the 18 genetically characterized temperature-sensitive paralytic mutants, viz. *no action potential*^{ts} and *paralysed*^{ts} (refs 16–18). This report on veratridine resistance of yet another ts paralytic mutant shows the need to screen other members of its class for their resistance to neurotoxins and insecticides.

Resistance to veratridine, DDT and pyrethroids is correlated with reduced neuronal sensitivity in *nap*^{ts} (ref. 17) which, in turn, is due to a reduction in the number of sodium channels[19]. There have been no neurophysiological studies on *stm A* to demonstrate either of these possibilities. There is evidence that delayed onset of lethality to toxins and pesticides, similar to that reported in *stm A*^{1} (Figure 3), is consistent with resistance mechanisms involving reduced target sensitivity[17,20,21]. This does not rule out the possibility of other mechanisms like efficient detoxification or reduced penetration of the toxin. However, because the behavioural paralytic phenotype and veratridine resistance of *stm A*^{1} are mutationally inseparable it is reasonable to expect a mechanism of resistance involving neuronal functions rather than one involving metabolic differences. This question needs more investigation.

In conclusion, this preliminary report on the resistance of *stm A* to veratridine, suggests that paralytic mutants of *D. melanogaster* should be increasingly used to study mechanisms of neurotoxin and insecticide resistance. The availability of P element alleles of *stm A*, in addition, open up the future possibility of its molecular cloning.

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Germ cell cytotoxicity in tumour-bearing mouse subjected to single therapeutic dose of *cis*-diamminedichloroplatinum (II)

Tanusree Biswas and Samar Chakrabarti
Department of Zoology, University of Burdwan, Burdwan 713 104, India

Cytotoxic potential of cisplatin on testicular germinal cells of sarcoma-bearing mice has been assessed at single therapeutic dose at certain chosen post-treatment time points. It was observed that the drug affected testicular germinal cells of both tumour and nontumour mice to a significant extent and cytotoxicity was manifested in the form of structural chromosome aberrations and precocious desynapsis of sex bivalent. It was further observed that both differentiating spermatogonia and primary spermatocytes were equally sensitive to the clastogenic action of cisplatin. The possible significance of the findings has been discussed.

DAMAGE to testicular germinal cells is a potential side effect of cancer chemotherapy. This is of particular concern to cancer patients of reproductive age who are subjected to prolonged course of chemotherapy. Information on the nature and extent of germinal cell damage in response to drug exposure will be of value for better understanding of drug action and better monitoring of chemotherapy. This can be achieved by employing tumour-bearing mouse as experimental model because of the similarity, in many respects, in the spermatogenesis of man and mouse[1,2]. It is known that any damage to spermatogonial stem cells would result in long term infertility and genetic risk[3,4]. Meiotic chromosome analysis at diak-meta. I constitutes an important parameter of evaluating germ cell damage at different phases of spermatogenesis depending on the time interval chosen between the time of drug exposure and the cell sampling.

Cisplatin, the commercially available preparation of *cis*-diamminedichloroplatinum (II), is an effective antineoplastic drug widely used at present to combat various forms of human malignancies[5]. The drug is at the same time a mutagen, a teratogen and a suspected carcinogen[6]. Naturally the potential hazards of its wide application cannot be ignored. Here we

report on the cytotoxic potential of cisplatin on the testicular germinal cells of tumour-bearing mouse. The main objective of the study was to determine the nature and extent of chromosome damage in the testicular cells and to find out if there is any meiotic stage specificity of cisplatin clastogenicity. So far as we are aware, there is no report on cisplatin mediated germ cell toxicity on a mouse tumour model.

Three-month-old outbred strain of Swiss albino mice (25–30 g bw) bearing ascites form of sarcoma 180 at log phase of tumour growth, constituted the experimental material for the present study[8]. Single therapeutic dose of cisplatin for this tumour model was determined by repeated trial of the different doses of the drug (viz. 1 mg, 2.5 mg, 4 mg, 7.5 mg and 11.5 mg kg^{-1} bw) in separate sets of sarcoma-bearing mice. A dose equivalent to 7.5 mg kg^{-1} bw yielded a maximum regression of tumour with a minimum rate of mortality. The mean survival time (MST) for cisplatin-treated tumour specimens increased up to 41 days against 32 days in control specimens.

Germinal cell toxicity was assessed from tumour specimens exposed *in vivo* to cisplatin (Choongwae Pharma, Seoul) at 7.5 mg kg^{-1} bw. Sampling of testicular cells was made, according to Oakberg time schedule for mouse spermatogenesis, on day 1 (to see the effect on spermatogonial metaphase), days 11 and 12 (to see if the spermatocytes are affected), and on days 18–20 (to see if the differentiating spermatogonia are the target of cisplatin action). The experiment was conducted with four tumour-bearing specimens at *each* post-treatment time point. A parallel control was maintained with specimens exposed to an identical amount of drug solvent. In a separate set, normal non-tumour mice of the same age group were exposed to the same concentration of cisplatin and the testicular cells were sampled at identical post-treatment time points for comparison.

Chromosomes from spermatogonial cells and from primary spermatocytes of both treated and control specimens were prepared by the following standard techniques[9,10]. Germ cell cytotoxicity was evaluated by assessing chromosome damage in the form of true breaks and exchanges which definitely involve DNA damage. The occurrence of premature desynapsis of sex bivalent was also recorded as additional parameter[3,11]. Gaps and lesions were scored but not pooled in final data because of the difficulty in their precise identification under light microscope.

Student's *t* test and analysis of variance were applied to compare the test value and the corresponding control.

An examination of spermatogonial metaphases sampled on day 1 from treated specimens revealed the existence of chromosome structural changes in the form of true DNA breaks and exchanges of robertsonian type (Figure 1 *b*). Examination of diak-meta. 1 spreads from spermatocytes sampled on days 11 to 20 revealed a high frequency of cells with translocation multivalents, true breaks and fragments (Figures 1 *c,d*). All the translocation multivalents observed were of autosomal type, and no X-autosome translocation was encountered in the present study. The occurrence of cells with X and Y univalency was recorded in high frequency in treated specimens at all post-treatment time points (Figure 1 *f*). Though no remarkable structural chromosome change was noted in solvent control series the occurrence of cells with X and Y chromosome univalency was always encountered. However, the frequency of such cells was always lower than that of the two treated series (Table 1).

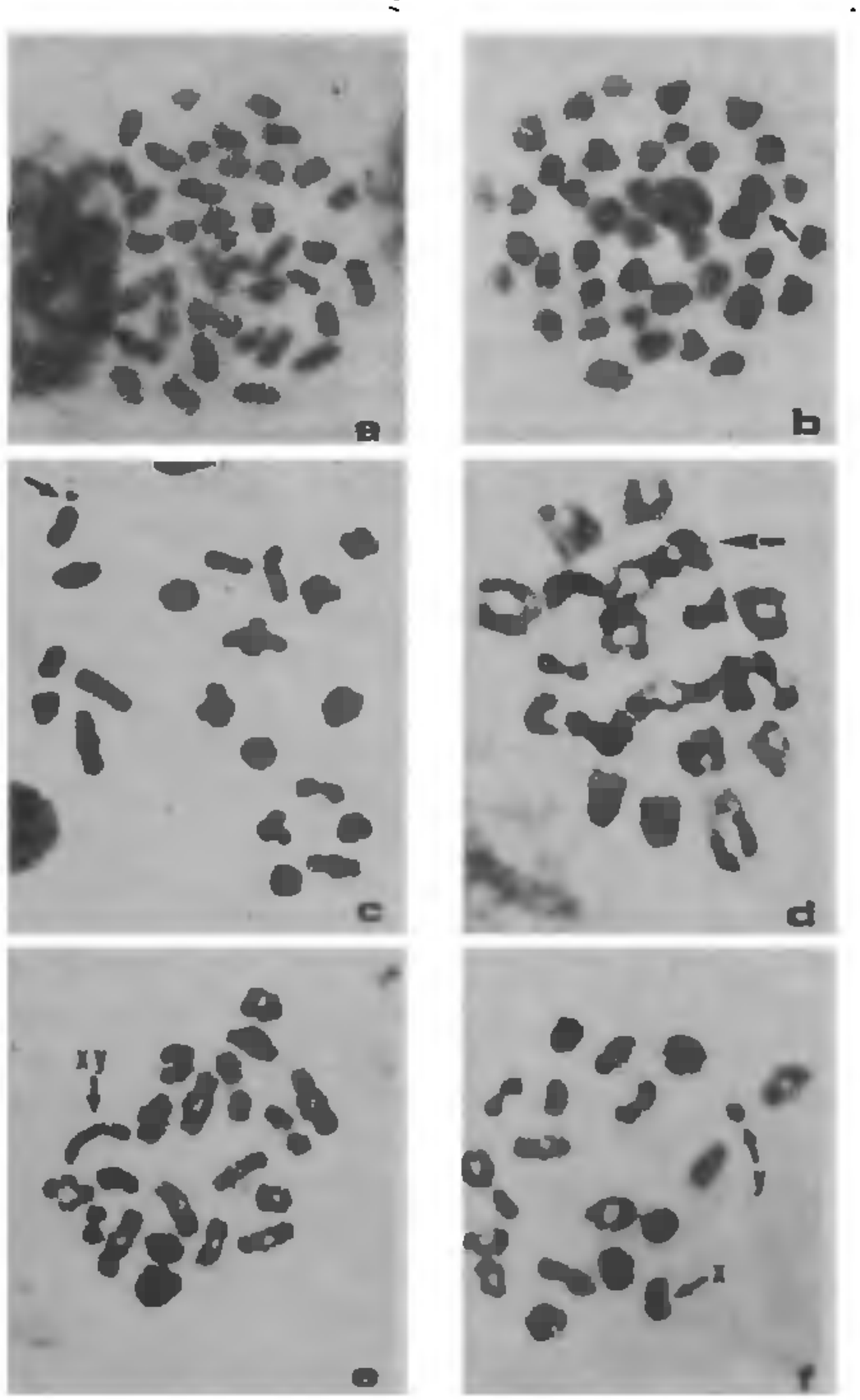

Figure 1. Chromosome spreads from testicular germ cells of S180-bearing mouse. *a*, Spermatogonial metaphase from solvent control specimen showing 40 telocentric chromosomes; *b*, Spermatogonial metaphase from cisplatin-treated specimen showing robertsonian fusion (arrow); *c & d*, Diak-meta. 1 spreads from drug-treated specimens showing fragment (*c*) and translocation multivalent (*d*); *e*, Diak-meta 1 spread from solvent control specimen showing 19 autosomal and one sex bivalents, arrow points the position of X Y bivalent; *f*, Diak-meta 1 spread from cisplatin specimen showing precocious desynapsis of X and Y chromosomes (arrow).

Table 1. Frequency distribution of affected testicular germ cells in normal and sarcoma-180-bearing mice after *in vivo* exposure to cisplatin at single therapeutic dose

Days between drug exposure and cell sampling	Affected cells (% ± SD)*		XY-univalency (% ± SD)*	
	Control[a]	Treated	Control	Treated
Day 1	–	12.0 ± 1.63[b] (9.75 ± 1.70)**	–	–
Day 11	–	14.75 ± 1.70 (8.0 ± 1.41)	6.0 ± 0.81	11.0 ± 1.82 (12.0 ± 1.41)
Day 12	–	12.25 ± 2.21 (10.0 ± 2.16)	6.0 ± 1.41	12.0 ± 2.58 (14.0 ± 2.16)
Day 18	–	11.50 ± 2.38 (8.50 ± 2.08)	7.0 ± 1.41	14.0 ± 2.94 (11.0 ± 2.44)
Day 19	–	15.75 ± 1.70 (9.50 ± 2.08)	7.0 ± 1.63	15.0 ± 2.44 (13.0 ± 3.55)
Day 20	–	14.25 ± 2.50 (11.0 ± 2.94)	6.0 ± 1.15	20.0 ± 2.16 (12.0 ± 2.16)

*Of 400 cells from four different specimens at each post-treatment time point.
**Figures in parenthesis indicate data obtained in normal (non-tumour) treated mice.
[a]Only gaps and lesions were recorded in control (not pooled in the table).
[b]Spermatogonial metaphases only.

A comparison of the data (Table 1) revealed that the frequency of cells with affected diak-meta. 1 was significantly higher both in tumour and nontumour specimens at all post-treatment time points with $P \leqslant 0.01$ by Student's t test. Although in two treated series, the frequency of cells with affected metaphases was almost identical (ranging from 12.0% to 15.7% in tumour and 8.0% to 11.0% in nontumour normal) at different time points, the frequency of cells with X-Y univalency showed a tendency of gradual increase in tumour mice with the increase in cell sampling time. However, such an increase was found to be insignificant in statistical analysis. When the results concerning X-Y univalency between treated and control series were compared it was found significant with $P \leqslant 0.01$ by Student's t test. Again when a comparison between the frequency of aberrations observed in tumour and nontumour mice was made it was noted that the difference was significant only at certain post-treatment time points, viz. days 11 and 19 with $P \leqslant 0.05$.

The present findings clearly pointed out that cisplatin at single therapeutic dose produced cytotoxic influence on testicular germinal cells of sarcoma-180-bearing mouse. The cytotoxicity was manifested in the form of chromosome aberrations and precocious desynapsis of sex bivalent. When the data were analysed with regard to Oakberg time schedule for mouse spermatogenesis[12], it became apparent that the drug affected testicular germ cells both at pre-leptotene–leptotene phase of meiosis I and at differentiating spermatogonial stage, because the cells exhibiting chromosome structural abnormalities on days 11 and 12 were, according to this time schedule, at leptotene–preleptotene stage of meiosis on day zero when the drug was initially administered. Similarly, cells sampled on days 18–20 were at differentiating spermatogonial stage at the time of cisplatin exposure. Since the frequency of affected cells showed no significant variation at different post-treatment time points, it has been assumed that both leptotene–preleptotene and differentiating spermatogonia were equally sensitive to the clastogenic action of cisplatin.

Cisplatin is known to inactivate DNA by forming intra-strand and inter-strand cross-links. The drug does not appear to possess significant cell cycle dependency[13]. The cell-killing activity of the drug has been tested on normal C3H mouse[15], and its potential to induce chromosome loss and dominant lethals in *Drosophila* has been well established[7,15]. In their studies Adler and El Tarras[14] documented a differential clastogenicity in primary spermatocytes and spermatogonial cells of normal C3H mouse treated with the different doses of cisplatin. They reported less sensitivity of differentiating spermatogonial cells compared to early prophase stage of spermatocytes. However, in the present study we noted an identical sensitivity of these two types of cells in response to *in vivo* cisplatin exposure at therapeutic dose. The discrepancy between the results might be due to difference in the mouse strains used and/or the presence or the absence of the tumour load.

The precise way by which cisplatin affected the pairing between the X and the Y chromosome and induced precocious desynapsis is difficult to ascertain. Precocious desynapsis of X–Y bivalent with respect to autosomes has been reported in different strains of mice[12,16,17]. In the present study we recorded the occurrence of X–Y desynapsis to a significantly higher rate in cisplatin-exposed specimens at all post-treatment time points. One possibility is the damage to the synaptinomal complex which holds the X and the Y chromosomes together, for a short sequence from pachytene to metaphase 1. Clastogens Mitomycin C, cyclophosphamide, etc. are known to cause SC damage in the meiocytes of mouse[18]. A similar effect of cisplatin on SC of mouse is not yet reported. However, like mitomycin C cisplatin is also known to interfere with protein and RNA synthesis[19]. It may therefore be assumed that the drug acted on the protein associated with the SC and thereby induced precocious desynapsis of sex bivalent which, unlike autosomal bivalents, remains synapsed only for a very short sequence during the pairing phase of meiosis.

It is evident that cisplatin at single therapeutic dose affected testicular germinal cells of sarcoma-180-bearing mouse and normal mouse to a significant extent. The cytotoxicity was manifested in the form of premature desynapsis of sex bivalent and chromosome structural alterations. While the former causes lowering of testis weight and thereby affects fertility, the latter may cause heritable chromosome alterations and

nonviability of spermatozoa[20]. Thus the present findings on cisplatin effect on germinal cells of tumour bearing mouse may constitute helpful guide for better monitoring of cisplatin-mediated chemotherapy, particularly to male cancer patients who are in reproductive age.

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Regulation of Hill reaction coupled to photoreduction of nitrate and nitrite in *Anabaena doliolum*

P. S. Bisen and S. Shanthy*
Department of Microbiology, Barkatullah University,
Bhopal 462 026, India
*Department of Biological Sciences, University of Warwick,
Coventry, CV4, 7AL, UK

The photoreduction of nitrate and nitrite with water as the electron donor was observed in *Anabaena doliolum*. Dicyclohexyl carbodiimide, a specific inhibitor of ATPase, inhibited O_2 evolution as this process involved ATP hydrolysis. Cyanide-*m*-chlorophenyl hydrazone, an uncoupler, abolished O_2 evolution by breakdown of the active energy dependent transport of nitrate and nitrite and of the proton gradient. NH_4Cl, methylamine and hydroxylamine inhibited O_2 evolution by acting as bases and cleaving high energy bonds. EDTA combined with the metal ions that were required for oxygen evolution, synthesis of photosynthetic units and photophosphorylation. This resulted in the blockage of the nitrate and nitrite photoreduction coupled to O_2 evolution by preventing transfer of electrons from water and reductant for nitrate and nitrite reduction.

A significant feature of all nitrate reductases is their tight association to membranes with photosynthetic activity[1–5]. The coupling of nitrate reduction with the photolysis of water in cyanobacteria has been observed and use reduced ferrodoxin as the physiological electron donor[1–6]. It is in the thylakoid membrane that the light energy is absorbed by the highly organized assemblies of photosynthetic pigments and electron carriers. The expulsion of high energy electrons from the chlorophyll molecules in the reaction centres and their flow down a redox potential gradient results in the formation of strongly electronegative electron carriers like ferredoxin and NADPH. Part of the released energy is incorporated during this electron transport into ATP in the process of photophosphorylation[1–9].

Uncouplers dissociate the electron transport from ATP formation while the electron transport inhibitors act on one or more of the intermediate electron carriers[10–13].

This paper reports that in *Anabaena doliolum*, there is a breakdown of the nitrate-dependent oxygen evolution due to the effect of inhibitors and uncouplers of the photosynthetic electron transport chain.

A. doliolum was grown axenically in Allen and Arnon medium[14] diluted eight times at $26 \pm 2^{\circ}$C in light at a photon flux density of 40 μmol m^{-2} s^{-1} incident at the surface of the culture vessel. The experiments were carried out with log phase cultures. The measurement of oxygen evolution coupled to nitrate reduction was carried out in a Clark-type oxygen electrode fitted with a circulating water jacket. The temperature was adjusted to 25°C. The light intensity on the surface of the cuvette was 10 Wm^{-2} (20,000 lux). The reaction