Determination of the Time of Fast Rejoining Processes after γ-Ray Fractionated Exposures in *Nigella* Chromosomes

In previous experiments[1-3] with *Nigella damascena* seeds, we demonstrated that fractionation of γ-ray exposures results in a very significant decrease of the frequency of the aberrations belonging to the chromosome class. This effect was suppressed or reversed when seeds were pretreated with a solution of chelating agent[2,3]. The following chelating agents such as 2–2′ dipyridil, 8-hydroxyquinoline, EDTA, Cupferron and diethyldithiocarbamate (DIECA) were tested, this last substance being in this respect the most efficient.

The drop observed in the control and its suppression or reversion after treatments with chelating agents suggested participation of ionic bonds in the fast rejoining process. This rejoining occurred approximately between 3 min 20 sec and 5 min 20 sec after the beginning of the irradiation. The present experiments are designed to bring some precision about the time at which this fast repair process occurs or its reversal by chelating agents takes place.

It would help in understanding the mechanisms by which the nuclear reactions interact in chromosome breakage and rejoining.

Material and methods. The material (*Nigella damascena* dry seeds) has been previously described in detail[4]. Dry seeds (about 8% water content) were irradiated in a γ-cell by a ^{60}Co γ-source. Two exposures of 4 Krads were selected for the facility of scoring chromosome aberrations without reaching the saturation level. The dose was acute (dose rate: 436,25 Krad/h), to avoid some of the investigated repairing processes appearing during the irradiation period. The 2 exposures were separated by intervals of 2 to 4 min with a constant increment of 10 sec in each case. The time necessary to move the samples up and down to the source was rigorously constant (8 sec) and considered as systematic error. During the period of irradiation, the temperature was 26 °C and the relative humidity 45%.

Seeds were sown immediately after irradiation to avoid after-effects. Diethyl-dithiocarbamate ($10 \times 10^{-2} M$/8 h) was selected as chelating agent for its great reversing power of the fractionation effect, and also its lack of toxicity and chromosome breaking activity. The parallel series of seeds treated with the chelating agent were put in the same container as the control so that the error on time estimation is exactly the same. This experiment was reproduced in the same experimental conditions with a different concentration of diethyldithiocarbamate ($1 \times 10^{-4} M$/8 h pretreatment). The manipulations of the material following irradiation and the cytological procedures have been reported elsewhere[3].

Results. In Tables I and II the modifications of frequency for each kind of aberrations are analyzed in detail without and with DIECA.

It can be seen that in the interval 2 and 4 min there is no slope in the decrease of the amount of aberrations. On the contrary, there is a significant decrease between 2′10″ and 2′20″. The decrease is such that at time intervals longer than 2′20″ (this interval included), the total effect is not significantly higher than the effect of the 4 Krad single exposure. All types of aberrations are involved in the decrease. On the other hand, Table II shows that the

[1] J. and M. Moutschen-Dahmen and J. Gilot, Experientia *24*, 843 (1968).
[2] J. and M. Moutschen-Dahmen and J. Gilot-Delhalle, Experientia *25*, 998 (1969).
[3] J. and M. Moutschen-Dahmen and J. Gilot-Delhalle, Medna Nucl. Radiobiol. lat. *12*, 17 (1969).

Table I. Effects of fractionated exposures (2 × 4 Krads) on dry seeds (20 metaphases scored per root tip)

Aberrations	Control 4 Krads	Control 8 Krads	Time interval (min′ and sec″)												
			2′	2′10″	2′20″	2′30″	2′40″	2′50″	3′	3′10″	3′20″	3′30″	3′40″	3′50″	4′
Breaks	8	17	21	19	16	7	12	4	13	0	1	2	0	1	13
Minutes	10	43	34	32	11	20	13	13	14	1	2	0	1	3	11
Exchanges	12	62	56	52	25	20	26	32	28	4	0	4	0	4	24
Totals	30	122	111	103	52	47	51	49	55	5	3	6	1	8	48
N. of metaphases analysed	200	200	200	200	200	200	200	200	200	20	20	20	20	20	200

Table II. Effects of fractionated exposures (2 × 4 Krads) on pretreated seeds (DIECA $1 \times 10^{-2} M$/8 h) (20 metaphases scored per root tip)

Abberrations	Control 4 Krads	Control 8 Krads	Time interval (min′ and sec″)												
			2′	2′10″	2′20″	2′30″	2′40″	2′50″	3′	3′10″	3′20″	3′30″	3′40″	3′50″	4′
Breaks	4	13	8	8	14	16	20	8	18	2	2	3	1	1	20
Minutes	22	14	9	10	26	23	16	18	17	3	6	4	12	7	42
Exchanges	26	44	39	39	57	51	53	55	61	5	5	6	5	4	51
Totals	52	71	56	57	97	90	89	81	96	10	13	13	18	12	113
N. of metaphases analysed	200	200	200	200	200	200	200	200	200	20	20	20	20	20	200

Table III. Statistical analysis of the data (variance analysis)

		Upper level Control-2'10"	Lower level 2'20"–4'	Total	Between 2'10" and 2'20"
Control	Expected	3.35 5.49	2.38 3.37	2.05 2.74	4.41 8.28
	Obtained	F^{2}_{27} 2.49	F^{5}_{54} 0.22	F^{8}_{81} 28.3	F^{1}_{18} 43.3
	Significance	no	no	highly	highly
DIECA		Lower level Control-2'10"	Upper level 2'20"–4'	Total	Between 2'10" and 2'20"
	Obtained	F^{2}_{27} 1.70	F^{5}_{54} 1.09	F^{8}_{81} 10.1	F^{1}_{18} 38.1
	Significance	no	no	highly	highly

Table IV. Differences between the data of 3 researchers for an independent experiment (200 metaphases analyzed in each case)

		1st	2d	3rd	mean	Confidence interval $\pm t$ 0.05
Control	2'10"	127	112	96	111.7	28.5
	2'20"	38	45	38	40.3	7.4
DIECA	2'10"	129	101	87	105.7	39.3
	2'20"	154	158	162	158	7.4

reversion of the effect also occurred exactly between 2 min 10 sec and 2 min 20 sec, confirming the data of Table I.

The curve of reversion due to the chelating agent parallels exactly the control curve. The statistical tests reported in Table III show that the differences are highly significant.

To avoid differences of observed aberration frequencies between different researchers, a kind of double blind test was realized respectively at the intervals of 2', 2'10" and 2'20" for the first and the second experiments. The data are given in Table IV. There is no significant difference in the results of the 3 researchers as evidenced in Table IV without or with chelating agent pretreatment.

The effects of the 2 concentrations ($1\times10^{-2}M$ and $1\times10^{-4}M$) are not significantly different. These results have been reproduced.

Discussion and conclusion. In previous experiments, the rejoining period was located between 3 min 20 sec and 5 min 20 sec but could not be defined more precisely. It included the time interval between the 2 exposures plus the duration of the first exposure, the dose rate being lower than in the present experiments. If the total time is calculated in the same manner, adding the first exposure time, the effect would occur between 2 min 43 sec and 3 min 3 sec. If the time of the second exposure is taken into account, the effect would occur between 3 min 16 sec and 3 min 26 sec which is of the same order of magnitude as in the previous experiments, keeping in mind the differences of dose rate, temperature and humidity.

The important finding is that the decrease of the amount of chromosome aberrations, as well as its increase after treatments with diethyldithiocarbamate, is occurring within 10 sec. It might be much shorter but no further precision could be obtained by the present method.

It can be remarked that all kinds of aberrations could be influenced in the same way, but it is likely that the distribution of the aberrations within the genome can vary with the time interval or the chelating agent. The slight difference between the effects of the 2 concentrations of diethyldithiocarbamate may be due to a relatively lower uptake at higher concentrations.

New experiments are designed to investigate if the reversion produced by other chelating agents occurs exactly at the same time.

Résumé. Des graines sèches de *Nigella damascena* ont été irradiées par les rayons γ du ^{60}Co à des doses fractionnées (2×4 Krads) séparées par des intervalles de temps compris entre 2 et 4 minutes et croissant de 10 en 10 sec. On a prouvé qu'une diminution très significative des taux d'aberrations chromosomiques se produit entre 2 min 10 sec et 2 min 20 sec. Lorsque les graines sont préalablement traitées par un agent de chélation (diéthyldithiocarbamate), on observe au cours d'une expérience strictement parallèle, un accroissement hautement significatif des taux d'aberrations se produisant exactement au même moment. Ces expériences confirment en les précisant les résultats d'expériences antérieures.

J. MOUTSCHEN, J. GILOT-DELHALLE[5] and R. THAKARE[6]

Laboratoire de Génétique,
Université de Liège,
15, rue Forgeur,
B-4000 Liège (Belgium), 2 August 1971.

[4] J. and M. MOUTSCHEN-DAHMEN, J. GILOT and M. REEKMANS, Cellule *66*, 83 (1966).

[5] Aspirant du Fonds National de la Recherche Scientifique.

[6] The author is thankful to the Belgian Government for awarding O.C.D. research fellowship. Present address: Bhabha Atomic Research Center, Trombay (Bombay) India.