleate cells and daughter binucleates. It appears that a binucleate cell often gave two mononucleate cells, one at each end, and one binucleate cell, in the middle of the parent cell. In the present study, a 1 parent:1 offspring ratio was not found and it suggested that the formation of two daughter binucleate cells occurred in a higher frequency than that found in Allium cepa, i.e. 5%. In addition, the production of tetraploid nuclei occurred; such division were noted in Allium cepa (Giménez-Martín et al., 1966), i.e. the formation of two mononucleate cells at either end and the fusion of two diploid nuclei in the middle resulting in the formation of a tetraploid nucleus.

2. Nuclear Growth in Binucleate Cells

It could be argued that the ability of nuclei to progress to G_2 into mitosis depends, to some extent, on the growth of the nuclei during interphase. Thus, there may be a certain minimum volume a nucleus must achieve before it can divide. In normal cells, nuclear growth appears to occur throughout interphase (Bansal, 1975; White and Davidson, 1976). But in binucleate cells, produced by treatment with EOC or IBMX, nuclei do not grow throughout interphase. On the contrary, they contract. A similar response has been reported in salivary glands of Drosophilia melanogaster treated with theophylline (Rensing et al., 1972); nuclear volumes decreased after exposure to the theophylline, which, like EOC and IBMX is a methylxanthine. Furthermore, Rensing reported that this response to theophylline was independent of changes in level of cAMP.

The interesting thing about the response of nuclei to treatment with methylxanthines is that although the nuclei show little or no growth for almost the whole of interphase, there is a sudden increase in volume just before the nuclei enter prophase. Thus between 1 + 13 and 1 + 15 hours, nuclear volumes increase in IBMX treated roots from 545 $\pm$ 131 μm^3 to 827 $\pm$ 151 μm^3. Also, volumes of prophase nuclei in IBMX treated binucleate cells and mononucleate cells are almost identical. It appears that restraints on nuclear growth are exerted throughout most of interphase but they are removed in nuclei that are about to divide. It would be interesting to know whether the nucleus grows because it receives a signal that initiates preparation for mitosis,

or, whether only the nuclei that have grown in late G_2 are able to enter mitosis. In EOC induced binucleate cells mitosis does not occur; these nuclei did not grow at the end of G_2.

Even though nuclear growth was inhibited by methylxanthine treatment, a large spread of volumes still occurs in G_1 nuclei. Within one hour of binucleate cell formation, i.e. telophase - early G_1, 7.0 and 7.4 - fold ranges existed in EOC and IBMX treated roots, respectively. The ranges of values were also large at 1 + 1 hours, i.e. 6.7 and 8.4-fold ranges. The spread of nuclear volumes may be the result of differential mitosis or of differences in rates of nuclear growth in G_1. In the latter case, part of the variability in nuclear volumes may be due to the presence of cycling and non-cycling cells in the population. In addition, both probit plots and tests using EDF statistics confirmed that in G_1, i.e. at 1 and 1 + 1 hours, nuclear volumes from binucleate cells do not form a single uniform population of values. Within the next 12 hour period of recovery, i.e. 1 + 1 to 1 + 13 hours, little or no nuclear growth occurred over the total nuclei population as the mean volumes slightly decreased from 649 μm^3 to 576 μm^3 and from 575 μm^3 to 545 μm^3 in binucleate cells induced by treatments of EOC or IBMX, respectively. However, some fundamental changes must have occurred as nuclear volumes had become much less variable, i.e. 3.4 and 3.7-fold ranges, as compared to those found at 1 and 1 + 1 hours (Tables 4 and 7). In the IBMX induced binucleate cell population, a differential behaviour of nuclei was found; while the larger nuclei of the population had contracted, many of the smaller nuclei of the total distribution showed growth (Figures 2 and 3). This suggests that

unequal growth rates existed within the population of nuclei; accompanying this pattern of differential growth there was a change in the distribution of nuclear volumes. From being non-normally distributed, the distribution of nuclear volumes was found to fit a single log-normal distribution. Nuclei from EOC induced binucleate cells had similar volumes, i.e. the range and its mean, to those found in binucleate cells induced with IBMX, but the changes within the distribution of nuclear volumes did not result in a uniform population of values.

Within the next two hours, i.e. 1 + 13 to 1 + 15 hours, all nuclei show large increments of growth. Interphase nuclei from EOC induced binucleate cells, however, have smaller increases in growth, i.e. a mean volume increase of 26% from 576 μm^3 to 726 μm^3, than interphase nuclei of binucleate cells induced with IBMX, i.e. a mean volume increase of 59.6% from 545 μm^3 to 827 μm^3; this suggests EOC induced binucleate cells do not achieve the same nuclear growth rate as IBMX induced binucleate cells. Within the remaining binucleate cell population induced by IBMX, a subpopulation of dividing binucleate cells was found; the mean volume of prophases was 1320 μm^3. This suggests that not all nuclei of binucleate cells induced by IBMX have grown at the same rate; those which enter mitosis grow disporportionally larger than those which remain in interphase at 1 + 15 hours. It may be that a nucleus has to have a minimum volume for entry into prophase; no prophase nuclei were seen that were less than 742 μm^3. However 71.3% of the interphase nuclei of IBMX induced binucleate cells overlapped with binucleate prophases, i.e. from 742 μm^3 to 1211 μm^3 and 80% of

the interphase nuclei of binucleate cells induced by EOC had similar volumes, i.e. from 742 μm^3 to 1575 μm^3, to the prophase nuclei from binucleate cells induced by IBMX. Therefore, nuclear volume per se does not seem to be the trigger for entry into mitosis. This further confirms the report (Bansal, 1975) that the age of a nucleus can not be determined by its position in either interphase or prophase. In addition, within the homogeneous population of cycling cells seen at prophase, variability in nuclear volumes, i.e. 3.3-fold range, still exists. Thus, this variability in volume is not confined to interphase.

3. Growth of Sister Nuclei

The two nuclei present in a binucleate cell are products of the same mitosis and, since they share a common cytoplasm, their growth should be affected to the same effect by cytoplasmic factors. A comparison of the volumes of sister nuclei showed that few pairs of nuclei had identical volumes; the frequency ranged from 3 to 12%.

Nuclear volumes were smaller in EOC induced binucleate cells than in IBMX induced binucleates, suggesting that EOC is a more potent inhibitor of nuclear growth. But even after EOC treatment, sister nuclei were generally of different sizes; the mean difference in nuclear volume was 78 μm^3 (Table 8) at the end of a one hour treatment cf. 178 μm^3 after IBMX. The existence of large differences in volumes of sister nuclei indicate that they are not completely identical. Since these differences are established very early in G_1, i.e. within one hour of the formation of binucleate cells, and since the two nuclei are in a common cytoplasm, it appears that sister nuclei differ in

some way soon after completion of mitosis.

The volume differences between sister nuclei are maintained throughout interphase and when nuclei enter prophase. This means that the mechanism that generates differences between sisters in early G_1 continues to operate throughout interphase. Further evidence for the differential growth of nuclei occurs at 1 + 1 and 1 + 13 hours after treatment with EOC or IBMX. At these times the larger nucleus of each pair contracts to a greater extent than the smaller nucleus (Table 9). Also from 1 + 13 to 1 + 15 hours, there is a differential increase in nuclear volume. Both results show that sister nuclei behave independently of each other throughout interphase. The one aspect in which pairs of sister nuclei behave in synchrony is entry into prophase. In all binucleate cells in mitosis, the two sister nuclei of a binucleate cell entered prophase simultaneously or were at the same stage of mitosis. In this regard, it appears that the stimulus to enter prophase affects both nuclei equally.

The increase in volume is not equal in sister nuclei of a binucleate cell throughout interphase. The greatest amount of growth occurs in the last two hours of interphase, i.e. from 1 + 13 to 1 + 15 hours. This result agrees with the observations on lateral roots of *V. faba* (Bansal, 1975) and on pollen grains of *Tradescantia paludosa*, in which most rapid growth occurs towards the end of G_2 (White and Davidson, 1976).

The mechanism underlying the differential growth of sister nuclei is not known. It could involve either: 1) chromosomal

constituents or 2) cytoplasmic constituents. An obvious cause of inequality between sister nuclei would be non-disjunctional segregation of sister chromatids. Such behaviour of chromatids would generate two nuclei with 2n + 1 and 2n -1 complements; this is unlikely to be the underlying mechanism since it would require a high frequency of non-disjunctional segregation and yet it was never observed. The second possibility is that sister chromatids differ in the amounts of either proteins or RNA that they carry. How such differences would affect nuclear growth is not known? The third possibility is that the cytoplasm of a binucleate cell is not homogeneous, in which case the two sister nuclei may be in somewhat different micro environments.

Whether or not the reason for the differential behaviour of sister nuclei in interphase lies in either of these possible explanations, it is clear that in some way, the two nuclei produced by mitosis are unequal. What the behaviour of sister nuclei in a binucleate cell indicates is that the mechanism generating this inequality may not be entirely cytoplasmic, which would be the most feasible explanation if we were dealing with two mononucleate sister cells. Rather, it seems, some chromosomal factors may also be involved.

4. Nuclear Morphology

In normal and treated roots, four classes of nuclear shape are found (Table 10). The frequencies of nuclei with different shapes change as the cells proceed through interphase. A similar trend in the changes in frequency of nuclei of different shapes was seen in

binucleate cells induced by treatment with EOC or IBMX, i.e. spherical and elongate nuclei were always the least frequent classes, while oval nuclei were almost the most frequent type. Furthermore, oval nuclei increased in frequency from G_1 to G_2 and prophase. A similar increase in the frequency of oval nuclei has been reported in pollen grains as they progressed from G_1 to G_2 (White and Davidson, 1976).

Sister nuclei in binucleate cells tended to have the same shape. This suggests that nuclear shape is regulated, either by cytoplasmic factors or as a result of interaction between the sister nuclei. What is not known is whether or not the oval shape confers some physiological advantage upon a nucleus.

Elongate nuclei were most frequent immediately after treatment, particularly in IBMX treated roots (Table 10). This may be a result of treatment. It has been reported that additions of prostaglandin E_1 or cholera toxin, both of which activate adenylate cyclase and lead to an increase in levels of cAMP, promote cell elongation in Chinese hamster cells immediately after mitosis; dbcAMP has the same effect (O'Neill et al., 1976). In addition, treatment with dbcAMP results in an increase in the number of microtubules per unit volume of cytoplasm and a change in their distribution from a random to an orderly arrangement. The microtubules lie parallel to one another and to the long axis of the cell (Porter et al., 1974). The cell elongation induced by dbcAMP is prevented by low concentrations of a divalent cation ionophore, though only if calcium is present in the culture medium (Henneberry et al., 1975); these observations suggest an interaction between Ca^{++} ions and cAMP in regulating

microtubule assembly and, or, orientation. Calcium ions are also known to prevent the assembly of tubulin into microtubules in vitro (Weisenberg, 1972). The cell elongation described here is sensitive to colchicine and vinca alkaloids; since these drugs prevent the assembly of microtubules and also prevent cell elongation, their effects provide further evidence that the assembly of microtubules is a requirement for cell elongation. Whether the assembly and orientation of microtubules also controls nuclear shape in normal and methylxanthine treated cells is not known.

5. Nucleo-cytoplasmic Interactions

A binucleate cell formed by the inhibition of cytokinesis of a mitotic cell provides a growth unit which possesses minimum biological variability. This is especially advantageous for the purpose of studying interactions between nucleus and cytoplasm, since two sister nuclei, products of a common mitosis, should be affected, to the same extent, by cytoplasmic factors. However, sister nuclei of binucleate cells induced by either EOC or IBMX rarely had identical volumes and in very early G_1, i.e. within one hour of binucleate cell formation, large differences in the volumes of sister nuclei indicated that sister nuclei differed in some way soon after the completion of mitosis. Such differences could be accounted for by (1) the presence of either fast, slow or non cycling sister nuclei within a binucleate cell; this suggests that heterophasic nuclei, i.e. nuclei of different stages of the cell cycle, could be found within a common environment; (2) a differential mitosis in which nuclear

components were not equally distributed to daughter nuclei or in which a gradient of some cytoplasmic factor was established and maintained through much of interphase.

The volume difference between sister nuclei was maintained throughout interphase and as sister nuclei entered prophase. This indicates that variation in growth rates occurs throughout the cell cycle. Further evidence of heterogeneity in nuclear growth has been reported in lateral root meristems of V. faba in which tetraploid nuclei showed a 14-fold range in volumes during G_1, and a 34-fold range during S (Bansal, 1975); in addition, sister diploid nuclei showed a 6-fold range in G_1 and a 8-fold range in G_2 (Davidson, 1975). Though the mean volumes of the larger and smaller nucleus of each pair changed during the cell cycle, the proportionate difference, i.e. volume ratio, between the means changed very little (Table 9). This suggests that the growth of the two sister nuclei is not absolutely independent; their growth must, to some degree, be coordinated, since the initial difference in their size is maintained. Interactions between nuclei and/or with their common cytoplasm is further suggested in this study. It appears that the stimulus to enter prophase affects both nuclei equally, since the two sister nuclei of a binucleate cell enter prophase together or were in the same stage of mitosis. Furthermore, although shape changes occurred in nuclei as they proceeded through interphase, sister nuclei tended to have the same shape; this suggested that nuclear shape is regulated, either by cytoplasmic factors or as a result of interaction between sister nuclei. Interaction between nuclei within a common environment has been

reported, particularly in regard to mitotic synchrony. In HeLa homokaryons, produced by cell hybridization, synchrony of DNA synthesis and mitosis is rapidly achieved in multinucleate cells, even when single cells from different stages of the cell cycle have been fused to form cell hybrids (Johnson and Harris, 1969). However, synchrony seen in binucleate cells is not always present in multinucleate cells. In multinucleate cells of Allium roots, for example, some cells show synchrony of entry into prophase but others are asynchronous (Hervás, 1976).

Furthermore, Rao and Johnson (1970) reported that the progress of two interphase nuclei can be altered depending on the type of cell fusions involved, i.e. depending on the stage of the cell cycle of the different nuclei used to produce dikaryon, for example, G_1 + S, G_1 + G_2 or S + G_2. Recent studies (Rao et al., 1975) have shown that in S + G_2 heterophasic binucleate cells, the S phase cell exerted complete dominance over the G_2 phase cell, so that these hybrids reached mitosis about the same time as the homophasic S + S cells. It was found, however, that the addition of Mg^{+2} to the medium stimulated the G_2 nucleus to enter mitosis ahead of the S phase nucleus; this resulted in premature chromosome condensation in the S phase nucleus. The authors suggest that the S phase component delayed progress of the G_2 nuclei towards prophase. They also observed that protein synthesis during G_2 may have a role in the G_2-mitotic transition. In addition to proteins, RNA may also be involved in the G_2-mitosis transition. Giménez-Martín et al. (1971) found that 3'-deoxyadenosine, which blocks RNA synthesis, arrests nuclei in

prophase. This study was made using multinucleate cells and the conclusion was reached that some RNA molecules are responsible for mitotic synchronization in these cells.

Other evidence for movement of proteins and RNA between two nuclei of a binucleate cell has come from studies using Amoeba proteus. Radioactively labelled proteins and RNA were shown (Legname and Goldstein, 1972; Goldstein, 1974) to shuttle backwards and forwards between the two nuclei. The evidence is that only certain protein and RNA fractions undergo this movement, i.e. it is not a random process. The studies by Goldstein, Rao and Johnson show that two or more nuclei in a cell are in chemical communication with each other and the cytoplasm; in certain aspects of their behaviour the nuclei behave in synchrony. But in one aspect, nuclear growth, the evidence reported here shows that even sister nuclei, products of the same mitosis do not behave identically; they are of different sizes through interphase and when they enter prophase.

In this respect, the mitosis from which they are formed must be a differential event. It may be that nuclear volume varies independently of cell cycle duration or that the volume of a nucleus at early G_1 varies independently of its rate of growth during interphase. Whether or not this variability at the level of nuclear growth is related to the heterogeneity of cell cycle duration is not known. But since the variability in nuclear volume shown by normal uninucleate cells also occurs in binucleate cells, the results reported here suggest that sister nuclei are not equal in all respects. If chromosomal constituents are distributed unequally between the two groups of sister

chromatids at anaphase, the molecules most likely to be involved are proteins and RNA. What is now needed, for a better understanding of how nuclear growth and cell cycle duration is regulated, is an analysis of (1) the chromosomal constituents present in very early G_1 and (2) the differences between sister nuclei in the rate of entry of cytoplasmic constituents in very early G_1.

SUMMARY

1. In primary root meristems of Vicia faba, the following effects were induced by methylxanthines:

   (i) Binucleate cells were formed; IBMX was more effective in inhibiting cytokinesis and producing binucleate cells than EOC.

   (ii) Chromatid stickiness and formation of tetraploid nuclei were induced by EOC but not IBMX.

   (iii) There was a reduction in the mitotic index after treatment; EOC appeared to be more effective at depressing mitotic activity than IBMX.

2. These results suggest that different methylxanthines affect cells by different mechanisms and that they operate on more than one sensitive point.

3. EOC induced binucleate cells did not come back into division; it converted proliferating cells into a non-proliferating condition, and it inhibited growth of the nuclei of the binucleate cells. These results suggest that EOC has exerted its effects on mitotic cells.

4. Binucleate cells induced by IBMX did divide; this suggests that IBMX and EOC have different effects on proliferating cells. In addition, the binucleate condition does not, per se, prevent a cell from completing a cell cycle. It appears that of the cells

in mitosis at any time, about 75% give rise to progeny that are fast cycling cells.

5. In binucleate cells induced by treatment with EOC or IBMX, nuclei did not grow throughout interphase, but at times, they contracted. Nuclei showed little or no growth for almost the whole of interphase, however, there was a sudden increase in volume just before the nuclei entered prophase. Volumes of prophase nuclei in IBMX treated binucleate cells and uninucleate cells were almost identical.

6. Although nuclear growth was inhibited by methylxanthine treatment, a large variation in volumes existed in G_1 nuclei. The spread of nuclear volumes may be the result of differential mitoses or of differences in rates of nuclear growth in G_1.

7. The variation in nuclear growth occurred during interphase and into prophase. An overlap of volumes of prophase nuclei with volumes of interphase nuclei indicate nuclear volume, by itself, does not seem to be the trigger for entry into mitosis.

8. Sister nuclei of binucleate cells rarely had identical volumes throughout interphase and even when they entered prophase. Sister nuclei behaved independently of each other, to some extent; this indicated that nuclei of binucleate cells, which are products of the same mitosis, were unequal and that this inequality may not have been entirely the result of cytoplasmic conditions.

9. A similar trend in the changes in frequency of nuclei of different shapes was found in both EOC or IBMX induced binucleate cells

throughout interphase. In addition, sister nuclei of binucleate cells tended to have the same shape. These results suggest that nuclear shape may be regulated, and that sister nuclei may influence each other or that both are influenced by cytoplasmic conditions.

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