3% and this suggests a negative mitoclasic activity of Haloperidol.

A significant feature was the elevated frequencies of behavioural aberrations[5] *viz* sex chromosomal and autosomal univalents following drug treatment, with a preponderance of the former. Lack of homologous segments and binding forces of chiasmata[6] may lead to an easy dissociation of the bivalent. Hence higher values of XY univalents are not surprising. Among the autosomal univalents the smallest pair was more often involved than others. The mechanism of induction of univalents and their consequences have been amply discussed and illustrated[2,5,7–13]. Spontaneous levels of these are known to be very high and vary widely[5,8–10]. Hence, the significance of induced univalency should be assessed by comparing them with the respective controls. Viewed from this angle XY and autosomal univalents induced by Haloperidol are significant only for some doses and periods, in either series. Besides these cells with more than one pair of univalents were also noted. The behavioural aberrations appear to be insignificant if a random or chance segregation leads to a normal distribution of univalents during metaphase, leading to the production of normal gametes. However, the possibility of its irregular distribution cannot be ruled out. The abnormalities have to be reckoned with as having some consequence and significance, if sex chromosomes are involved. In general presence of abnormalities till the fifth week in single dose series indicates that the drug or its metabolite/s may affect the various stages of spermatogenesis. Moreover the drug is known to be found in small quantities for several weeks following administration[14]. Overall percentages of aberrations induced by haloperidol show only marginal variation between single and cumulative treatments for any specific type of aberration.

Negative findings with haloperidol with respect to clastogenic and mitoclasic activity indicates non-hazardous nature of the drug at therapeutic levels. These observations made on germ cells corroborate the earlier report on somatic chromosomes of mouse[1].

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DOSE RESPONSE RELATIONSHIPS FOR CHROMOSOME ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES AFTER CYTOSTATICS *IN VITRO*

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VINBLASTINE sulphate, an antineoplastic agent is often used in combination with other drugs in the treatment of Hodgkin's disease and a wide variety of carcinomas[1–4]. In the present investigation, an attempt

has been made to study the dose response and time-dependent relationships for chromosome aberrations in peripheral blood lymphocytes after introduction of the cytostatic drug *in vitro*.

Human lymphocyte cultures were initiated from the whole blood samples according to the modified method of Moorhead *et al*[5]. For studying the effect of the antineoplastic drug, vinblastine sulphate (prepared by dissolving 5 mg of the drug in 10 ml sterile distilled water and filtered by millipore filtration prior to use), on human lymphocyte cultures the cells were treated for periods of 24, 48, and 72 hr by adding the drug at the respective time intervals, *i.e.* 24 hr before termination, 24 hr after initiation and at the time of initiation respectively. Four different concentrations were used for each period, *i.e.* 1.25, 2.5, 3.75 and 5.0 μg ml respectively. Cultures were harvested in the usual way and the cells were observed under the microscope. All cultures were maintained in duplicate. Control cultures without the addition of the drug were maintained simultaneously.

The results obtained after the treatment of the human lymphocyte cultures with VLB are given in table 1.

Our observations revealed that the mitotic index was considerably reduced after the treatment of the human lymphocytes for longer periods (72 hr), especially at the highest concentration (5 μg/ml). Extreme condensation of chromatin was observed at concentrations of 5 μg/ml after 24 hr treatment. Stickiness of chromosomes was common for all the three time periods—24 hr (5 μg/ml), 48 hr (5 μg/ml) and 72 hr (3.75 μg/ml and 5 μg/ml). D-D and G-G acrocentric associations were observed at concentrations of 3.75 μg/ml after 72 hr treatment. Polyploidy was observed at 5 μg/ml for both 48 hr and 72 hr treatment. An interesting observation was the terminal deletion of one arm of a C group chromosome and the subsequent translocation to another chromosome of the same group after 48 hr treatment at 3.75 μg/ml. No other chromosomal abnormalities were observed.

The percentage of pooled abnormalities ranged from 0.33 to 1.66 at 24 hr treatment, from 1.00 to 1.66 at 48 hr treatment and from 1.00 to 1.66 at 72 hr treatment. The results were significant at the higher concentrations of 3.75 μg/ml and 5.0 μg/ml for all the three time periods and also at 2.5 μg/ml for 72 hr.

Mitotic poisons may roughly be grouped into (a) preprophase poisons (b) radiomimetic or chromosome poisons and (c) spindle or metaphase poisons[6,7]. VLB falls under the last category and produces C-mitosis *in vitro*. It has no action on the structure of centrioles or kinetochores and the spindle tubules were lacking in arrested metaphases, thereby indicating that they interfered with the normal assembly of spindle fibre proteins into an oriented tubular structure. Without organization of structural units, functional spindle tubules are not found and the normal chromosome movements are prevented[8].

Previous reports on the action of VLB indicate no evidence of chromosome damage after the treatment of normal and neoplastic human cells[7]. Kucerova and Polivkova[9] indicated that VLB (10^{-3} to 10^{-7} mg/ml) induces a few, if any, mutagenic effects even at high

Table 1 *Analysis of human lymphocytes after treatment with vinblastine sulphate* (VLB). *(No of Cells analysed 500)*

Cytostatic drug	Time (hr)	Concentration (μg/ml)	% of blast cells	% of dividing cells	% of abnormalities	χ^2 values (control versus concentration)
Control	—	—	33.33	10.89	0.00	0.00
Vinblastine sulphate	24	1 25	33.33	14.58	0.33	1.00
		2.50	32.50	15.68	1.00	3.01
		3.75	28.86	16.72	1.33	3.86*
		5.00	26.84	18.91	1.66	5.04*
	48	1.25	24.20	20.00	1.00	3.01
		2.50	23.40	18.00	1.00	3.01
		3.75	21.26	14.40	1.33	3.86*
		5 00	20.86	12.00	1.66	5.04*
	72	1.25	22.28	12.00	1.00	3.01
		2.50	18.20	10.00	1.33	3.86*
		3.75	16.60	8.00	1.66	5.04*
		5 00	12.40	4.00	1.66	5.04*

* $P < 0.05$

concentrations. In Don lung cells from Chinese hamster, vinblastine sulphate produced a dose- and time-dependent increase in various chromosomal aberrations including chromatid breaks and sister chromatid exchanges[10]. A study of the relative mutagenicity and cytotoxic effects of vinblastine and other antineoplastic drugs revealed that the drugs did not significantly increase the frequency of $TG^{R}HGPRT^{-}$ mutants, but were highly cytotoxic[11]. No dose response relationship was reported by Hartwich *et al*[12] in lymphocytes of three of the five patients treated with VLB but only slight increase in percentage of aberrations in the other two.

In a review of the genetic and related effects of *Vinca rosea* alkaloids, Degraeve[13] indicated that the induced lesions may often be related to an individual action on the spindle rather than to a direct action on chromosomes. VLB blocks spindle formation at low concentrations with doubling of chromosomes but failure of chromatid separation or formation of macro or micro nuclei with lagging chromosomes in the interpolar regions. Higher doses have a direct effect on the chromosomes, resulting in their shrinkage and coalescence to form a dense pycnotic mass[14]. The *in vivo* time effect relationships in studying chromosome aberrations by VLB has been worked out by Georgian *et al*[15]. Kubock *et al*[16] observed some nuclear abnormalities, breaks and dicentric chromosomes in patients suffering from Hodgkin's disease or chronic myelosis and treated with Velbe which could be related to a prolonged arrest in metaphase rather than to a direct action on chromosomes.

The dominant toxic effect of VLB is leucopenia[17] which limits the therapeutic dose that can be given. Finally, all the published data agree about the absence of demonstrable mutagenicity of VLB in both micro organisms and mammals and the chromosomal effects in humans[18,19]. The primary effect of VLB is metaphase arrest, by interference with, or inhibition of, mitotic spindle formation with very little interaction with DNA.

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