A Thymidine-Induced Sex-Linked, Sex-Limited Temperature-Sensitive Lethal and the Significance of Modifier Genes in *Drosophila melanogaster*

In an earlier report, the author[1] investigated a group of eight sex-linked lethals induced in *D. melanogaster* by feeding it on thymidine-containing culture medium[2]. One out of these eight behaved as a temperature-sensitive lethal. This has been maintained in a balanced stock (against *y* Sc^{S1} *In* 49 Sc^{8}) in our laboratory. A pure stock of this lethal (denoted as 1^{ts}) has been established and kept at 26 °C. The fecundity and fertility are relatively good. The homozygous females are viable at 16° and 26 °C, the hemizygous males at 26 °C only. The present note is a complete analysis of this lethal (see also SUZUKI and DUCK[3] and SUZUKI, PITERNICK et al.[4]).

The stock employed for the localisation carried the markers *w sn B* in the *X*-chromosome. By the usual crossing-over technique the lethal factor 1^{ts} was found to be located at $52 \pm 1\%$. An examination of the salivary gland chromosomes stained with acetic-orcein revealed no gross changes and deletion of the size of one band could not be ruled out. It seems very probable that the lethal factor is a point mutation. Further, as the lethal effect of 1^{ts} is found to set in in the late third instar larvae/early pupae stage, this has been termed as a L/Pr-boundary lethal.

To explain the difference in the survival-behaviour of the males and females, as mentioned above, one might presume (see HADORN[5]) that in the females the activity of the ring-gland hormones produced is strong enough to make them cross the L/Pr threshold and let them survive at both temperatures. In males it is insufficient at 16° but strong enough for survival at 26 °C. However, this is just a conjecture.

The rest of the genotype being identical for both of the sexes, the temperature-sensitivity of the present lethal factor may be a result of its interaction with some modifying factor (denoted as '*m*') located on the *X*-chromosome. In males, the modifier is present in a single dose, whereas in females the dose is doubled (2 *X*-chromosomes) and the effect might be potentiated (HERSKOWITZ[6]).

To verify the above hypothesis, the homozygous females 1^{ts} *m*/1^{ts} *m* were mated to normal Oregon males and the emerging females 1^{ts} *m*/+ + in turn mated to *y* Sc^{S1} *In* 49 Sc^{8} males. The resulting recombinant and non-recombinant females were individually mated to *y* Sc^{S1} *In* 49 Sc^{8} males and a large number of cultures started at 26 °C. Out of 154 cultures checked for the presence or abscence of normal males, 13 were without normal males. Assuming the estimated number of the + *m* genotype to be equal to 1^{ts} + genotype, this would give a crossing-over rate of the order of 26/154 or 17%. The 13 cultures, corresponding to the genotype 1^{ts} + (devoid of *m*) behave as lethal both at 26° and 16 °C, being no longer temperature-sensitive. This observation is important from general biological considerations as it points out that the deleterious effects of certain genes might automatically be compensated by others. Further, the terms such as 'sub-vital', 'semi-lethal' and 'penetrance' win new significance in the light of the present observations. More experiments are under progress and will be reported elsewhere[7].

Zusammenfassung. Bei *Drosophila melanogaster* wurde ein temperaturabhängiger Letalfaktor gefunden. Die Temperaturabhängigkeit der Manifestation wird mit dem Vorhandensein von Modifikationsgenen erklärt.

O. PARKASH

Institut für Allgemeine Biologie der Universität, A-1090 Wien (Österreich), 3 February 1969.

1 O. PARKASH, Drosoph. Inf. Serv. *42*, 109 (1967).
2 O. PARKASH, Experientia *23*, 859 (1967).
3 D. T. SUZUKI and P. DUCK, Abst. Genetics Soc. of America, Stanford University, Stanford, California. 31 Aug. – 2 Sept. (1967).
4 D. T. SUZUKI, L. K. PITERNICK, S. HAYASHI, M. TARASOFF, D. BAILLIE and U. ERASMUS, Proc. natn. Acad. Sci. *57*, 907 (1967).
5 E. HADORN, *Advances in Genetics*, *45*, 53 (1951).
6 I. H. HERSKOWITZ, *Genetics*, 2nd Ed. (Little, Brown and Co., Boston 1965), p. 487.
7 The author is grateful to Prof. Dr. F. MAINX and Doz. Dr. D. SPERLICH for providing facilities for this work and useful suggestions.

Modifications with Chelating Agents of the Effects of γ-Ray Fractionated Exposures on Chromosome Aberrations

In previous experiments[1] with *Nigella damascena* seeds, we showed that fractionation of γ-ray doses results in a decrease of chromosome aberration frequencies when intervals of time between the two exposures are longer than 3 min 20 sec and shorter than 5 min 20 sec. In this plant material, the effect of fractionation was concerned with aberrations of the chromosome class only in which exchanges are much more affected than breaks. In this research, it was clear that we were dealing with fast rejoining processes in which ionic bonds are generally imputed[2,3] which excludes the participation of some active metabolism. In view of these experiments, chelating agents were tested to modify the effects of ionizing radiations and found to be active on chromosome breakage as well as cation starvation[4–6]. In this respect, ethylene diamine tetra-acetic acid (EDTA)[7,8], 2, 2′-dipyridyl[9] and sometimes Cupferron[10] were thought to act by a mechanism of chelation of nuclear components.

The present experiments are designed to test whether, in our biological system, chelating agents can influence

1 J. and M. MOUTSCHEN-DAHMEN and J. GILOT, Experientia *24*, 843 (1968).
2 D. MAZIA, Proc. natn. Acad. Sci. U.S.A. *40*, 521 (1954).
3 D. STEFFENSEN, Proc. natn. Acad. Sci. U.S.A. *41*, 155 (1955).
4 D. STEFFENSEN, Radiat. Res. *5*, 597 (1956).
5 D. STEFFENSEN, Genetics *42*, 239 (1957).
6 R. A. NILAN and L. L. PHILIPS, NW. Sci. *31*, 139 (1957).
7 S. WOLFF and H. E. LUIPPOLD, Proc. natn. Acad. Sci. U.S.A. *42*, 510 (1956).
8 A. L. DELONE, Dokl. Acad. Nauk SSSR *119*, 800 (1958).
9 N. S. COHN, Expl Cell Res. *24*, 596 (1961).
10 B. KIHLMAN, J. biophys. biochem. Cytol. *2*, 543 (1957).

Modifications of effect for each class of aberrations

Aberrations	Control	2,2'-Dipyridyl		8-Hydroxyquinoline		Cupferron		EDTA		Diethyldithiocarbamate	
	a	a	b	a	b	a	b	a	b	a	b
Breaks	0.597	0.789	3.043	0.982	1.565	1.348	2.162	0.895	0.753	1.426	2.522
Exchanges	0.669	0.925	1.347	1.162	1.253	1.561	1.011	1.310	1.138	1.628	1.744
Minutes	0.417	1.542	1.597	1.408	2.421	2.334	2.196	1.228	3.000	1.917	3.228
Total	0.581	0.971	1.692	1.208	1.615	1.636	1.387	1.232	1.538	1.677	2.261

a, ratios of the means of the 3 final levels to the 3 initial levels of the same series. b, ratios of the means of the 3 final levels of each chelating agent to the final control levels.

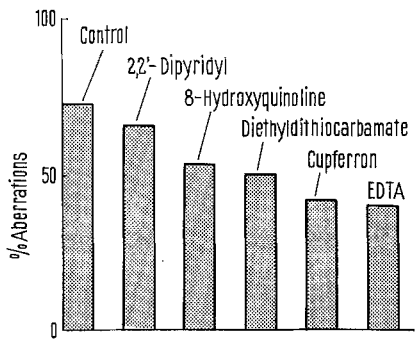

Fig. 1. Percentage of aberrations after unfractionated exposures (4 krad) of dry seeds treated with several chelating agents (1×10^{-4} M/8 h). (Analysis of 200 metaphases in each case.)

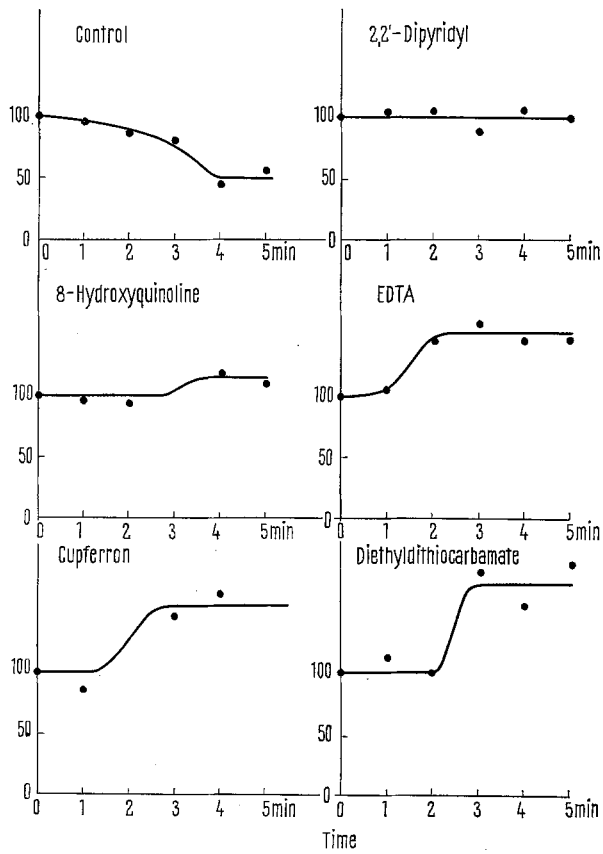

Fig. 2. Percentage of aberrations after fractionated exposures (2 × 2 krad) expressed in percent of the initial level (unfractionated exposure). (Analysis of 200 metaphases in each case.)

the effects of γ-ray fractionation. If so, it would be an indication of their action at the nuclear level. Therefore the localization of such cations at some important sites within the nucleus could be inferred. Since it was also found that chelating agents can themselves be chromosome breakers[11], proper concentration should first be carefully choosen.

Material and methods. The material (*Nigella damascena* dry seeds) has previously been described in detail[1]. They were treated with 1×10^{-14} M solutions for EDTA, Cupferron, 2,2'-dipyridyl, 8-hydroxyquinoline, diethyldithiocarbamate or distilled water (control) for 8 h and then redried to their initial level (8%). A preliminary experiment has shown that the concentration chosen was free of effect on chromosomes.

Seeds were irradiated with ^{60}Co γ-rays (Picker of 4200 Ci without filter) at the dose rate of 3 krad/min. Exposures were 2 times 2 krads separated by time intervals of 0, 1, 2, 3, 4, 5 min. Longer exposures, which could result in saturation of aberrations, were avoided. Intervals longer than 5 min, which did not show any difference in previous experiments, were not investigated. Technical manipulations after irradiation and cytological procedures have previously been reported in detail[1].

Results. The effects of all kinds of aberrations (in %) after chelating agents were compared with the control level (Figure 1) without dose fractionation. After all chelating agents, the effect was found to be lower. This is in agreement with the occurrence of a protective effect first described in mammals[12] after EDTA and diethyldithiocarbamate. The protective effect increases in the order 2,2'-dipyridyl, 8-hydroxyquinoline, diethyldithiocarbamate, Cupferron and EDTA.

On the other hand, it can be seen (Figure 2) that the decreased amount of aberrations normally occurring at intervals higher than 4 min is suppressed by all chelating agents. With 4 out of 5 investigated agents, there was a clear increase at intervals longer than 3 min. The intensity of this increase was in the order 2,2'-dipyridyl, 8-hydroxyquinoline, EDTA, Cupferron, diethyldithiocarbamate. For 2,2'-dipyridyl, the effect can be considered as positive since there is no significant decrease, as is the case in the control. The amount of aberrations scored after the increase was higher than the control level after the decrease each kind of aberrations being involved.

Data are given in the Table, in which final levels (mean amounts of aberrations after the increase) are compared with the initial levels (mean amount before the increase)

[11] B. Kihlman, Adv. Genetics *10*, 1 (1961).

[12] Z. M. Bacq, A. Herve and P. Fischer, Bull. Acad. r. Méd. Belg. *8*, 226 (1953).

(a) and the final levels (after the increase) with the control final level (after the decrease) (b). Comparison is made for each kind of aberration. Minutes have been separately classified, the major part of it consisting probably in 2 hit aberrations. In the control series all kinds of aberrations decrease. This decrease is higher for exchanges which confirms previous findings[1].

With all chelating agents investigated the ratios are significantly increased for all kinds of aberrations (b). The slight exception noted after EDTA for breaks was probably due to some sample error. Compared with the control, the absolute increase for the total aberrations is ordered: Cupferron, EDTA, 8-hydroxyquinoline, 2, 2'-dipyridyl, diethyldithiocarbamate.

Discussion and conclusions. The fractionation effect was clearly suppressed by all chelating agents, the efficiency of which being different for each kind of agent, which may depend on the stability constant of the chelating complexes. The present finding is a strong indication that ionic bonds are involved in the first rejoining processes of broken chromosomes suggesting a direct effect at the chromosome level. However, the protective effect after unfractionated doses or doses separated by small intervals could arise from an indirect mechanism as in mammals. New experiments designed to see the part played by different ions, as well as induced specific chromosome modifications, are in progress[13].

Résumé. Des graines sèches de *Nigella damascena* ont été traitées par les agents de chélation suivants: l'EDTA, le 2, 2'-dipyridyl, le Cupferron, la 8-hydroxyquinoléne et le diéthyldithiocarbamate avant d'être irradiées par les rayons γ du ^{60}Co à des doses fractionnées (2×2 krads) séparées par des intervalles de 0 à 5 min. On a constaté une suppression de l'effet de fractionnement et une augmentation absolue des fréquences de tous les types d'aberrations des chromosomes. Par contre, pour des intervalles courts ou nuls, un effet radioprotecteur est observé par rapport au témoin.

J. and M. MOUTSCHEN-DAHMEN and J. GILOT-DELHALLE[14]

Laboratoire de Génétique, Université de Liège (Belgium), 28 April 1969.

[13] The authors wish to express their gratitude to Dr. J. GARSOU and his collaborators for radiation facilities.

[14] Aspirant au Fonds National Belge de la Recherche Scientifique

A Correlation Between the Ability to Withstand High Temperatures and Radioresistance in *Drosophila melanogaster*

Single inseminated founder females of *D. melanogaster* collected in the wild have been shown to lead to strains which differ for several quantitative traits, such as scutellar and sternopleural chaeta numbers[1,2], mating speed and duration of copulation[3], ability to withstand temperature shocks[4], and resistance to irradiation with Co^{60} γ-rays[5]. As argued in these publications, this is indirect evidence that the wild populations are polymorphic for genes (or polygenes) controlling these traits[6]. The polymorphism for scutellar chaeta number has been exploited by HOSGOOD, MACBEAN and PARSONS[7] who found that when directional selection for high chaeta number was based on those strains which had a high scutellar chaeta number, extremely rapid responses were obtained.

In this paper, we discuss the ability of populations to withstand high temperatures in relation to their level of radioresistance to Co^{60} γ-rays. Referring first to the ability to withstand high temperatures, newly hatched larvae were placed into vials for each of 18 strains all derived from single inseminated females collected at Leslie Manor (Victoria)[1-5], and the number of adults that emerged at the extreme temperature of 30.5 °C was scored. This is a temperature at which it is difficult to culture *Drosophila*. The experiment was done twice, 2 generations apart, and the percentages that did not emerge are given in Table I. The data in Table I were transformed using the angular transformation to avoid a correlation between the raw percentages and their variances. The correlation coefficient between the angular values for the 18 strains in Table I came to 0.690, which is significantly > 0 at $P \approx 0.001$ (17 degrees of freedom). Thus, even though fewer flies emerged in the first than in the second experiment, there is a high degree of repeatability between experiments in spite of limited data. It is relevant that strain 2 is very sensitive to a

Table I. Percentage of flies that did not emerge as adults when grown at 30.5 °C

Strain	First experiment[a]	Second experiment[b]
1	92.5	32
2	89.5	64
3	53	12
20	57.5	12
21	48	28
22	68	15
23	88.5	28
24	68.5	16
25	64	17
26	74.5	17
27	72.5	33
28	61.5	32
29	54	7
30	64.5	18
31	86	37
32	88.5	32
33	77.5	30
34	75.5	24

[a] Percentages based on 4 replicates of 50 larvae. [b] Percentages based on 4 replicates of 25 larvae.

[1] P. A. PARSONS and S. M. W. HOSGOOD, Genetica *38*, 328 (1967).
[2] P. A. PARSONS, Aust. J. biol. Sci. *21*, 297 (1968).
[3] S. M. W. HOSGOOD and P. A. PARSONS, Aust. J. biol. Sci. *20*, 1193 (1967).
[4] S. M. W. HOSGOOD and P. A. PARSONS, Experientia *24*, 727 (1968).
[5] P. A. PARSONS, I. T. MACBEAN and B. T. O. LEE, Genetics *61*, 211 (1969).
[6] P. A. PARSONS, I. T. MACBEAN and B. T. O. LEE, Molec. gen. Genet. *99*, 165 (1965).
[7] S. M. W. HOSGOOD, I. T. MACBEAN and P. A. PARSONS, Molec. gen. Genet. *101*, 217 (1968).