Effects of Some Chemicals on the Facet Number Variation in Bar-Eyed Mutant of *Drosophila melanogaster*[1)]

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Seyster found in 1911 that the facet number of the Bar-eyed mutant in *Drosophila melanogaster* varied under the influence of temperature. Then the Bar-eyed mutant was used by several investigators as a valuable tool in the study of physiological genetics. In addition to the temperature, numerous factors were found to modify the phenotype of the Bar-eyed mutant : they are X-rays (Luce, Quastler & Chase '51), oxygen (Margolis '39), nutrition (Krafka '20), extract of insect (Ephrussi, Khouvine & Chevais '38), and various chemicals (Hinshow '41, and Khouvine, Chevais & Grégoiré '43). Further it was found that the chemicals such as amino acids, imidazol derivatives, ureids, and acid amides strongly increased the facet number of Bar-eyes, and that in some cases they converted the phenotype of Bar-eyes into the wild or the heterozygotic type (Kaji '54). On the other hand, there are reports that the facet number of Bar-eye is reduced through the treatment of formalin (Hinshow '41, Luce '41). It is of interest to investigate whether any chemicals other than formalin influence or not to reduce the facet number of Bar-eye from the viewpoint of physiological genetics. In this paper, the author wishes to report the results of some preliminary experiments undertaken from the above viewpoint.

Material and methods

The *Bar* and *Ultra-Bar* strains of *Drosophila melanogaster* employed as material in the present study were derived from the stocks maintained at the Kyôto University. A few pairs of *B* or *BB* flies were cultured in the glass tube, 14 cm in length, 2.5 cm in diameter, containing 5 cc of the culture medium with living yeast. Chemicals which are to be examined were added in definite concentration to the ordinary culture medium. Percentages shown in Tables 1 and 2 denote the rate of chemicals to the culture medium, which consists of white flour 65 g, sugar 25 g, agar 7 g, and water 550 cc. In oxyquinolline, scarlet and Sudan III, the concentrations were indicated by cc of the alcoholic saturated solutions in the culture medium, and then the letter S is used to designate the con-

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centration in Tables 1 and 2.

In each experiment, the culture medium without chemicals was prepared to use as a control. Throughout the experiments the temperature was kept at 25° to 28°C. The facet number of the left eye was counted in the next progeny. In some exceptional cases the facet number of the right eye was counted. Flies for observation were sampled randomly from early hatched flies.

Table 1. Facet numbers of Ultra-Bar flies fed on various chemicals.

Chemicals	Concentration	Obs. no.	♀♀ Facet number Min.-Max.		Mean	Obs. no.	♂♂ Facet number Min.-Max.		Mean
a Formalin	Control	10	34	45	39.2	10	40	69	53.6
	0.08%	20	22	35	28.4*	20	26	49	38.5*
b Potassium cyanide	Control	10	34	53	41.2	10	43	66	52.6
	0.04%	20	34	53	41.7	16	32	69	43.7
c Thiosulphate sodium	Control	10	29	78	49.6	10	37	74	60.5
	0.05%	12	44	75	53.3	5	41	76	62.6
d Dinitrophenol	Control	9	32	47	40.2	10	35	61	45.2
	0.00002M	9	31	49	40.4	3	43	51	45.6
	0.0001 M	10	23	39	30.3*	10	38	72	46.5
	0.0002 M	20	19	40	30.9*	19	25	75	49.7
e Cresol	Control	42	23	57	38.6	38	44	79	57.2
	Ort- 1%	5	26	45	38.6	2	59	77	68.0
	Ort- 2%	14	34	58	43.0	12	47	74	59.4
	Ort- 3%	35	20	49	32.7	14	44	68	53.2
	Ort- 4%	39	27	63	35.7	25	33	68	45.5
	Ort- 5%	18	28	43	33.1	21	41	62	48.3
	Meta- 1%	9	24	42	31.5	7	32	70	47.3
	Meta- 2%	18	22	50	31.5	24	39	72	58.8
	Meta- 3%	10	34	54	44.5	10	39	66	52.9
	Meta- 4%	9	24	44	32.8	4	36	48	41.6
	Meta- 5%	24	24	38	30.8	15	37	56	44.4
	Para- 1%	13	26	46	36.0	11	40	75	59.8
	Para- 2%	15	23	40	29.4	13	31	67	51.8
	Para- 3%	5	37	46	42.0	7	42	85	61.3
	Para- 4%	30	25	41	33.6	30	36	77	47.5
	Para- 5%	25	25	44	35.6	48	37	63	51.3
f Oxyquinolline	I Control	10	34	53	41.2	10	43	66	52.6
	I 0.5 S	10	34	50	40.3	10	33	61	50.8
	I 1. S	10	30	48	37.2	10	25	74	40.2
	II Control	8	35	53	42.5	5	38	62	51.8
	II 1. S	7	28	31	29.7	5	33	70	40.4
g Piridin	Control	3	34	40	36.3	6	34	65	51.8
	0.05%	10	35	56	43.3	10	41	81	57.1
	0.2 %	7	42	57	47.7	5	42	63	51.4

* means that difference from its control is statistically significant with more 95% of confidence.

Results of experiments

Effects on Ultra-Bar flies: Examination of reducing effect of formalin on *Ultra-Bar* was made in a 0.08% solution using the *BB* strain. The results obtained are shown in Table 1, a. The facet number of *BB* flies fed on 0.08% formalin are reduced significantly in comparison with the control flies.

Table 2. Facet number of Bar flies fed on various chemicals.

Chemicals		Concentration	♀♀ Obs. no.	♀♀ Facet number Min.-Max.	♀♀ Mean	♂♂ Obs. no.	♂♂ Facet number Min.-Max.	♂♂ Mean
a Phenol		Control	17	25 79	63.2	17	45 105	75.2
		0.1%	15	35 67	45.9	10	64 102	82.7
		0.2%	6	36 62	49.5	8	64 76	70.2
b Hydroquinnone		Control	17	25 79	63.2	17	45 105	75.2
		0.2%	15	49 68	54.6	8	60 73	68.4
c Phloroglucinol		Control	17	25 79	63.2	17	45 105	75.2
		0.1%	3	51 56	53.3	7	64 89	73.2
		0.2%	6	30 46	36.3*	12	49 78	60.6
d Ethanol		Control	10	38 74	58.0	5	54 115	73.2
		19%	10	32 47	39.4*	10	43 111	62.5
e Boric acid		Control	20	46 103	65.1	19	50 92	72.7
		0.01%	2	32 43	37.5	11	44 77	60.2
f Oxyquinolline		Control	4	56 87	66.5	4	75 110	92.5
		1S	7	35 52	44.0	1		50.0
g Acriflavine	I	Control	9	53 87	64.4	6	60 85	74.5
		0 01%	10	39 50	42.7*	11	35 66	50.3*
	II	Control	20	46 103	65.1	19	50 92	72.7
		0.08%	12	28 48	41.6*	8	53 77	66.2*
h Butter yellow		Control	20	46 103	65.1	19	50 92	72.7
		0.02%	10	39 80	55.7	10	51 98	75.1
		0.05%	11	27 46	33.8*	9	39 60	47.6*
i Scarlet	I	Control	9	53 87	64.4	6	60 85	74.5
		0.01S	4	35 39	37.0	11	51 96	70.2
		0.03S	3	37 42	39.3	10	44 87	60.7
		0.05S	10	27 45	33.2*	10	26 55	45.4*
	II	Control	10	38 74	58.0	5	54 115	73.2
		0.01S	17	41 65	51.5*	7	75 116	87.4
		0.05S	10	27 45	33.2	8	41 94	56.2*
j Sudan	III	Control	20	46 103	65.1	19	50 92	72.7
		0.03S	7	58 75	66.4	5	65 75	70.0
		0.05S	10	44 61	52.7	9	58 80	70.3
		0.1 S	5	33 42	38.1*	3	44 65	55.6*

* means that difference from its control is statistically significant with more 95% of confidence.

Potassium cyanide and 2–4, dinitrophenol, both of which were commonly known as inhibitors of oxidative enzymes, were used to observe reducing effect of the facet number of *BB* flies. The reducing effect on the facet number was not clearly indicated by the treatment with 0.04% potassium cyanide (Table 1, b), but the facet numbers of female *BB* flies were reduced significantly in 0.0002 M and 0.0001 M of dinitrophenol (Table 1, d).

Thiosulphate sodium, ort-, meta-, and para-cresol, oxyquinolline, and piridin were examined to learn the reducing effect on the facet number of *BB* flies. The results obtained here are shown in Table 1, c,e,f, and g. They gave no clear effect of reduction on facet number, except oxyquinolline. Through the treatment with oxyquinolline (1 S), the facet number of *BB* flies was reduced in comparison with the results of the controls in both of I and II experiments, though not statistically significant.

Effects on Bar flies: Since the reducing effect of dinitrophenol on the facet number of *BB* flies was seen, phenols (such as phenol, hydroquinnon and phloroglucinol), ethyl alcohol, and oxyquinollin were examined to see a similar effect on *Bar* flies. The results of these experiments are as shown in Table 2, a to e, 0.2% phloroglucinol and 19% ethanol showed a slight reducing effect, especially in female flies. No clear effects were given by treatment with other chemicals.

Boric acid and carcinogenic dyes such as acriflavin, butter yellow, scarlet and Sudan III come under examination for reducing effect on the facet number of *Bar* flies. From the results of these experiments as indiacted in Table 2, f to j, it is possible to say that all of carcinogenic chemicals give a remarkable reducing effect on the facet number of *Bar* flies, with exception of boric acid, since the flies examined with the latter chemical are too small in number. A similar reducing effects were shown in the repeated experiments of the examination with acriflavine and scarlet.

Remarks and conclusion

Reviewing the data presented in this study, two points call attention for discussion. The first deals with a comparison between the action of so-called facet increasing substances (B^+-substances) and those of chemicals examined here. B^+-substances act more intensely on the male than on the female (Kaji '51). On the contrary, the reducing effect was more manifest in the female than in the male. The second point is a fact that carcinogenic dyes show a reducing act on the facet number of the Bar-eyed mutant like nitrogen mustard. Kaji and Ogaki ('51) found that phenocopies of eyeless or Bar-eyed mutants in the wild flies were induced by the treatment of nitrogen mustard.

It seems probable from the preliminarly experiments reported here that carcinogenic dyes such as acriflavine, scarlet and Sudan III, and some kinds of phenols such as dinitrophenol and phloroglucinol have a similar reducing effect as formalin on the facet number of Bar-eyed mutant, though the effects were not very remark-

able, in comparison with the effects of B^+-substances such as acid amides. Since these chemicals affect indirectly the process of Bar-eye formation, it is necessary to find chemicals with more direct action on the reducing facet number of Bar-eyed mutant in the future.

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