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Mutagenicity of nickel sulphate in *Drosophila melanogaster*

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Summary

Nickel sulphate was injected into *Drosophila melanogaster* males at different concentrations in order to test the chemical for the induction of SLRL and SCL in germ cells. Nickel sulphate induced SLRL at concentrations tested, with the peak of activity at premeiotic and postmeiotic stages. It failed to produce SCL except at the highest concentration tested, where induction of XO males was significant for the pooled data.

Nickel compounds were shown to induce chromosome aberrations (Nishumura and Umeda, 1979; Umeda and Nishimura, 1979) and morphological transformations (Saxholm et al., 1981) in mice. In vitro exposure of mammalian cells to nickel inhibited cellular uptake of [^{3}H]thymidine and somatic mutations (Sunderman, 1981). Nickel reacts readily with DNA and decreases the fidelity of DNA synthesis (Sirover and Loeb, 1976; Miyaki et al., 1979). The carcinogenicity, mutagenicity and teratogenicity of nickel have been reviewed recently by Léonard et al. (1981).

It has been claimed that soluble nickel compounds are in general not carcinogenic in animals while sparingly soluble ones have tumorigenic properties (Nishimura and Umeda, 1979).

Nickel is a human carcinogen (Doll et al., 1970; Saknyn and Shabynina, 1970; Sunderman, 1977; Høgetveit and Barton, 1976). In workers occupationally exposed to nickel, increments in chromosome aberrations or SCEs have not been found, although gaps were significantly increased (Waksvik and Boysen, 1982). Nickel sulphate is a soluble salt which is not carcinogenic in rats (Kazarantzis and Lilly, 1979) but induces SCE in Don Chinese hamster cells (Ohno et al., 1982) and in human lymphocytes in culture (Newman et al., 1982). In *Saccharomyces cerevisiae*, $NiSO_4$ did not induce reverse mutations and gave a weak positive response in tests for conversion (Singh, 1984). In Syrian hamster embryo cells, Rivedal and Sanner (1980) observed a mutagenic synergism between nickel sulphate and benzo[*a*]pyrene.

We performed the following experiments in order to learn whether nickel sulphate produces mutagenic effects in *Drosophila melanogaster*. Male germ cells were treated with nickel sulphate and assayed for the inductions of sex-linked recessive lethals (SLRL) and sex-chromosome loss (SCL).

Materials and methods

Strains. For SLRL, white males were treated and crossed to BASC females. For SCL, ring X males of the genotype X^{c2} y B/sc^8 Y were treated and crossed to y^2 w^a females.

Stocks were maintained in mass cultures at 25 ± 1°C.

Culture medium. Food medium was prepared with 9.2% agar-agar, 15.4% D-glucose, 21.5% sugar,

TABLE 1

INDUCTION OF SEX-LINKED RECESSIVE LETHALS IN DROSOPHILA MALES INJECTED WITH NICKEL SULPHATE

Concentration (ppm)	A		B		C		D		A+B+C+D [a]	
	(0–2 days)	%	(2–4 days)	%	(4–7 days)	%	(7–10 days)	%	(0–10 days)	%
Control	4/1204	0.33	4/1436	0.28	2/926	0.22	2/1230	0.16	12/4796	0.25
200	8/1102	0.73 [b]	2/1024	0.19	2/1100	0.18	12/1582	0.76 [b]	24/4818	0.50 [b]
300	6/708	0.85 [b]	2/1078	0.19	2/1028	0.19	20/1376	1.45 [b]	30/4190	0.72 [b]
400	10/1226	0.82 [b]	3/1006	0.30	2/1140	0.17	28/1700	1.65 [b]	43/5072	0.85 [b]

[a] Sum of 2 Expts.
[b] Significant at the 5% level (Kastenbaum-Bowman test).

38.5% corn meal, 9.2% yeast, 3.1% propionic acid, 3.1% nipagin and 1000 ml distilled water.

Chemicals. $NiSO_4 \cdot 6H_2O$ (Merck) was dissolved in 5% sucrose solution at 200, 300 and 400 ppm. In a preliminary test the LD_{50} was found to be at 400 ppm. Adult males 0–48 h old were injected intraperitoneally with the salt. Control males were injected with 5% sucrose solution.

Mutagenicity test. (1) SLRL test. White males 0–2 days old (treated and control) were crossed with BASC virgin females 1–3 days old, for two days at a ratio of one male × two females. The progeny of each treated male was kept separately to identify possible clusters of lethals. To test the sensitivity of different germ cell stages, a brood pattern analysis was performed (Broods A, B, C, D) by transferring the males to fresh virgin females at intervals of 2–2–3–3 days. F_1 females auto-

TABLE 2

FREQUENCIES OF OFFSPRING RESULTING FROM SEX-CHROMOSOME LOSS IN DROSOPHILA MALES INJECTED WITH NICKEL SULPHATE

Concentration (ppm)	Brood	Total offspring [a]	Exceptional			
			Males XO	%	Females XXY	%
Control	A	3560	3	0.08	2	0.06
	B	3137	1	0.03	1	0.03
	C	3230	3	0.09	1	0.03
Total	A+B+C	9927	7	0.07	4	0.04
200	A	3269	8	0.24	2	0.06
	B	3557	4	0.11	4	0.11
	C	3421	7	0.20		
Total	A+B+C	10247	19	0.19	6	0.06
300	A	3415	8	0.23	2	0.06
	B	3156	3	0.10	6	0.19
	C	3260	8	0.24	2	0.06
Total	A+B+C	9831	19	0.21	10	0.10
400	A	3337	8	0.24	2	0.06
	B	3138	10	0.32	7	0.22
	C	3212	8	0.25	2	0.11
Total	A+B+C	9687	26	0.27 [b]	11	0.11

[a] Sum of 3 Expts.
[b] Significant at the 5% level on chi square test.

fertilized by F_1 males were intercrossed in vials and the F_2 scored for sex-linked recessive lethals. F_3 was retested.

(2) SCL test. 0–2 day old ring X males, treated or kept untreated as controls, were mass-mated in bottles with 1–3 day old virgin y^2 w^a females for 2 days (A) followed by 1 or 2 successive broods (B, C), at a ratio of 3 females per male. The F_1 offspring were scored for exceptional XO males and XXY females.

Statistical calculations. The sample size of each exposure was 100 males treated/concentration and control. (1) SLRL were calculated by the Kastenbaum–Bowman (1970) tables. (2) SCL chi square was applied to test the significance.

Results and discussion

Table 1 summarizes the results for the SLRL test. The data clearly show a linear concentration–effect relationship. Also a marked difference in mutational response between postmeiotic, meiotic and premeiotic stages can be noted. In broods A (0–2 days) and D (7–10 days) the mutation rates are significantly enhanced at all concentrations at the 5% confidence level (Kastenbaum–Bowman test) (1970). For broods B (2–4 days) and C (4–7 days) mutagenic activity is not apparent.

Table 2 shows the results obtained for sex-chromosome loss (SCL). Nickel sulphate did not significantly increase the number of XXY females at all concentrations employed. Regarding the induction of XO males a positive response was found at the highest concentration (400 ppm) tested, which represents the LD_{50}, where the pooled data of the treated group (broods A + B + C) are significant at the 5% confidence level in chi square test.

In summary, induction of sex-linked recessive lethals by $NiSO_4$ occurred at all concentrations tested while total sex-chromosome loss was only detectable at significant numbers at the highest concentration. Thus we can conclude that nickel sulphate is mutagenic in the SLRL array in *Drosophila melanogaster* and gives a weak response in the assay for chromosome loss.

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