

Hence there is a need for synthesis of this disulphide-linked peptide and to investigate further the nature/specificity of the antibodies produced on immunization with such synthetic peptides in animal models. Data obtained in this and other papers<sup>7,8</sup> using cold modification method have clearly indicated importance of disulphide bonds in the integrity of epitopes and explains why most of the linear peptides show very low cross reactivity. Thus, it appears that a viable substitute for proteins in vaccine programmes or for diagnostic utility can only be a disulphide constrained peptide, which can be obtained at present by reoxidation of linear peptides synthesized by chemical methods or genetic engineering procedures. These data clearly demonstrate the importance of understanding the reoxidation of peptides/proteins with a view for field application.

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## Combined effect of cisplatin and $\alpha$ -tocopherol on mice bearing Dalton's lymphoma

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**Inhibitory effect of cisplatin and  $\alpha$ -tocopherol on the growth of murine Dalton's lymphoma was studied *in vivo*. Subtherapeutic dose (3 mg/kg) of cisplatin was combined with different concentrations of  $\alpha$ -tocopherol in order to increase the therapeutic benefit of this antitumour compound. This combination therapy not only increased the survival time of tumour-bearing mice but also resulted in tumour-free survivors. Such a treatment of Dalton's lymphoma is safer compared to cisplatin alone at higher doses due to its dose-related nephrotoxicity.**

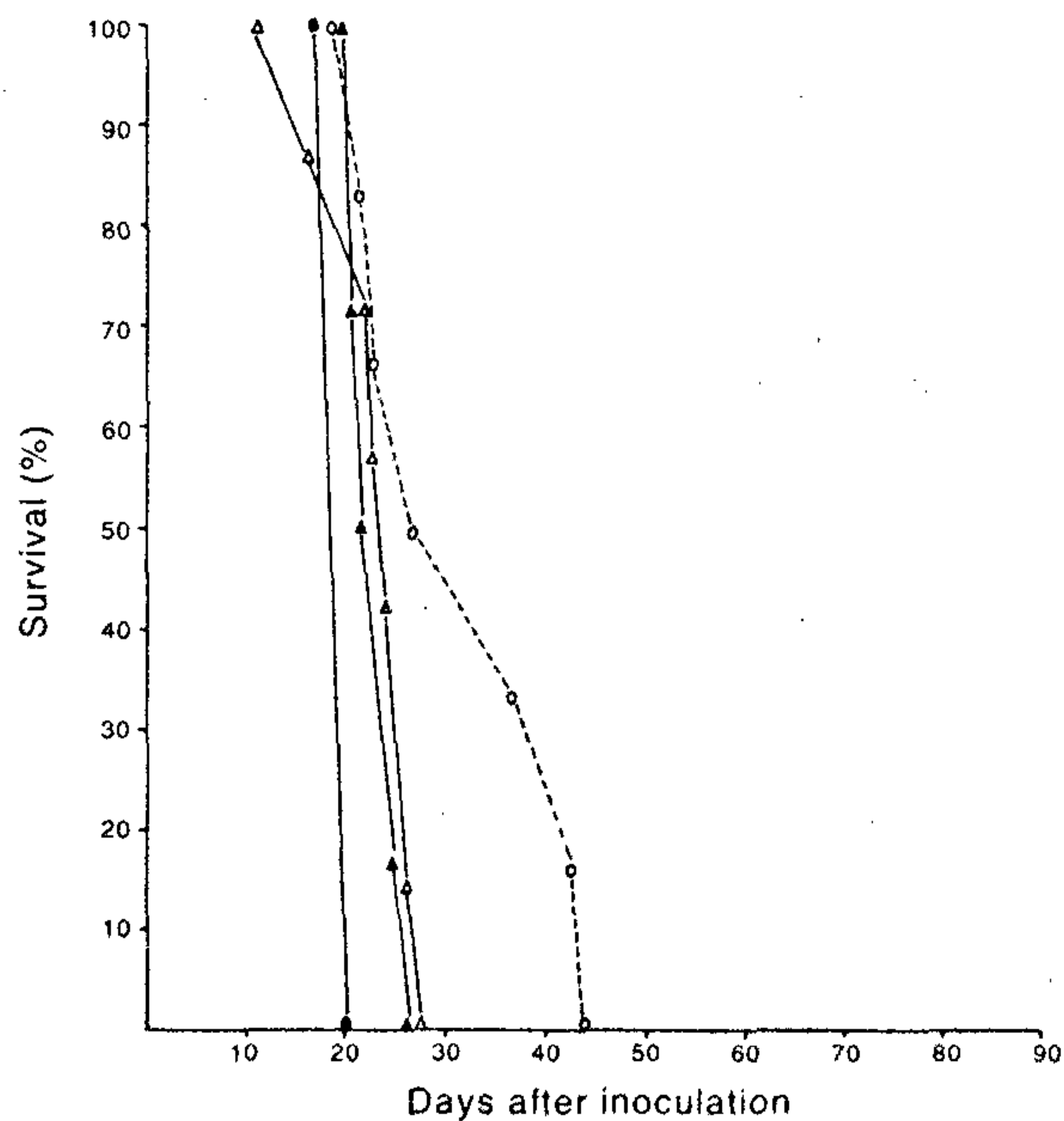
CISPLATIN is effective in the treatment of a number of tumours if used at a therapeutic dose<sup>1-4</sup>. Despite its antitumour effects, nephrotoxicity and other side effects have been reported, limiting cisplatin's long term administration to experimental animals or humans<sup>5-8</sup>. Cisplatin-induced renal damage has been reported to be induced by oxygen-free radical reaction<sup>9</sup>. The cells can be protected against free radical mediated damage by antioxidants like ascorbic acid, vitamin E, glutathione and enzymes like superoxide dismutase, catalase and peroxidases. Sometimes ascorbic acid and  $\alpha$ -tocopherol as well as ascorbic acid and glutathione function as partners in defence<sup>10-14</sup>. Besides antioxidant property, ascorbic acid and  $\alpha$ -tocopherol have been reported to be protective against cancer<sup>15-17</sup>. It has been reported that incubations of tumour cells with sodium selenite or  $\alpha$ -tocopherol succinate for 24 h prior to exposure to X-rays or chemical carcinogens resulted in an inhibition of transformation by each of the antioxidants<sup>18</sup>. Additive or synergistic effect of  $\alpha$ -tocopherol with several anti-tumour agents has been reported on the growth inhibition of murine neuroblastoma cells<sup>19,20</sup>. In the present investigation we studied the combined effect of subtherapeutic dose of cisplatin with different concentrations of  $\alpha$ -tocopherol on Dalton's lymphoma bearing mice to observe whether  $\alpha$ -tocopherol can enhance the antitumour effect and tumour growth inhibition induced by cisplatin.

Transplantable Dalton's lymphoma in C3H/He mice was used in all experimental groups. Tumour ( $2 \times 10^7$  cells/mice) was transplanted i.p. on day zero. On day 6 post-transplantation, mice bearing palpable tumour received a single i.p. injection of 3 mg/kg cisplatin alone or in combination with two repeated injections either 20, 40 or 60 mg/kg  $\alpha$ -tocopherol on alternate days. Control (without any treatment) was run for each group. High dose of  $\alpha$ -tocopherol (60 mg/kg) was administered



as single or repeated injections along with cisplatin in different groups. Increase in the mean survival time of tumour-bearing mice, percentage of increase in the life span of tumour-bearing mice and percentage of tumour-free survivors were recorded in each experimental and corresponding control groups. Each experimental and control group comprised of ten mice. Every set of experiment was repeated thrice and results are expressed as arithmetic mean  $\pm$  SD. Statistical significance was analysed by Student's *t*-test.

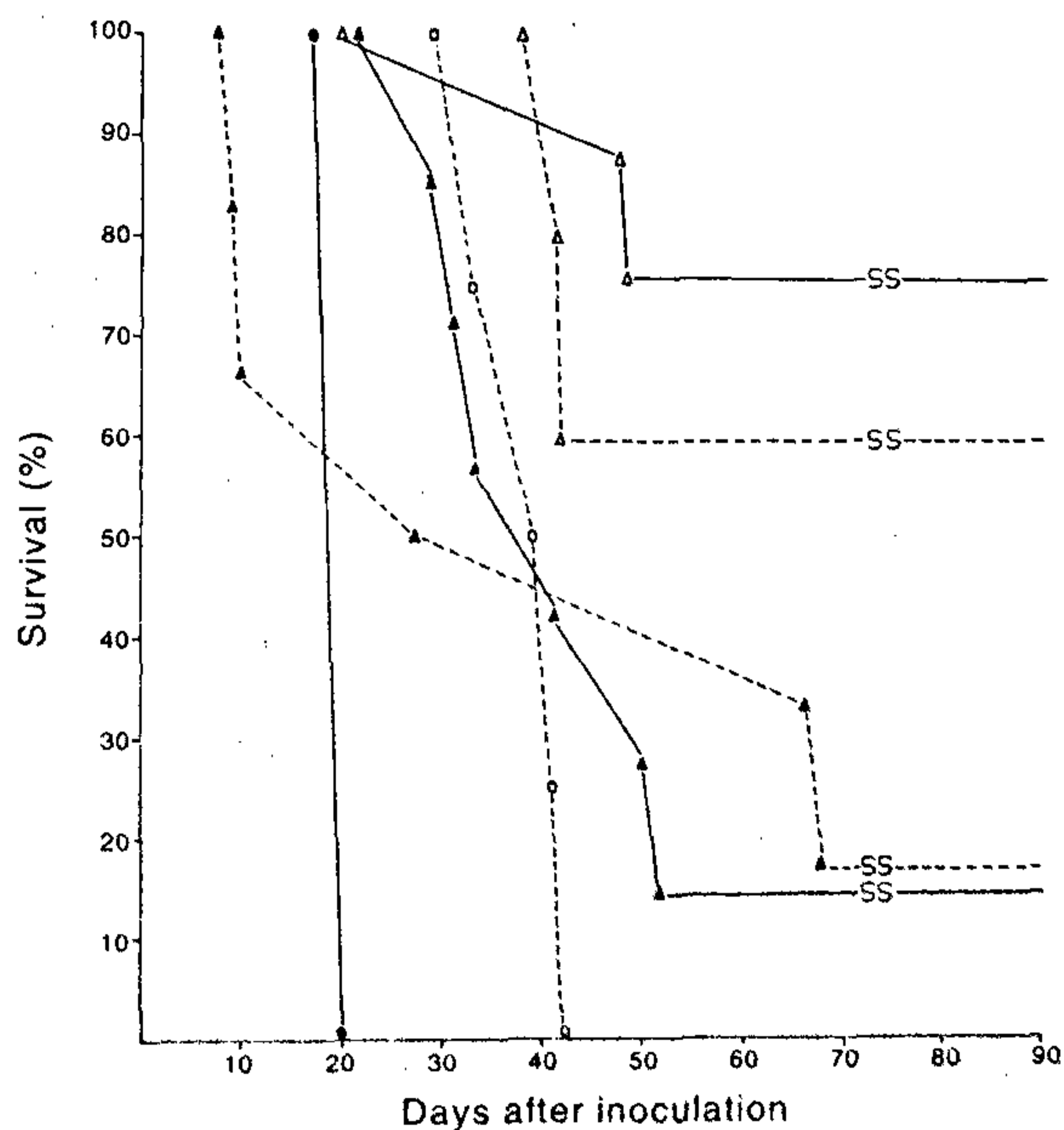
Vitamins are antioxidants as they prevent toxic effects of free radicals and due to this property are frequently used in cancer therapies<sup>15</sup>. The present study has shown none or little effect of different doses of  $\alpha$ -tocopherol on the growth of Dalton's lymphoma (Figure 1). Sub-therapeutic dose of cisplatin (3 mg/kg) resulted in a significant increase ( $P < 0.001$ ) in the survival time of tumour-bearing mice but without tumour-free survivors. Combination therapy of cisplatin with  $\alpha$ -tocopherol not only increased the survival time of tumour-bearing mice but also resulted in tumour-free survivors (Table 1). The most effective dose of  $\alpha$ -tocopherol in combination with cisplatin was found to be 40 mg/kg resulting in 75% tumour-free survivors. Even 20 mg/kg of  $\alpha$ -toco-



**Figure 1.** Effect of different concentrations of  $\alpha$ -tocopherol on the survival period of tumour-bearing mice. ●—● Control; ○—○ 20 mg/kg  $\alpha$ -tocopherol  $\times$  2; ▲—▲ 40 mg/kg  $\alpha$ -tocopherol  $\times$  2; △—△ 60 mg/kg  $\alpha$ -tocopherol  $\times$  2.

pherol was able to increase the survival time of tumour-bearing animals with 60% tumour-free survivors if administered with cisplatin (Figure 2). It has been reported earlier that  $\alpha$ -tocopherol significantly enhances the uptake of cisplatin into tumour cells but not in kidney and other organs<sup>20</sup>. One of the causes for the enhancement of cisplatin-induced tumour growth inhibition by  $\alpha$ -tocopherol might be increased uptake of cisplatin into tumour cells. It has been reported that electropermealization of cells transiently increase the influx and efflux of cisplatin or any other cytotoxic intermediate and cell survival increase if they are electropermeabilized shortly after treatment with cisplatin<sup>21</sup>.  $\alpha$ -tocopherol modulates the permeability of the tumour cell membrane by altering the level of peroxidation<sup>22</sup>. Further, DNA is the critical target for cisplatin-induced cytotoxicity and DNA-platinum adduct inhibits the DNA synthesis<sup>23</sup>.  $\alpha$ -tocopherol may act at the site on DNA or RNA where cisplatin binds<sup>19</sup>.

Low concentrations of cisplatin result in significant potentiation of antitumour cytotoxicity *in vitro*<sup>24</sup>. In the present system low dose of cisplatin increases the survival time of tumour-bearing mice, indicating enhancement of antitumour immunity.  $\alpha$ -tocopherol possibly uplift the



**Figure 2.** Effect of cisplatin alone or in combination with  $\alpha$ -tocopherol on survival period of tumour-bearing mice. ●—● Control; ○—○ 3 mg/kg cisplatin; △—△ 3 mg/kg cisplatin + 20 mg/kg  $\alpha$ -tocopherol  $\times$  2; ▲—▲ 3 mg/kg cisplatin + 40 mg/kg  $\alpha$ -tocopherol  $\times$  2; ▲—▲ 3 mg/kg cisplatin + 60 mg/kg  $\alpha$ -tocopherol  $\times$  1; ▲—▲ 3 mg/kg cisplatin + 60 mg/kg  $\alpha$ -tocopherol  $\times$  2.

## RESEARCH COMMUNICATIONS

Table 1. Increased life span of tumour-bearing mice and percentage of tumour-free survivors after treatment with cisplatin/ $\alpha$ -tocopherol

Treatment	Mean survival time in days <sup>a</sup> $\pm$ SD	Increased life span of tumour-bearing mice <sup>b</sup> (%)	60/more than 60 day tumour bearing survivors <sup>c</sup> mean $\pm$ SD (%)	Tumour-free survivors <sup>c</sup> mean $\pm$ SD (%)	P
Control	18.0 $\pm$ 2.5	-	-	-	
DMSO	19.2 $\pm$ 2.4	-	-	-	NS*
20 mg/kg $\alpha$ -tocopherol $\times$ 2	28.0 $\pm$ 2.3	55.5	-	-	< 0.001*
40 mg/kg $\alpha$ -tocopherol $\times$ 2	21.5 $\pm$ 3.4	19.5	-	-	< 0.01*
60 mg/kg $\alpha$ -tocopherol $\times$ 2	20.7 $\pm$ 2.1	15.0	-	-	< 0.05*
3 mg/kg cisplatin	35.4 $\pm$ 6.8	96.6	-	-	< 0.001*
3 mg/kg cisplatin + 20 mg/kg $\alpha$ -tocopherol $\times$ 2	39.5 $\pm$ 5.3	119.0	-	60 $\pm$ 2.5	< 0.001* < 0.02**
3 mg/kg cisplatin + 40 mg/kg $\alpha$ -tocopherol $\times$ 2	36.5 $\pm$ 4.5	102.7	-	75 $\pm$ 3.5	< 0.001* < 0.02**
3 mg/kg cisplatin + 60 mg/kg $\alpha$ -tocopherol $\times$ 1	28.0 $\pm$ 3.4	55.5	10 $\pm$ 1.2	17 $\pm$ 2.7	< 0.001* < 0.05**
3 mg/kg cisplatin + 60 mg/kg $\alpha$ -tocopherol $\times$ 2	34.0 $\pm$ 5.7	89.0	-	14 $\pm$ 1.5	< 0.001* < 0.05**

<sup>a</sup>Including 60-day survivors.

<sup>b</sup> $[(T - C)/C] \times 100$ ; where  $T$  is the mean survival days of treated mice and  $C$  the mean survival days of untreated control mice.

<sup>c</sup>Percentage of three groups each consisting of 10 mice.

\*vs control.

\*\*vs cisplatin.

level of immunity. It stimulates helper T cells which might enhance the antitumour activity of cisplatin<sup>20</sup>. Like combination with cisplatin, administration of  $\alpha$ -tocopherol has been reported to enhance both lymphoproliferative reactions and antitumour effect of adriamycin<sup>25-26</sup>. Further, vitamins as antioxidants may have potential anticancer activity. They possess chemical properties which allow them to regulate the oxidation reduction potential of the cell and are involved in cellular metabolism<sup>15</sup>. Vitamin C has been reported to exert its antineoplastic effects by increasing cytolytic and autophagic activity, membrane disruption and increased collagen synthesis, thus inhibiting cancer cell metabolism and proliferation<sup>27</sup>. Possibly  $\alpha$ -tocopherol may somehow interfere with cellular metabolism to check the tumour cell proliferation. High dose of  $\alpha$ -tocopherol (60 mg/kg) in combination with cisplatin was found to be comparatively less effective causing total tumour regression only in 14-17% of animals (Figure 2). No effect of excess  $\alpha$ -tocopherol<sup>28</sup> or increased incidence of tumour has been reported when  $\alpha$ -tocopherol was administered at very high doses<sup>29</sup>. The animals receiving combined treatment of cisplatin and high dose of  $\alpha$ -tocopherol became inactive after 24 h with decreased survival period as compared to animals in other groups. This effect is possibly due to renal toxicity caused by high dose of  $\alpha$ -tocopherol. Earlier, a slight glomerular change and tubular dilation has been reported at a dose of 20 mg/kg of  $\alpha$ -tocopherol<sup>20</sup>. Further, no difference in the amount of platinum in kidney was reported in animals treated

either with cisplatin alone or in combination with tocopherol. Since  $\alpha$ -tocopherol did not increase the uptake of cisplatin into the kidney and no significant effect was observed microscopically<sup>20</sup>, a combination thereof of Dalton's lymphoma with low dose of cisplatin along with  $\alpha$ -tocopherol is more safer compared to cisplatin alone at a therapeutic dose.

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## Ammonium sulphate induced stress related alterations in the opercular epidermis of the live fish *Heteropneustes (Saccobranchus) fossilis* (Bloch)

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Histopathological analysis of the sublethal toxicity induced by 0.2 g/l (10% of 96 h  $LC_{50}$  value) of the inorganic fertilizer ammonium sulphate to the outer and inner opercular epidermis of *Heteropneustes fossilis* has been made. Density and dimension of the goblet mucous cells (MCs) of the outer opercular epidermis increase enormously in the initial stages of exposure. Perinuclear vacuoles appear in the necrotic epithelial cells (ECs) which also bear pyknotic nuclei before their shedding at several stages of treatment. The club cells also exhibit great vacuolization. The damage becomes more extensive in later stages of exposure when severe wear and tear of the epidermis take place. The inner opercular lining however does not show such massive necrotic changes. Hyperplasia of the ECs and great vacuolization at various stages of exposure are the main histopathological alterations.

HISTOPATHOLOGICAL studies pertaining to the toxicity of ammonia ( $NH_3$  and  $NH_4^+$ ) are mainly concerned with the gills and certain other important organs like kidney,

gonads, liver, alimentary canal<sup>1-6</sup>. While majority of the above organs are situated in the body cavity, the gills remain enclosed in the opercular cavity, which however allows the gills the direct exposure to the external environment. The operculum remains guarded by a protective lining of epidermis on its external and internal surfaces. Although the main function of the operculum is to facilitate irrigation of the gills, the epidermal linings play a vital role in maintaining the milieu interior of the fish by testing the quality of water. To understand the role played by the opercular epidermis of *H. fossilis* in combating the toxicity of the inorganic fertilizer, ammonium sulphate, this work was undertaken. Ammonium sulphate readily dissolves in water to form ionized ( $NH_4^+$ ) as well as unionized ( $NH_3$ ) ammonia<sup>7</sup>.

Healthy individuals of *H. fossilis* (length 16-18 cm; body weight 35-40 g) collected from a single population at Varanasi were acclimated in large plastic aquaria for 3 weeks. Fish were fed with minced goat liver on every alternate day. Water was renewed after every 24 h, leaving no faecal matter and unconsumed food. For histopathological analysis, five groups of ten fish each were exposed separately to 50 l of 0.2 g/l [10% of 96 h  $LC_{50}$  value (2 g/l) determined by trimmed Spearman-Kärber (with 5% trimming) method<sup>8</sup> and 24 h renewal bioassay system] ammonium sulphate solution prepared in tap water having pH 7.5 dissolved oxygen 6 mg/l, waterhardness 23.2 mg/l and water temperature  $22 \pm 1^\circ C$ . In the appropriate control groups, no ammonium sulphate was added. Experimental and control media were renewed after every 24 h. Feeding was allowed for control and experimental groups for 3 h before the renewal of the media. Five experimental and five control fish each were sacrificed after 5, 10, 20, 30 and 45 days of exposure. Opercula were fixed in 10% neutral formalin, Bouin's fluid and Helly's fluid. Six  $\mu m$  paraffin sections were stained with Ehrlich's haematoxylin/eosin (H/E) for routine histopathological analysis, periodic acid Schiff (PAS)<sup>9</sup> for neutral glycoproteins, alcian blue pH 1.0 (AB 1.0)<sup>10</sup> for sulphated mucopolysaccharides, alcian blue pH 2.5 (AB 2.5) for acidic glycoproteins, AB 2.5/PAS dual staining for neutral and acidic glycoproteins<sup>11</sup> and bismarck brown (BB) for water-resistant mucoproteins<sup>12</sup>. Morphometric measurements were taken using an oculometer and stage micrometer. Standard statistical procedures based on random sampling from 10 different sections from all the five fish of each stage of all the experimental and control groups were performed. One way analysis of variance (ANOVA) followed by Duncan's multiple range test were done for multiple comparison (Figures 1 and 2). Since the differences between the measurements taken from various control groups at different time intervals of the exposure were not significant, average of all the control groups was taken into consideration.