4. N. N. Trapeznikov, V. V. Yavorskii, A. B. Surkin, et al., Vestn. Vses. Onkol. Nauch. Tsent. Akad. Med. Nauk SSSR, No. 1, 31 (1990).
5. S. N. Bykovskaya, I. F. Abronina, T. A. Kupriyanova, et al., Biomed. Pharmacol., **44**, 263 (1990).
6. M. Eura, T. Machara, T. Ikawa, et al., Cancer Immunol. Immunother., **27**, 147 (1988).
7. B. Mukherji, S. A. Wilhelm, A. Guha, et al., J. Immunol., **136**, 1888 (1986).
8. S. A. Rosenberg, M. T. Lotze, L. M. Muul, et al., New Engl. J. Med., **16**, 889 (1987).
9. T. Tage, K. Kuroi, Y. Kininobu, et al., Clin. Exp. Immunol., **74**, 409 (1988).
10. F. Vanky, A. Peterffy, K. Böök, et al., Cancer, Immunol. Immunother., **16**, 17 (1983).
11. J. Wang, A. Wall, B. Gordon, et al., Am. J. Med., **83**, 1016 (1987).
12. W. J. Urba, J. W. Clark, R. G. Steis, et al., J. Nat. Cancer Inst., **89**, 602 (1989).
13. A. Yu, H. Watts, N. Jaffle, et al., New Engl. J. Med., **297**, 121 (1977).

SPECIFICITY OF THE TOXIC GENETIC ACTION OF CARCINOGENIC AROMATIC COMPOUNDS ON MUS-MUTANT STRAINS OF *Drosophila*

V. S. Shpigel'man, S. Yu. Fuks, R. D. Safaev, and G. A. Belitskii

UDC 615.918:582.28].015.4:]612.052:575.224.23

KEY WORDS: *Drosophila*; genetic toxicity; specificity; metabolism; precarcinogens

Genetic toxic effects of chemical carcinogens are revealed in *Drosophila* in the form of mutations in sex or somatic cells [1] or as their lethal action on larvae. The toxic effect increases to the level of hypersentivity in specially created mutant strains, defective for DNA repair [7]. Among these strains, the mus-mutants of *Drosophila melanogaster* obtained by Henderson and co-workers, and characterized by sensitivity to several direct and indirect action carcinogens [5], are of great interest. Since precarcinogens are activated to genetic toxic metabolites by microsomal monooxygenases [6], it was important to discover if correlation exists between the sensitivity of individual strains to precarcinogens and the activity of these enzymes in them. To use mus-mutants as the test object for screening carcinogens, it is also necessary to know how specific their sensitivity to these agents may be, i.e., to study their effects parallel with the action of noncarcinogenic analogs.

EXPERIMENTAL METHOD

Reagents. Benz(a)pyrene [B(a)P] was obtained from "Fluka AG," Switzerland; benz(e)pyrene [B(e)P] from "Schuchardt," West Germany; 2-acetylaminofluorene(2-AAF)and dimethyl sulfoxide (DMSO) from "Serva," West Germany; pyrene and fluorene were of USSR origin and of the chemically pure grade.

Strains of *D. melanogaster* mus210, mus208^{B1}, mus208^{B2}, and mus205^{B1}, and also the original strain b pr cn/Cy 0, not containing the mus locus, were generously provided by Dr. Henderson.

For the experiments on a standard nutrient medium, 10 homozygous pr cn*/b pr cn* females and 7 or 8 heterozygous b pr cn*/Cy 0 males were transferred into each glass container. After 48 h the parents were removed and 200 μl of an emulsion of the test substances in 10% DMSO solution was applied to the surface of the 2-day *Drosophila* cultures. The

Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 11, pp. 521-523, November, 1991. Original article submitted April 25, 1991.

TABLE 1. Toxic Action of B(a)P on Larvae of *D. melanogaster* mus-mutants and on Aryl Hydroxylase Activity in S15 Fractions of Homogenates of Their Imagos

Strain	Survival rate					Aryl hydroxylase activity, pmoles 3-OH-BP/mg protein/min***
	Control (DMSO), homozygotes*	B(a)P, 0.05%		b(a)P, 0.1%		
		homozygotes*	heterozyg.**	homozygotes*	heterozygotes	
b pr cn/Cy O	1961/2081 (0,94)	1824/1880 (0,97)	90,3	1433/1905 (0,76)	91,5	53±5
mus205^{B1}	852/1942 (0,44)	366/1846 (0,20)	95,1	287/1752 (0,16)	90,2	49±9
mus208^{B1}	2253/2620 (0,86)	564/2562 (0,22)	97,8	714/2551 (0,28)	97,4	60±4
mus208^{B2}	1126/1808 (0,62)	96/1372 (0,07)	75,9	4/1210 (0,003)	66,9	53±6
mus210	2159/2918 (0,74)	90/2994 (0,03)	101	104/2603 (0,04)	89,2	61±4

Legend. *) ratio of homozygotes/heterozygotes; absolute number of imagos flying off and ratio between them in parentheses; **) ratio of number of heterozygotes flying off in groups to their number in control (in percent); ***) mean values of activity (M ± m) calculated for 5 experiments.

genetic toxic effect was estimated by the ratio of the number of homozygotes flying off in F_1 to the number of heterozygotes in the comparable control group, treated with 10% DMSO solution [5]. The nonspecific toxic action of the test compounds was assessed by the ratio of the number of heterozygotes in the experimental and control groups.

Aryl hydroxylase activity was determined in the S15 fraction obtained by centrifugation of homogenates of 7-9-day adult flies at 15,000*g* in 0.1 M phosphate buffer, pH 7.4, by the fluorometric method of Dehnen and co-workers [3] on a "Hitachi-850" spectrophotometer (Japan). The protein concentration was determined by Albro's method [2].

EXPERIMENTAL RESULTS

Larvae of homozygotes of all the mus-strains investigated proved to be more sensitive to B(a)P than the original strain (Table 1); the genetic toxic effect on strains mus208^{B2} and mus210, moreover, was much stronger than in strains mus205^{B1} and mus208^{B1}, in good agreement with the results of Henderson et al. [5]. Meanwhile, no differences between the strains were found for aryl hydroxylase activity in S15 fractions obtained from homogenates of adult flies. Most probably there are no such differences in these larvae, for the hormonal background, which determines expression of microsomal monooxygenases is the same in the imago and the young larva [4]. This suggests that the principal role in the mechanism of sensitivity of the test strains to B(a)P is played, not by the characteristics of its metabolism, but by disturbances of DNA repair. This result was not evident, since ethyl methanesulfonate, by means of which the mutations disturbing the work of DNA repair enzymes were obtained, could also induce other mutations, including those relating to the cytochrome P-450 system. The results show that the level of metabolic activation of B(a)P in somatic cells of *Drosophila* larvae was high enough to obtain a well marked genetic toxic action, although, however, it is not manifested in flies with a normal level of repair processes because of their high efficiency.

As Table 1 shows, in strain mus208^{B2} not only the homozygotes, but also the heterozygotes were sensitive to B(a)P, evidence either of the incomplete recessiveness of its mus-mutation or of the presence of a second dominant, with weak manifestation. Accordingly, subsequent experiments to study specificity of the genetic toxic effect of the precarcinogens were limited to larvae of the mus210 strain, in which only homozygotes are sensitive to B(a)P (Table 1).

It will be clear from Table 2 that analogs of B(a)P, unable to induce tumor growth in mammals, namely pyrene and B(e)P, a promotor of skin cancer [8], did not increase mortality among the homozygous larvae, i.e., they had no genetic toxicity. Fluorene, known as a cocarcinogen in skin carcinogenesis, induced by 3-methylcholanthrene in mice [8], in a concentration of 0.1% had a toxic effect on the level of LD50 in homozygotes and on the level of LD25 in heterozygotes. So far as the second carcinogen (2-AAF) is concerned, just as in the experiments of Henderson and co-workers [5], it proved to be highly toxic for homozygotes and sufficiently toxic also for heterozygotes (Table 2).

TABLE 2. Toxic Action of Carcinogenic and Noncarcinogenic Aromatic Compounds on Larvae of Strain mus210 of *D. melanogaster*

Compound (concentration)	Carcinogenicity for mammals	Survival rate	
		homozygotes*	heterozygotes**
Control			
a		1478/1797 (0,82)	100
b		874/1131 (0,77)	100
c		2206/2918 (0,74)	100
Pyrene 0,05 %[b]	—	761/999 (0,76)	88,3
Pyrene 0,10 %[b]		680/841 (0,81)	74,4
B(e)P 0,10 %[a]	—	1492/2071 (0,72)	115
B(a)P 0,10 %[c]	+	90/2994 (0,03)	101
B(a)P 0,10 %[c]		104/2603 (0,04)	89,2
Fluorene 0,10 %[a]	—	581/1315 (0,44)	73,2
2-AAF 0,10 %[a]	+	0/423 (0,00)	23,5

Legend. a, b, c) control groups for corresponding experimental groups. Remainder of explanation as in Table 1.

From the practical point of view strain mus210, which is highly sensitive to the action of precarcinogens and resistant to the action of their analogs, can be used as a battery for screening carcinogens, as a test system providing information in addition to that obtained by mutagenicity tests in *Drosophila.*

LITERATURE CITED

1. S. Yu. Fuks and G. A. Belitskii, Vopr. Onkol., **8**, 911 (1990).
2. P. W. Albro, Analyt. Biochem., **64**, 485 (1975).
3. W. Dehnen, R. Tomingas, and J. Roos, Analyt. Biochem., **53**, 373 (1973).
4. W. Gering and R. Nothüger, Developmental Systems: Insects, ed. by S. Counce and C.H. Waddington, Vol. 2, New York (1973), p. 211.
5. D. S. Henderson, A. Bailey, D. A. R. Sinclair, and T. A. Grigliatti, Mutat. Res., **177**, 83 (1987).
6. J. A. Miller, Cancer Res., **30**, 569 (1970).
7. T. D. Nguyen, J. B. Boyd, and M. M. Green, Mutat. Res., **63**, 67 (1979).
8. "Polynuclear aromatic compounds," IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 32, Lyon (1983), pp. 211, 225, 365, 431.