Effects of Gamma-Ray Fractionated Doses on Chromosome Aberration Frequencies

Changes of radiation intensity have been shown to modify the amount of some chromosomal aberrations.

In *Tradescantia* a decrease was obtained for two-hit aberrations in definite experimental conditions[1].

Fractionated doses were found to be more convenient than variation of radiation intensity and the same conclusion was reached[2]. These results were confirmed with different biological materials[3–5]. As far as chromosome rejoining is concerned, the interpretation of the effect was that the aberrations produced by a first dose do not interact with those of a second one, when time intervals between 2 irradiations are sufficiently long. From this interpretation the occurrence of 2 chemically different types of chromosomal breaks was postulated in *Vicia*[6]. A first kind of aberrations can rejoin within a short time, whereas some others can remain open for a much longer time. An alternative interpretation is that a first dose of radiation sensitizes the cell against a second one by way of physiological changes[7].

The occurrence of 2 kinds of aberrations was also demonstrated in *Allium*[8] and *Tradescantia*[9]. Fast rejoining breaks were sometimes imputed to ionic bonds[10,11], whereas slow rejoining ones were rather attributed to covalent bonds[6].

Several questions remain unsolved. Are the results obtained with microspores reproducible in very slow metabolizing systems like dry seeds (in contrast with presoaked seeds of previous experiments)? Do they have the same implications for different kinds of aberrations?

Material and methods. Nigella damascena seeds (var. Miss Jekyll blue double) were irradiated with Co^{60} γ-rays (Picker, of 4200 c without filter). Two sets of experiments were successively performed at the same dose rate 3 krad/min. In the first set seeds were given 2 exposures of 4 krad separated by increased times (from 1–64 min). These treatments yielded saturation of aberrations so that the exposure was reduced to 2×2 krad, given in exactly the same experimental conditions (22 °C, 85% relative humidity). In these conditions the water content was 8%. Immediately after irradiation seeds were germinated in Petri dishes on wet filter paper at 19 °C. Root tips were fixed (Carnoy) about 60–70 h after the onset of germination and slides were prepared from Feulgen squashes. It is well known that in these conditions the only aberrations belong to the chromosome class[12].

Results. The data of the first set is given in Table I. All exchanges (comprising dicentrics + acentrics, rings + acentrics and symmetrical translocations) have been pooled. Minutes (microfragments, conventionally < 1 μ) were classified separately owing to some uncertainty of origin.

It is quite plausible that the majority of such small aberrations consist in interstitial deletions.

Each analysis is made on 5 roots (10 in dose of 4 krad). Twenty metaphases were analysed in each root.

These data show a decrease of the effect when the interval of time between the 2 exposures is 4 min or more. Since the effects reach a saturation level, no attempt was made to distinguish the difference between the 3 components. Variance analysis in the 3 first groups (0–2 min) does not show any significant difference $F^2_{12} = 0.35$. This is also the case for the 5 remaining groups (4–64 min) $F^4_{20} = 0.171$.

[1] K. Sax, Proc. natn. Acad. Sci. USA *25*, 225 (1939).
[2] U. Fano and L. D. Martinelli, Proc. natn. Acad. Sci. USA *29*, 59 (1943).
[3] G. R. Lane, Heredity *5*, 1 (1951).
[4] K. Sax, E. D. King and H. Luippold, Radiat. Res. *2*, 171 (1955).
[5] F. de Serres and N. H. Giles, Genetics *38*, 407 (1953).
[6] S. Wolff and H. Luippold, in *Progress in Radiobiology* (Eds. Mitchell, Holmes and Smith; Oliver and Boyd, 1956), p. 217.
[7] G. R. Lane, Heredity (Suppl.) *6*, 23 (1953).
[8] N. S. Cohn, Genetics *43*, 362 (1957).
[9] M. Iwabuchi, S. Tanifuji and H. Ochiai, Jap. J. Genet. *41*, 395 (1966).
[10] D. Mazia, Proc. natn. Acad. Sci. USA *40*, 521 (1954).
[11] D. Steffensen, Proc. natn. Acad. Sci. USA *41*, 155 (1955).
[12] J. Moutschen, M. Moutschen-Dahmen, R. Woodley and J. Archambeau, Radiat. Res. *15* (1968), in press.

Table I. Effects of a fractionated dose of 8 krad (2 exposures of 4 krad)

Aberrations	Control 4 krad	Control 8 krad	Interval (min)						
		0	1	2	4	8	16	32	64
Breaks	10.5	27	21	24	13	18	19	21	21
Exchanges	38	78	71	81	66	70	60	55	59
Minutes	27.5	118	115	89	38	45	46	34	40
Total	106	223	207	194	117	133	125	110	120

Table II. Effects of fractionated exposure (2×2 krad) (analysis on 10 roots, 20 metaphases/root)

Aberrations	Control 2 krad	Control 4 krad	Interval (min)					
		0	1	2	4	8	16	64
Breaks	4.5	14	13	13.5	6	7	8	9
Exchanges	7	30	26	22	11	11.5	18	13
Minutes	2	12.5	18	8.5	7	4	7.5	4
Total	13.5	56.5	57	44	24	22.5	33.5	26

Table III. Variance analysis for the 3 components

Interval of time (min)	Degrees of freedom	Breaks	P	Exchanges	P	min	P
0.1.2	F^2_{27}	0.25	n.s.	1.18	n.s.	2.55	n.s.
4.8.16.64	F^3_{36}	0.5	n.s.	0.15	n.s.	1.43	n.s.
Total	F^6_{63}	3.52	< 0.01	4.67	< 0.01	5.82	< 0.01

On the other hand, variance analysis for all groups (0–64) is highly significant $F^7_{32} = 4.41$ ($P < 0.01$).

The experiment was repeated with 2 exposures of 2 krad (Table II).

The same decrease of effect appeared as was the case at higher exposure. This decrease occurs for the 3 types of aberrations. After intervals longer than 4 min the level of effect of 2×2 krad is practically twice as high as 1×2 krad. In the 2 experiments, the results obtained for the common controls are of the same order of magnitude. Variance analyses were carried out the same way as with the higher exposure but for the 3 components (Table III).

From the analysis of all groups, it can be safely concluded that heterogeneity exists among the different groups for each kind of aberrations. On the other hand, no significant difference exists within the 3 first groups and within the 4 last ones.

Thus there is a decrease of the effect at intervals longer than 4 min. Adding the duration of 2 irradiations in the case of the second experiment, it can be derived that the interval for break rejoining is longer than 3 min 20 sec and shorter than 5 min 20 sec. There is no further decrease at longer intervals and, according to some more recent experiments, for intervals as long as 4 h.

Discussion and conclusion. The effect obtained in our experiments is similar to that obtained in *Allium*[8], but in this last case seeds had been immersed before irradiation. This was also the case of *Vicia*[6].

It can be concluded that the fall of the amount of aberrations is independent of the water content. The majority of the experiments realized by previous workers did not rule out the participation of metabolic processes in break rejoining since germination was started before irradiation.

This is in contrast with the present findings in which a very slow metabolizing system was utilized.

Previous workers[6,8,9] observed a second fall which was attributed to metabolic repair. This metabolism would be needed for the second kind of aberrations.

As expected this second fall did not occur in the present experiments.

Another point worth mentioning is that all kinds of aberrations are concerned in the process and not only exchanges as in other materials. Since in none of the present experiments active metabolism is involved, it would be tempting to adopt the hypothesis[10,11] that ion bonds can play a role in the rejoining of the first class of breaks which is here the only existing one. Experiments designed to verify this assertion are in progress[13].

Résumé. Des graines sèches de *Nigella damascena* ont été irradiées par les rayons γ du Co^{60} à des doses fractionnées de 2×4 krads et 2×2 krads séparées par des intervalles de temps allant de 1–64 min. Les résultats des 2 expériences se confirment et montrent qu'à des intervalles de temps de 4 min et plus, la fréquence de tous les types d'aberrations diminue de manière significative. Ces faits impliquent l'existence de mécanismes réparateurs où interviennent peu ou pas du tout les processus métaboliques.

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Die Identifizierung von Ligninspaltstücken anhand von Papier- und Dünnschichtchromatographie

Neun phenolische Verbindungen, deren Auftreten beim Abbau des Lignins möglich ist, sind auf ihr Verhalten in 6 Laufmitteln für Papier- und 2 für Dünnschichtchromatographie untersucht worden[1–3]. Ferner wurden ihre Reaktionen mit 4 Farbreagenzien[4] und ihre Fluoreszenz im UV-Licht verschiedener Wellenlänge verglichen. Zur Charakterisierung ihrer UV-Absorptionsspektren in Methanol wurden deren Maxima und Minima angegeben. Folgende Substanzen wurden untersucht:

Vanillin (I), Vanillinsäure (II), *p*-Hydroxybenzaldehyd (III), *p*-Hydroxybenzoesäure (IV), Ferulasäure (V), Cumarsäure (VI), Protocatechusäure (VII), Kaffeesäure (VIII), Gallussäure (IX). Bei den Verbindungen mit *cis-trans*-Isomerie[5] ist jeweils nur die dominierende *trans*-Form berücksichtigt worden. Die Rf-Werte ergeben sich

[1] H. MAEDER, Diss. Giessen (1960).

[2] J. M. BRAND, J. Chromat. *21*, 424 (1966).

[3] C. F. VAN SUMERE, G. WOLF, H. TEUCHY und J. KINT, J. Chromat. *20*, 48 (1965).

[4] E. MERCK AG, Darmstadt: Anfärbereagenzien für Dünnschicht- und Papier-Chromatographie.

[5] H. BÖRNER, Beitr. Biol. Pfl. *33*, 33 (1957).