in nine hours. At 25°C, mitotic indices did not reach the control level even after 30 hours in both experiments.

Ram S. Verma

10. The effect on the nuclear cycle of mitomycin C and the production of chromosomal aberrations.

The purpose of the present study was to examine the effect of mitomycin C (MC) on the nuclear cycle and to record from metaphases of similar experiments the frequency of chromosomal aberrations assignable to specific chromosomes. Seeds of "Seneca 60" were germinated and grown at 24 ± 1°C on filter paper kept moist with distilled water. Germinated kernels with roots about 2 cm. in length were used for these experiments. Intact roots were exposed to H^3-thymidine (1 μc/ml final concentration) for 30 min., washed thoroughly, and treated with 0.001% MC for 2 hrs., following which the roots were washed in water again. After the last washing, the root tips were returned to the germination chamber for further growth and fixed at 2-hour intervals up to 36 hrs. post-treatment. Liquid emulsion autoradiographs (Kodak NTB-2) were prepared from Feulgen squashes of this material. Slides were scored for the frequency of labelled mitotic figures and the mitotic index was calculated. For each collection period, 3 or 4 root tips were examined to give a population of $6 - 8 \times 10^3$ cells.

In the experiments with MC, we did not accumulate metaphases by addition of colchicine, any other chemical, or cold; consequently, normal anaphase cells were also observed. Apparently, MC does not affect spindle formation during mitosis. However, a reduction in the mitotic index was recorded after MC treatment, suggesting that there was an immediate delay in the rate at which cells entered mitosis (Table 1). The relation between mitotic index and prophase labelling at various times from the beginning of MC treatment indicated that mitotic index was affected immediately even during the incubation period. Within the 14 to 18 hour period after the beginning of MC treatment, the mitotic index had decreased to about 1% (9.53% in control, Table 1), which in turn indicated that those nuclei were in a very early S period.

Table 1

Relation between mitotic index and proportion of prophase labelled at various times from the beginning of MC (0.001%) treatment (2 hrs.)

Time after the beginning of MC (hr.)	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Mitotic index (%)	6.7	5.5	2.9	3.1	5.7	4.6	1.0	0.8	1.4	5.0	6.1	8.7	3.0	4.3	9.5	12.1	9.1	10.3
Prophase labelled (%)	4.3	16.7	33.1	51.1	68.5	90.5	93.3	92.9	62.1	40.1	33.4	32.6	27.6	25.0	39.0	21.4	22.1	16.9

*Control 9.53%

The duration of the nuclear cycle with a 2 hr. treatment with MC indicates (Table 2) that the nuclei which pass through a complete S to G_1 cycle under the influence of MC were delayed by the drug. The increase in mitotic index between 18 hrs. and 24 hrs. post-treatment is an indication of the entry into a new mitosis of cells which were in G_1 and M phases at the time of treatment. The mitotic index reached the control level after 30 hrs.

Table 2

The duration of the nuclear cycle with or without a 2 hr. treatment with MC (0.001%) in Zea mays root tips

treatment / phase	hours	
	control*	mitomycin C
G_2 + P	2.1	7.0
S	5.0	11.5
Total	9.9	22.0

*Verma's data

In a second experiment, roots were exposed for 30 min. to H^3 - thymidine and then for 2 hrs. to MC (0.0005%). Fixations were made at 2 hr. intervals up to 32 hrs. after the removal of MC. The information on both aberration yield and labelling index was obtained at all fixation times. In order to make the chromosomes spread well and to study the chromosomal aberrations, a 0.03% solution of 8-hydroxyquinoline was employed for pretreating the intact root tips for 2.5 hrs. before fixation. At each fixation, the metaphase cells on each of three or four slides were scored, both for aberration yield and presence of labelling. In some cases very few metaphase cells were obtained due to the mitotic depression resulting from MC treatment.

In Table 3, the data on aberration yields show clearly that very few aberrations were observed from the first post-treatment mitosis of those cells in G_2 at the time of MC treatment. The aberrations which

Table 3

Yields of the various aberration types obtained at various times after a 30 min. exposure of roots to H^3-thymidine followed by 2 hr. MC (0.0005%)

Time after treatment (h.)	Total metaphase scored	% labelled metaphase	% metaphase aberration	No. of metaphase nuclei		No. of chromosome breaks		No. of chromosome interchanges and rings		No. of chromatid breaks		Aberration frequencies per cell
				L*	U*	L	U	L	U	L	U	
2	202	0.99	4.45	2	200	0	8	0	0	0	1	0.04
4	212	15.56	8.49	33	179	2	14	0	1	0	3	0.09
6	188	42.55	12.77	80	108	7	11	0	0	4	3	0.13
8	203	80.30	15.76	163	40	30	2	2	0	1	1	0.18
10	221	94.57	24.43	209	12	44	2	4	0	2	0	0.24
12	54	94.44	38.88	51	3	25	0	3	0	0	0	0.57
14	45	95.56	64.44	43	2	38	0	3	0	0	0	0.91
16	59	79.66	62.71	47	12	41	9	2	2	0	1	1.00
18	55	63.64	65.45	35	20	31	24	0	1	0	0	1.03
20	124	41.94	75.00	52	72	42	76	3	6	5	6	1.11
22	151	46.36	40.40	70	81	21	39	0	10	0	7	0.56
24	201	45.27	42.79	91	110	32	77	0	8	1	4	0.64
28	222	55.40	55.85	123	99	75	60	6	4	8	12	0.78
32	148	37.84	52.70	56	92	29	67	2	4	2	6	0.78

*L = labelled; U = unlabelled.

Table 4

"Goodness of fit" test for random distribution of chromosome breaks in various chromosomes induced by MC, based on percentage of unit chromosome lengths

Chromosome number	1	2	3	4	5	6	7	8	9	10	Total
% of unit chromosome length	14.4	12.0	11.1	11.0	10.4	9.0	8.4	8.6	8.0	7.1	100
observed	66	38	39	12	42	89	22	36	18	7	369
expected	33.1	44.3	40.9	40.6	38.3	33.2	31.1	31.7	29.5	26.3	369
x^2	3.1	0.9	0.1	20.1	0.4	94.2	2.7	0.6	4.5	14.1	140.5**

**P > 0.01

were observed occurred in labelled cells and the results in general indicate that there is an increase in sensitivity with increasing fixation time, i.e., the cells in early S show a higher aberration yield than the cells in late S at the time of treatment. The aberration frequency at 20 hr. after MC treatment is higher than other treatments; these cells would have been in late G_1 or early S at the time of treatment.

MC-induced chromosomal aberrations among the 10 pairs of chromosomes showed an apparent non-random distribution. If the drug was acting randomly per unit of chromatin, then the distribution of breaks should be according to the length of the chromosomes. The relative lengths in percentage of the complement (long arm plus short arm using Chen's data, 1969) of metaphase chromosome are shown in Table 4. The observed versus expected values were tested by Chi-square and a highly significant value was obtained. We attempted to assign the location of each aberration not only to a specific arm, but in most cases to a segment within arms. While admittedly somewhat arbitrary, our distribution analysis suggests that the frequency of breaks in certain arm segments is higher than in other arm segments. Future work to identify the locations of heterochromatin or knobs on the chromosome may lead to the study of the relationship between breakage and heterochromatin in maize chromosomes. At this time we can report only that the greatest concentration of breaks induced by MC appears at the secondary constriction and centromeres.

M. S. Lin

11. The identification of chromosome segments by their replication behavior using principal components analysis.

Previously we described the DNA replication behavior of maize chromosomes using ^{3}H-TdR autoradiography in different genetic stocks and at two temperatures (MGCNL 44:195-198, 1970). Replication behavior was described by plotting the mean number of silver grains for each chromosome arm at hourly intervals of the S period (a 'replication profile'). The data have been extended to include a chromosome 3 tester stock containing one B chromosome. The experimental input now includes: