Effects of Pre- and Simultaneous Treatment with Urea on the Induction of Chromatid Aberrations in *Vicia faba*

I. SCHUBERT and R. RIEGER

Zentralinstitut für Genetik und Kulturpflanzenforschung der Akademie der Wissenschaften der DDR, Correnssstrasse 3, DDR-4325 Gatersleben (German Democratic Republic, DDR), 15 January 1976.

Summary. Pre- and simultaneous treatments of *Vicia faba* root tip meristems with urea and ethyl alcohol, mitomycin C, maleic hydrazide, ^{3}H-thymidine and X-rays, respectively, were found to result in mutagen-specific changes of the spectrum and yield of induced chromatid aberrations.

The present investigation was aimed at determining possible effects of pre- and combination treatments with urea on the patterns of intrachromosomal distribution of chromatid aberrations induced by X-rays, ethyl alcohol (EA), mitomycin C (MI), maleic hydrazide (MH), and ^{3}H-thymidine (^{3}HT) in the main root meristems of a reconstructed karyotype (symbolized ACB) of *V. faba*[1]. This karyotype allows one to distinguish all 6 chromosome pairs and thus to locate induced chromatid aberrations in any of the 28 segments into which the chromosome set was subdivided. Treatment with the clastogens mentioned above resulted in patterns of preferential aberration distribution. Aberration clustering was found to occur in heterochromatin containing chromosome segments and to be, in part at least, mutagen-specific[2–5]. Since urea treatment has been reported to result in uncoiling of metaphase[6] and polytene chromosomes[7], we presumed that urea-induced decompaction of heterochromatin during interphase might possibly modify the intrachromosomal distribution patterns of induced chromatid aberrations and therefore tested pre- and combination treatments with urea on aberration induction by the clastogens (for details of clastogen treatments see Table; preparation of slides scoring and localization of aberrations have been described elsewhere[8,9]).

No chromosome structural changes were induced by treatment with the urea concentration which has been used for pre- and simultaneous treatments with the clastogens.

Neither pre- nor combination treatment with urea resulted in any significant change of the intrachromosomal distribution patterns of chromatid aberrations induced by X-rays, EA, MI, MH, or ^{3}HT. The aberration yields obtained after treatment with EA, MI or ^{3}HT remained unaffected by urea and corresponded to those found without urea pre- or combination treatment. As compared with X-raying alone, additional pretreatment with urea was found to result in a significantly higher yield of induced chromatid aberrations. The opposite observation was made after treatment with urea and MH.

The most interesting effect of pre- or simultaneous treatment was a differential influence of urea on the ratio of isochromatid breaks and chromatid translocations induced by EA, MI, MH, and ^{3}HT, on the one hand, and X-rays on the other (Table). In the case of EA, MI, MH, and ^{3}HT urea resulted in a significant decrease of the number of induced chromatid translocations (t) and a

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Effects of pre- and simultaneous* treatment with urea on the yield of chromatid aberrations and the ratios of isochromatid breaks and chromatid translocations (i:t) after treatment with ethyl alcohol (EA), maleic hydrazide (MH), mitomycin C (MI), X-rays and ^{3}H-thymidine

Treatment	Metaphases		No. of aberrations (=100%)	Types of aberrations (%)				i	intra-/ interchanges
	Scored	Damaged (%)		i	t	dd	d	t	
EA	4,000	18.9	814	47.3	26.0	10.0	16.7	1.82	2.84
Urea + EA*	3,200	19.0	649	59.9	12.0	14.8	13.2	4.99	7.32
MI	2,450	29.3	786	48.3	29.5	11.4	9.3	1.64	2.34
Urea + MI	2,450	29.6	808	60.0	16.7	12.7	9.4	3.62	4.96
MH	1,600	24.1	413	51.1	29.3	10.2	9.4	1.74	2.41
Urea + MH	1,700	18.4	324	68.2	12.0	8.6	11.1	5.66	7.31
^{3}HT	1,700	23.2	446	43.3	35.2	9.4	6.5	1.23	1.84
Urea + ^{3}HT	4,100	19.6	890	52.8	22.8	8.3	9.4	2.32	3.38
X-rays	5,050	25.7	1,401	46.0	30.6	7.7	13.1	1.50	2.27
Urea + X-rays	1,200	36.9	522	36.0	40.6	9.6	11.7	0.89	1.46

Similar i:t ratios were found for all recovery times tested and, therefore, data from all recovery times have been pooled in the Table.
Treatment conditions: Urea: 2.5×10^{-1} mole, 4 h, 24 °C. EA: 2×10^{-1} mole, 4 h, 30 °C; recovery times 18, 21, 24, 27 h. MI: 10^{-5} mole, 1 h, 24 °C (recoverly times as with EA). MH: 3×10^{-4} mole, 1 h, 24 °C (recovery times as with EA). ^{3}HT: 25 µCi/ml, specific activity about 15 Ci/m*M*, 4 h, 24 °C; recovery times 6, 9, 12 h. X-rays: 150 r, 90 r/min; recovery times 0, 3, 6, 9, 12 h.
Symbols: i, isochromatid breaks; t, chromatid translocations; dd, duplication deletions; d, intercalary deletions.

corresponding increase of the yield of isochromatid breaks (i). The opposite effect, i.e., an increase in the yield of chromatid translocations accompanied by a decrease in the yield of isochromatid breaks, was found after combined treatment with urea and X-rays. All results summarized in the Table are significant at the 0.1% level (χ-square test). Similar i:t ratio shifts have been observed for all recovery times tested (see Table) and also after prolonged urea pretreatments (6 and 8 h).

Three conclusions may be drawn from the present results: 1. Urea pre- or combination treatments remain without influence on the patterns of intrachromosomal distribution of chromatid aberrations induced by the clastogens mentioned above. 2. Urea exerts a differential clastogen-dependent (EA, MI, MH and ^{3}HT on the one hand, X-rays on the other) influence on the i:t ratios found after treatment with the clastogens: The quantitative change of the yield of one of the two aberration types (i or t) was found to be regularly accompanied by an opposite change of the frequency of the other type which completely compensated for the first. 3. In the case of treatment with EA, MI and ^{3}HT, urea is without effect on the total yield of induced chromatid aberrations. The yield of X-ray induced chromatid aberrations becomes increased with urea pretreatment; a decreased yield of MH-induced chromatid aberrations is obtained after urea pretreatment.

We are presently unable to explain these mutagen-specific effects of urea. Very similar results have, however, been obtained by GEBHART[10] after combined treatments of human lymphocytes with Trenimon and various amino acids. We hope that further experiments will provide a more detailed insight into the mechanisms underlying the differential, mutagen-specific modifications by urea of aberration yield and aberration spectrum.

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Stimulation of Growth by Insulin in *Drosophila* Embryonic Cells in vitro

GIULIANA MOSNA and C. BARIGOZZI

Istituto di Genetica dell'Università, Via Celoria 10, I-20133 Milano (Italy), 24 December 1975.

Summary. For obtaining a better yield of established lines of embryonic *Drosophila* cells, insulin proved to be a useful substance to be added to the culture medium. 10% of lines became established, showing a predominantly diploid chromosome number.

The establishment of cell lines of *Drosophila* in vitro is obviously an important step for the study of several problems of genetics such as cell differentiation and gene action; therefore the possibility of obtaining a sufficiently high proportion of cell lines in a relatively short time is of fundamental importance.

Sor far the establishment of lines from embryonic cells obtained in the same conditions as ours has been very irregular[1–5] and the number of lines obtained from primary cultures is still low. Moreover, it is still impossible to predict (even roughly) the time needed by primary cultures to start growing continuously and the frequency of continuous lines. To improve the production of continuous lines from primary cultures, we took advantage of a paper by SEECOF and DEWHURST[6], in which it is briefly mentioned that insulin added to the medium seems to facilitate the initiation of continuous cell lines. The aim of the present work was to increase this frequency by supplementing the medium with different doses of insulin. The experiment consisted in setting up 80 primary cultures from the wild stock Varese of *D. melanogaster* according to the method of growing cells in vitro of ECHALIER and OHANESSIAN[2]. Thus 20 primary cultures were set in a medium without insulin, and 60 cultures in a medium supplemented with insulin in the following doses: 1.85 mU/ml, 3.70 mU/ml, 7.4 mU/ml, each dose being tested on cultures.

Insulin was obtained from BDH (bovine, crystalline, 24.4 U/mg). The three doses were established with reference to the dose used by SEECOF and DEWHURST[6]. As cell lines we considered those primary cultures which required to be subcultured owing to the excessively high cell concentration. Within a fixed period (3 months), we counted the lines which required subculturing. Those which failed to show any growth at the end of the 3rd month were discarded. To date, the majority of the lines have undergone several passages.

To obtain preliminary information on the caryotype, slides of each line were prepared and conventionally stained with orcein. The experiment was performed twice.

The effect of insulin on growth of *D. melanogaster* embryonic cells in vitro

Insulin concentration (mU/ml)	No. primary cultures	No. continuous lines	Continuous lines (%)
1st experiment started in November 1974			
0.002[a]	20	1	5
1.85	20	5	25
3.70	20	–	–
7.40	20	2	10
2nd Experiment started in May 1975			
0.002[a]	20	–	–
1.85	20	2	10
3.70	20	2	10
7.40	20	2	10

[a] In unsupplemented medium traces of insulin may derive from fetal calf serum.

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