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# Cross-linking between histones and DNA following treatment with a series of dimethane sulphonate esters*

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**Summary.** The cross-linking of the nucleohistone to the associated DNA by a series of methanesulphonates of the busulphan series ($CH_3SO_2O(CH_2)_nOSO_2CH_3$) from $n=4-9$ has been studied by gel electrophoresis, by the use of $^{35}S$ labelling and by pyrenemaleimide competition. It has been shown that all the dimethanesulphonates react with the Cys 114 of histone $H_3$, and that octamethylene dimethanesulphonate ($n=8$) cross-links histone $H_3$ with DNA via the sulphydryl group, as well as forming $H_3$-$H_3$ dimers.

## Introdcution

Members of the homologous series of dimethanesulphonic acid esters of general formula $H_3C \cdot SO_2O \cdot (CH_2)_nO \cdot SO_2 \cdot CH_3$ ($n=1-10$) have been shown to be active against a number of tumours in experimental animals, including the Walker 256 rodent carcinoma [19] and the Yoshida lymphosarcoma [3]. Busulphan ($n=4$) demonstrated maximal biological activity when the series was assayed for selective neutrophil-depressing activity [11], immunosuppressive activity [4] and haemosuppressive properties [10]. Busulphan was subjected to clinical trials against chronic myeloid leukaemia [17], for which it is still a drug of choice today. Methylene dimethanesulphonate (MDMS, $n=1$) was found to be almost as efficient as busulphan in depressing the levels of circulating neutrophils and CFU/femur in rats [10], and the drug has a marked antitumour activity in the Walker [15] and the Yoshida [14] rat tumours. Ethylene dimethanesulphonate ($n=2$) is ineffective in depressing the haemopoietic system in rats [11], but has a potent action on interstitial cell function in spermatogenesis [7, 21]. Nonane ($n=9$) is active against a variety of experimental tumours [30] and has been used clinically against chronic myeloid leukaemia [31] and a variety of other malignancies, including chronic lymphocytic leukaemia, Hodgkin's disease and bronchial carcinoma [24].

The series of dimethanesulphonates provides a valuable system for the study of bifunctional reactivity with key intracellular target sites. Their chemistry is well documented, and following alkylation of nucleophilic sites their breakdown products are usually non-toxic. It has been recognized for some time that the relative position of the two alkylating centres within such bifunctional agents are of importance with regard to their antitumour activity [13], and the dialkanesulphonates provide a useful tool for examining such structure-activity relationships within cells. Thus, the ability of the series to span only selected target nucleophilic distances coupled with the availability and reactivity of these sites could be an important factor in determining the relative ability of these esters to produce cross-links which interfere with specific biochemical reactions.

On the basis of limited chromatographic evidence it was suggested that busulphan produced cross-links in DNA through a four-carbon bridge [6]. A subsequent investigation, however, did not yield any definite physical or chemical evidence for the formation of interstrand cross-links [26], but this lack of detection may have been due to the insensitivity of the techniques employed. Tong and Ludlum, using high-pressure liquid chromatography, were able to isolate a diguanyl derivative after busulphan [33]. However, no distinction could be made as to whether this was derived from inter- or intrastrand cross-linking. The use of alkaline elution technology has made it possible to identify the presence of a proteinase-resistant filter retention in Yoshida lymphosarcoma cells treated with busulphan [2] or with MDMS [1]. This retention was concluded to be due to DNA–DNA interstrand cross-links. In addition, following MDMS treatment a large amount of proteinase-sensitive DNA-protein cross-linking was also observed, which was attributed to the action of formaldehyde produced on hydrolysis of the drug. In a further study the ability of the entire series of dimethanesulphonates ($n=1-9$) to induce DNA–DNA interstrand and DNA-protein cross-links was examined [3]. With the exception of EDMS ($n=2$), DNA–DNA interstrand cross-links were detected with all members of the series, with maximal activity on an equimolar basis with the hexam-

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*Abbreviations:* MDMS, methylene dimethane sulphonate; EDMS, ethylene dimethane sulphonate; YS, Yoshida sarcoma parent line; YR/BUS, Yoshida sarcoma resistant to busulphan; YR/MDMS, Yoshida sarcoma resistant to MDMS; DMSO, dimethyl sulphoxide; SDS, sodium dodecyl sulphate; NPM, *N*-(3-pyrene)maleimide

ethylene ($n=6$) derivative. DNA-protein cross-links were observed with MDMS and with the higher members of the series ($n=7-9$), with maximal cross-linking occurring with the octamethylene ($n=8$) derivative. An approximate correlation between the ability to form DNA–DNA interstrand cross-links and in vitro cytotoxicity was observed with most members of the series. The octamethylene derivative, however, appeared to be more highly cytotoxic than its ability to produce DNA–DNA interstrand cross-links suggests. The nature of the proteins involved in this DNA-protein cross-linking, the presence of any protein-protein cross-links, and the importance of protein cross-linking to cytotoxicity were not studied. In the present study the cross-linking of histone proteins with the DNA in Yoshida cell chromatin following treatment with higher members of the series of dimethanesulphonates ($n=4-9$) was investigated.

## Materials and methods

*Chemicals.* Busulphan was synthesized by the reaction of 1, 4 butanediol and methanesulphonyl chloride [20]. The other esters were synthesized by the reaction of the appropriate dibromalkane with silver methanesulphonate in acetonitrile solvent [12]. The products were recrystallized from hot ethanol and their purity verified by melting point and infrared spectral absorption measurements.

*Cell culture.* Yoshida wild-type sensitive (YS), and busulphan-resistant (YR/BUS) cell lines were derived from the solid rodent sarcoma as described previously [16] and were grown in suspension cultured in the presence of antibiotics and glutamine in an atmosphere of 5% $CO_2$: 95% air in Fischer's medium (Flow Laboratories) supplemented with 20% horse serum (Gibco Bio-cult Ltd). Cloned cells were routinely screened for mycoplasmal contamination prior to use.

*Preparation of nuclei.* A modification of the method of Berkowitz [5] was used. Cells were harvested by centrifugation at 1000 *g* for 5 min and suspended in solution A (0.32 *M* sucrose, 2 m*M* magnesium chloride, 1 m*M* potassium phosphate, pH 6.8). The suspension was homogenized in a hand-held Teflon homogenizer of low clearance surrounded in ice for 5 min. The homogenate was centrifuged at 1000 *g* for 10 min. The pellet was suspended in solution B (1 m*M* sodium chloride, 1 m*M* potassium phosphate, pH 6.8) and left to stand on ice for 20–30 min. The suspension was rehomogenized and centrifuged. The pellet was resuspended in solution C (0.32 *M* sucrose, 1 m*M* magnesium chloride, 0.3% Triton X-100, pH 6.3), homogenized and recentrifuged. The crude nuclear pellet was then resuspended in a small volume of 0.15 *M* sodium chloride and overlayered onto 20 ml 0.25 *M* sucrose, 3 m*M* calcium chloride and centrifuged at 1100 *g* for 10 min; the pellet was washed twice in 0.15 *M* sodium chloride. The nuclear pellet was collected by centrifugation at 1660 *g* for 10 min and stored at −20°C.

*Isolation of chromatin.* Chromatin was solubilized by brief digestion of nuclei with micrococcal nuclease (E. C. 3.1.4.7., Worthington, 100 units/ml, 15 min, 37°C) in 0.3 *M* sucrose, 0.01 *M* Tris-HCl, 0.75 m*M* $CaCl_2$, pH 7.2. Digestions were terminated by the addition of 0.1 *M* EDTA to give a final concentration of 10 m*M*. Digested nuclei were pelleted at 12000 *g* for 15 min, followed by resuspension at 4°C. Nuclear debris was pelleted at 3000 *g* for 10 min. Supernatants from both the 12000 *g* and 3000 *g* fractionations were pooled, dialysed against 10 m*M* Tris-HCl, 0.25 m*M* EDTA, pH 7.8, and fractionated on a Sepharose-4B column equilibrated with the same buffer at 4°C. Void peak fractions were pooled and contained high-molecular-weight chromatin.

*Histone cross-linking studies.* Nuclei suspended in phosphate-buffered saline (1 mg/ml) were treated at 37°C with appropriate concentrations of drug in DMSO. The latter did not exceed a final concentration of 5%. Reaction was stopped by the addition of 2 *M* hydrochloric acid to give a final concentration of 0.2 *M* and cooling to 4°C on ice. Samples were sonicated (3 × 20 s, MSE ultrasonic disintegrator) and centrifuged at 20000 *g* for 15 min at 4°C. The acid-soluble free histone-containing supernatants were removed and dialysed against double-distilled water at 4°C (28-place microdialysis system, Bethesda Research Labs. USA, 6000–8000 mol. wt. cut-off). Samples were diluted 4:1 with 5-times-concentrated sample solvent and analysed by polyacrylamide gel electrophoresis.

The acid-insoluble pellets remaining after the removal of unbound histones were incubated in 50 m*M* Tris, 0.1% SDS, pH 8 at 37 °C for 48 h, diluted with sample solvent and analysed directly by electrophoresis. This procedure is known to reverse DNA-histone cross-links [22] and allow those histones that were bound to DNA to be analysed.

*Polyacrylamide gel electrophoresis.* The electrophoretic conditions are a modification of the SDS gel system of Laemmli 1970), consisting of a 15% acrylamide, 0.4% bisacrylamide, 0.1% SDS, 0.375 *M* Tris, pH 8.8 running gel and a 4.5% acrylamide, 0.12% bisacrylamide, 0.1% SDS, 0.125 *M* Tris, pH 6.8, stacking gel. The running buffer was 0.1% SDS, 0.025 *M* glycine, pH 8.4 and the running time at 25 mÅ was approximately 15 h. Samples in sample solvent 2.3% SDS, 0.76% Tris, 10% glycerol, pH 6.8) were loaded at 10 µl per channel. Gels were stained with 0.1% Coomassie blue R-250 (Bio-rad, Richmond USA) in 10% acetic acid, 45% methanol for 6 h at room temperature. Destaining was by diffusion in 5% acetic acid, 25% methanol. Gels were scanned using the Beckman DU-8 spectrophotometer fitted with a gel scanning accessory at 575 nm at 10 m*M*/min with an external slit width of 0.1 mm, and the information was transferred on-line to an Apple (Apple Computers Inc.) microprocessor for analysis. Area calculations were achieved using an Apple graphics tablet.

*Fluorescence probe studies. N*-(3-pyrene) Maleimide (NPM) was synthesized as described by Weltman et al. [36]. Prior to use, a stock solution of 0.1 m*M* NPM in ethanol was prepared. Following drug treatment and dialysis to remove excess drug, chromatin samples (0.2 mg/ml protein) were treated with 500 nm NPM for 1 h and the resulting fluorescence was monitored using a Perkin-Elmer fluorescence spectrophotometer employing 1.0 cm quartz cells at an emission wavelength of 377 nm and excitation 342 nm.

*$^{35}$S-Cysteine labelling.* Yoshida cells were grown in culture from $5 \times 10^3$/ml to $2.5 \times 10^5$/ml in Fischer's medium containing *L*-($^{35}$S)-cysteine (1100 Ci/mmol, Radiochemical

Centre, Amersham International Ltd) harvested by centrifugation, and chromatin was prepared as outlined above. Following drug treatment of nuclei, histones were extracted as before and the acid-soluble fractions assayed for total protein content (Bio-rad protein assay kit) and total radioactivity (0.2 ml samples counted in 10 ml phosphor II solution).

*Chromosome analysis.*

Treated cells (25 ml, $1 \times 10^5$/ml) were centrifuged down, resuspended in fresh medium and grown for a further 24 h at 37°C. Vinblastine (Velbe, Eli Lilly, UK) was added to give a final concentration of 1 μ*M* and the cultures grown for a further 2 h. The cells were harvested by centrifugation (1000 *g*, 5 min), and the cell pellet suspended in 10 ml 75 m*M* potassium chloride for 10 min followed by recentrifugation. The resulting pellet was fixed by the careful addition of methanol : acetic acid (3 : 1). After 30 min the pellet was resuspended and centrifuged. The fixed cells were washed several times in the fixative solution and stored at 4°C. A drop of fixed cells was run down the surface of an ice-cold slide, air dried, and stained by incubating the slides in 50 ml 20% Giemsa (Gurr Ltd, London) in Gurr's buffer, pH 6.8, for 8 min followed by washing in distilled water and drying. Slides were dipped in xylene and coverslips applied with Euparol adhesive. Slides were scored for mitotic index and aberration frequency.

## Results

*Histone cross-linking*

The effect of six members of the series of the dimethanesulphonate esters ($n=4$–9) on the histone component of Yoshida call nuclei was studied. Treatment of nuclei with busulphan ($n=4$), 1,5 pentamethylene dimethane sulphonate (penta-DMS, $n=5$), 1,6 hexamethylene dimethane sulphonate (hexa-DMS, $n=6$), and 1, 9 nonamethylene dimethanesulphonate (nona-DMS, $n=9$) with doses up to 500 μg/ml and analysis of the histones on SDS gels did not produce any significant dose-dependent difference the histone pattern compared with untreated controls. The resulting gel scan profiles following treatment with nona-DMS can be seen in Fig. 1. Following treatment with 1,8-octamethylene dimethanesulphonate (octa-DMS, $n=8$), however, a marked decrease in the histone H3 component of the extracted histone was reproducibly observed after treatment for 1 h with doses up to 500 μg/ml (Fig. 2). The distribution of the four-core histones as a percentage of the control can be seen in Fig. 3 and clearly demonstrates the preferential dose-dependent removal of histone H3 to approximately 50% of control at 500 μg/ml compared with the other core histones. The effect was accompanied by a small but significant increase in the first peak coincident with histone Hl, considered to be due to the formation of histone H3-H3 dimers which are known to co-migrate in this region of the gel [22]. The increase in this band could not be attributed wholly to the decrease in the histone H3 observed, however, and the acid-insoluble pellets remaining after the initial histone extraction were examined for histone-DNA cross-linked products. The resulting gel scans (Fig. 4) revealed an increase with dose of two bands, one of which corresponded to histone H3, indicating the formation of DNA-histone H3 cross-links produced by this drug. The increase in material in the H1 region of the gel may be due to the formation of H3-H3 DNA cross-linked products, but this idea requires more direct proof. A similar study with the heptamethylene derivative ($n=7$)

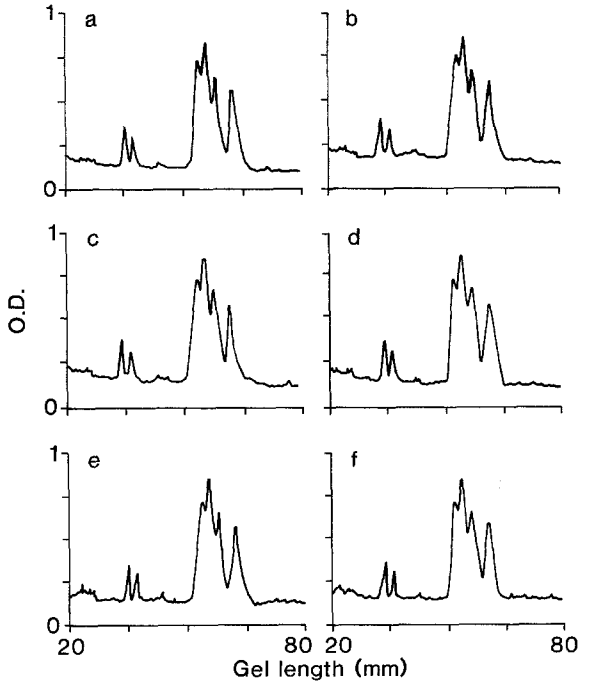


**Fig. 1a–f.** Effect of nona-DMS on YS histones. Gel scans of extracted histone samples following treatment of nuclei with **a** 0; **b** 50 μg/ml (0.16 m*M*); **c** 100 μg/ml (0.32 m*M*); **d** 200 μg/ml (0.63 m*M*); **e** 350 μg/ml (0.95 m*M*); **f** 500 μg/ml (1.58 m*M*) nona-DMS

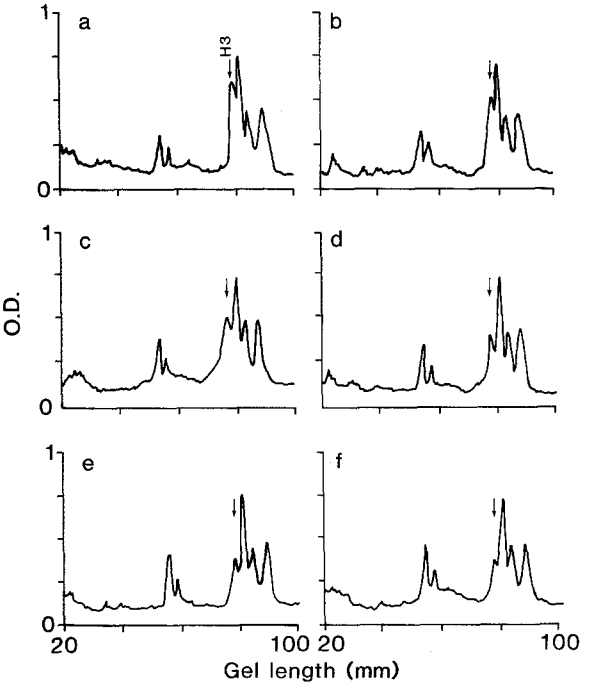


**Fig. 2a–f.** Effect of octa-DMA on YS histones. Gel scans of extracted histone samples following treatment of nuclei with **a** 0; **b** 50 μg/ml (0.17 m*M*); **c** 100 μg/ml (0.33 m*M*); **d** 200 μg/ml (0.66 m*M*); **e** 350 μg/ml (1.66 m*M*) Octa-DMS

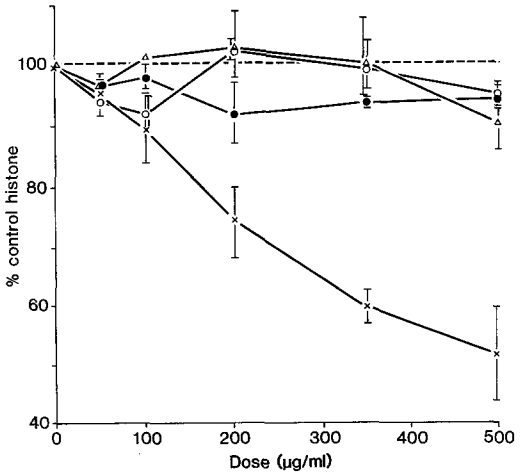


**Fig. 3.** Percent decrease in the individual core histones following treatment with octa-DMS. ○, H2a; ●, H2b; X, H3; △, H4. Results are means ±SD of three separate readings in each case

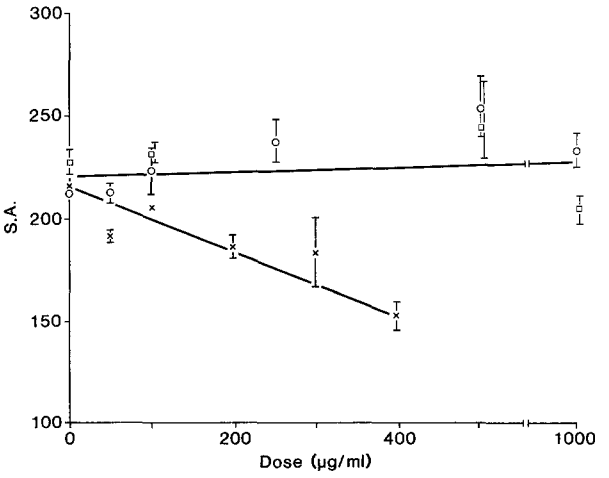


**Fig. 5.** Specific activity of $^{35}$S-labelled histone fractions following treatment with MDMS (○), octa-DMA (X), and hexa-DMS (□)

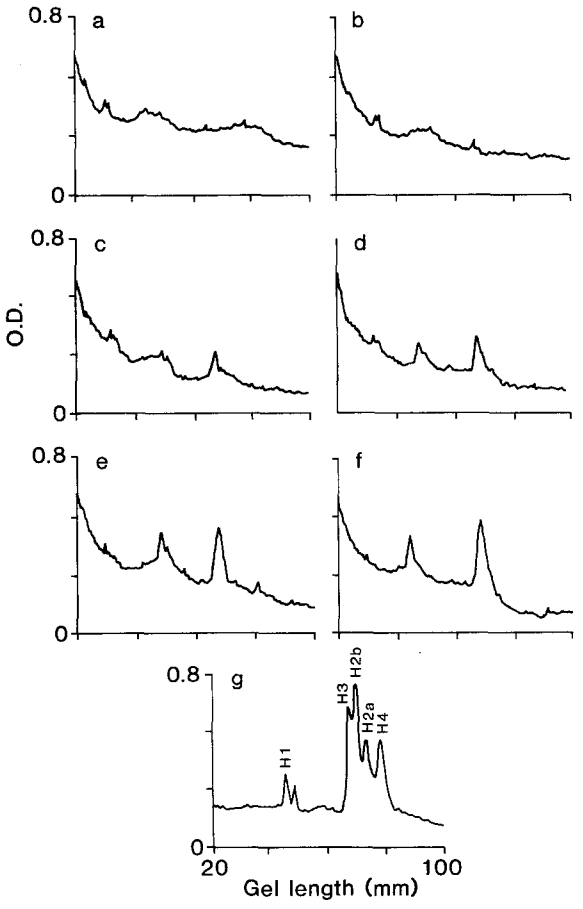


**Fig. 4a–f.** Histones cross-linked to DNA by octa-DMS. Gel scans of acid insoluble pellets remaining after the removal of unbound histones and following incubation with 0.1% SDS, 50 m*M*, Tris, pH 8, 37°C for 48 h. Samples had been treated with doses of **a** 0; **b** 50 μg/ml (0.17 m*M*); **c** 100 μg/ml (0.33 m*M*); **d** 200 μg/ml (0.66 m*M*); **e** 350 μg/ml (1.16 m*M*); **f** 500 μg/ml (1.66 m*M*) octa-DMS

revealed a similar interaction to that occurring with the octamethylene derivative, but at a much lower level (data not shown).

A comparison of the effect of octa-DMS on the YS and YR/BUS cell lines did not reveal any significant differences in the histone cross-linking between the two cell lines.

### $^{35}S$ *Labelling studies*

The unique property of histone H3 to contain cysteine residues in its primary sequence was used to examine the effects of this histone in more detail. Cells grown in medium containing *L*-($^{35}$S)-cysteine were shown to incorporate the label into histone H3. Labelled nuclei were treated with MDMS (0–1000 μg/ml), and the unbound histone was extracted as outlined previously. In each case samples were assayed for total radioactivity and protein content. The resulting specific activities with increasing dose are shown in Fig. 5. In the case of MDMS and hexa-DMS the specific activity remains essentially constant with increasing drug dose, whereas following treatment with octa-DMS the resulting specific activity decreases with drug doses up to 400 μg/ml. (Drug treatments at higher dose levels were not

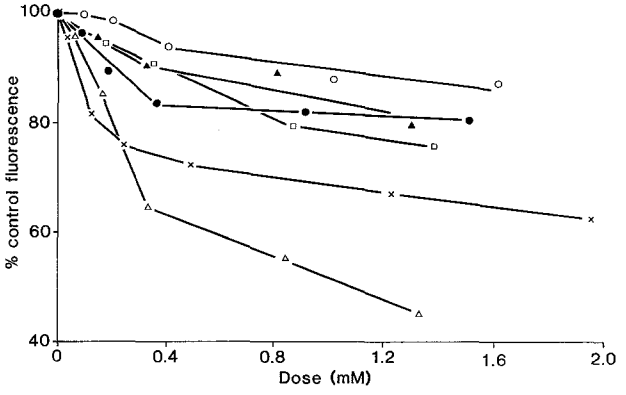


**Fig. 6.** Binding of NPM (500 nm) to Yoshida chromatin following treatment with MDMS (X), busulphan (○), hexa-DMS (●), hepta-DMS (□), octa-DMS (△), and nona-DMS (▲)

**Table 1.** Chromosome aberrations of YS and YR-BUS cells following treatment with MDMS, busulphan and octa-DMS

| Sample | Drug | Dose (μg/ml) | M.I.[a] | Aberration frequency[b] | % Survival (approx.) |
|---|---|---|---|---|---|
| YS | – | 0 | 0.155 | 6 | 100 |
| | MDMS | 1.0 | 0.068 | 62 | 50 |
| | | 2.5 | 0.050 | 78 | 30 |
| | | 12.5 | 0.005 | 100 | <1 |
| | | 20 | 0.002 | 100 | <<1 |
| | BUS | 7.5 | 0.083 | 60 | 52 |
| | | 25 | 0.048 | 88 | 15 |
| | | 50 | 0.009 | 100 | 2 |
| | OCTA | 0.5 | 0.071 | 62 | 48 |
| | | 2.0 | 0.038 | 98 | 2 |
| | | 4.0 | 0.004 | 100 | <1 |
| YR-BUS | – | 0 | 0.150 | 4 | 100 |
| | MDMS | 1.0 | 0.114 | 8 | 96 |
| | | 2.5 | 0.083 | 18 | 90 |
| | | 12.5 | 0.047 | 64 | 47 |
| | | 20 | 0.003 | 98 | 10 |
| | BUS | 7.5 | 0.121 | 8 | 98 |
| | | 25 | 0.082 | 24 | 80 |
| | | 50 | 0.041 | 84 | 25 |
| | OCTA | 0.5 | 0.118 | 10 | 95 |
| | | 2.0 | 0.077 | 48 | 70 |
| | | 4.0 | 0.039 | 72 | 50 |

[a] Mitotic index (= no. of mitoses divided by no, of cells scored)
[b] Percentage of cells containing chromosome aberrations. In each case, 50 metaphase cells were analysed for chromosome aberrations

possible in the case of this drug, due to its poor solubility in aqueous media.) In the case of MDMS the resulting constant specific activity obtained was the result of a simultaneous and proportional decrease in the protein content and the total radioactivity and is consistent with our previous findings, which indicate that following MDMS the whole histone component of the nucleosome is bound to the DNA (J. A. Hartley and B. W. Fox, 1986, in preparation). With hexa-DMS the specific activity is constant because the histone component is unaffected by drug doses up to 1 mg/ml. With octa-DMS, however, the resulting decrease in specific activity is due to a preferential removal of the labelled histone H3 from the acid-extractable unbound histone component.

*Fluorescent binding studies*

The use of *N*-(3-pyrene)maleimide, a fluorescent sulphydryl probe [35], made it possible to study the interaction of six members of the series ($n=1,4,6-9$) with the histone H3 in more detail. In all cases, following drug treatment of chromatin a dose-dependent decrease in binding of the fluorescent probe was observed, with maximal effect with the octamethylene derivative (Fig. 6). The decreased fluorescence was shown to be due to decreased binding of NPM, as no additional eximer peaks at 420–480 nm were observed, which indicated that there was no interaction between the pyrene rings of adjacent labelled histone H3 molecules. It was therefore concluded that after each drug the sulphydryl group of H3 was attacked to varying degrees, but at least in the case of $n=4$, 6, and 9 where no DNA-histone or histone-histone cross-linking was detected on polyacrylamide gels, the products of the drug interaction must be primarily either monofunctional, or alternatively of the 'sulphur-stripping' type, as shown previously [27] in the mechanism of detoxification of busulphan in vivo.

*Chromosome analysis*

The mitotic indices, aberration frequency and approximate cell survival values obtained from growth curves following treatment with MDMS ($n=1$), busulphan ($n=4$) and octa-DMS ($n=8$) in the YS and YR-BUS cell lines can be seen in Table 1. The frequency of metaphase aberrations was much greater in the Yoshida sensitive cell line than in the busulphan-resistant cell line following equimolar treatments with the drugs. This difference was reflected in the different cytotoxicity of the drugs at this dose level.

## Discussion

Evidence is presented that cross-linking interactions occur between histone proteins and between DNA and histone protein within Yoshida cell chromatin following treatment with some members of a series of straight-chain dimethanesulphonates. Busulphan ($CH_3SO_2O(CH_2)nOSO_2CH_3$) ($n=4$) was found not to bind histones to DNA or to interfere with the normal histone pattern as observed by electrophoresis. A similar result was observed with the penta-,

hexa- and nona-methylene derivatives. The results presented, however, indicate that nuclei treated with the octamethylene derivative show a preferential interaction with the histone H3 to form DNA-histone H3 cross-links and some H3-H3 cross-links, both unbound and bound to DNA. Covalent linkages of DNA to protein can only occur when the interacting molecules are relatively close and their reactive groups are within a few bond lengths apart. Following UV irradiation, histone H3-DNA cross-links have been shown to occur at levels many times higher than for other core histones following UV radiation [9] and following *cis*-platinum [32]. This suggests that in nuclei and native chromatin the histone H3 (or at least a component of it) is exposed to the DNA. Using three different chemical cross-linking agents [25] it has been shown that histone H3 was the most favoured core histone to form cross-links with histone H1. With increasing knowledge with respect to the position of the non-core histone H1 to the rest of the nucleosome structure it is suggested that histone H3 interacts with DNA in a similar site or sites near the origin of the DNA path around the octamer core. The cross-linking of histone H3 via disulphide bonds to a number of non-histone proteins in close proximity has also been reported after prior oxidation of nuclei with hydrogen peroxide [18] and when, within the nucleosome, the two cys-110 residues in the histone H3 molecules are arranged close to each other [8]. Histone H3 is one of the proteins conserved to a very high degree during evolution [29] and cys-110 is conserved in organisms more evolved than yeast. The reduced binding of *N*-(3-pyrene) maleimide to histone H3 following treatment with members of the series of dimethanesulphonates indicates that all these agents have targets at the cysteinyl binding site of histone H3. Alkylating agents owe their activity to the alkylation of intracellular nucleophilic centres, while thiol-containing constituents are known [34] to be amongst the most nucleophilic.

In an earlier study [3] the ability of these agents to produce DNA–DNA cross-links varied according to the number of carbon atoms in the alkyl chain and was related to their ability to span nucleophilic target distances. In both the earlier and the present studies, maximal DNA-protein cross-linking with the higher members of the series was observed with the octamethylene derivative. This suggests that the favoured protein-interacting domain and the nucleophilic site on the DNA lie within about 10 Å of each other. This agent is nearly as cytotoxic as the hexa derivative in vitro [3], but induces only half the number of DNA–DNA cross-links at equimolar concentrations. There are at least two possible explanations for this. If the type of DNA–DNA interstrand cross-links produced by this agent are particularly cytotoxic to the cell, then less lesions would be required to produce the same lethality. Alternatively, the high level of DNA-protein cross-links produced by this agent also contributed to the cytotoxicity, particularly if unrepaired. In contrast to the relatively non-cytotoxic DNA-protein cross-links produced after MDMS and formaldehyde, those induced by this agent could play an important role in the cytotoxic effect of this drug. The supralethal doses used in these studies to produce observable effects is based on the assumption that the levels of cross-linking and histone interaction observed were higher than, but proportional to, those that could be measured at the lower dose levels if the techniques were sensitive enough to detect them.

Chromosomal studies indicated that the frequency of metaphase aberrations was much greater in the Yoshida sarcoma parental cell line than in the busulphan-resistant cell line following treatment with MDMS, busulphan and the octamethylene derivative. Correlation between metaphase aberration frequency and differential survival of YS and YR/MDMS-resistant cells after sulphur mustard [28] and between anaphase chromosome aberrations and differential survival in YS and YR-BUS cells after busulphan [2] have been reported previously. In both cases the differential levels of aberrations were thought to be due to differential level of DNA interstrand cross-linking. Since the three agents used in the present study induced DNA–DNA interstrand cross-links as determined by alkaline elution [3], but only MDMS and the octamethylene derivative were shown to produce DNA-histone cross-links, it was concluded that the former type of cross-linking, namely DNA–DNA interstrand cross-linking and not DNA-histone cross-linking, was primarily responsible for the chromosome aberrations observed. However, the possibility remains that DNA-histone H3 cross-links could contribute to the antitumour action of those drugs that were capable of matching the internucleophilic distances involved, in this case $n=7-9$.

In conclusion, higher members of the series of dimethanesulphonates were shown to interact with histone protein, resulting in the case of the octamethylene (and to a lesser extent the heptamethylene) derivative in the formation of both DNA-histone H3 cross-links and histone H3-H3 dimers. Such cross-linking, however, was found not to be related to the level of chromosome aberrations observed in the sensitive and resistant cell lines.

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