Brit. J.Pharmacol. (1965), **24**, 24–28.

IN VIVO EFFECTS OF METHYLENE DIMETHANESULPHONATE ON PROLIFERATING CELL SYSTEMS

BY

B. W. FOX AND H. JACKSON

From Experimental Chemotherapy, Paterson Laboratories, Christie Hospital, Manchester, 20

(*Received May* 19, 1964)

The chemical and pharmacological properties of the series of dimethanesulphonates of straight chain diols, $CH_3.SO_2.O.(CH_2)_n.O.SO_2.CH_3$, with values of " n " ranging from 2 to 10, have been described (Ross & Davis, 1957; Timmis & Hudson, 1958). Two of these compounds, busulphan (Myleran; n=4; Roberts & Warwick, 1961; Trams, Nadkarni & Smith, 1961) and " Nonane " (n=9; Miller, 1961) have been investigated in detail in both animals and man; the former has an established role in the treatment of chronic myeloid leukaemia (Galton, 1956; Haut, Abbott, Wintrobe & Cartwright, 1961). The simplest homologue (n=1) has been prepared (Emmons & Ferris, 1953) but nothing appears to be recorded of its pharmacological action. The fact that the first member of a homologous series may exhibit chemical and physical properties which differ from its successors suggested that an examination of the pharmacological effects of this methylene diester ($CH_3.SO_2.O.CH_2.O.SO_2.CH_3$) should be carried out.

The compound was prepared as a colourless, crystalline material (melting point, 76 to 78° C) by the method of Emmons & Ferris (1953), after repeated crystallization from benzene and intermediate clarification using charcoal. Hydrolysis of the ester followed a first-order reaction ($K=5{,}250\times10^{-4}$, at 37° C and *p*H 7 in 0.017 M-sodium chloride solution), the time of 50% hydrolysis under these conditions being 22 min. The material was administered by the intraperitoneal route in arachis oil suspension. In the rat, the toxicity of the compound (LD50, 25 mg/kg, intraperitoneally) was rather less than that of busulphan and contrasted with the homologue n=2 (LD50, about 150 mg/kg).

Tumour inhibitory activity

Against the Walker tumour a single dose (15 mg/kg) given 6 days after transplantation produced 95% inhibition, assessed at 14 days. A similar result followed administration of daily doses (10 mg/kg) on the 7th and 8th days after transplantation, or five daily doses (5 mg/kg) commencing on day 5. Cross-resistance with a tretamine-resistant Walker carcinoma (Jackson, 1954) was also noted, since only 60% inhibition was obtained with this tumour. Busulphan (10 mg/kg) on the 6th day following transplantation produced 65% inhibition, so that the methylene dimethanesulphonate has comparable antitumour activity.

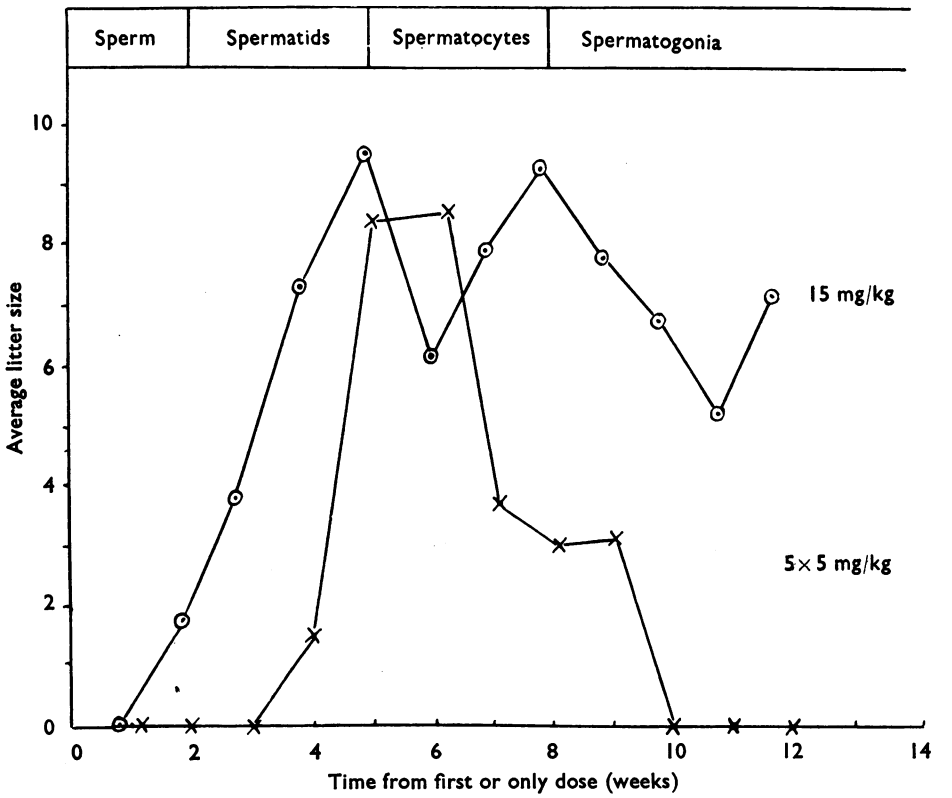

Fig. 1. The antifertility effects of intraperitoneal injections of methylene dimethanesulphonate into the rat as revealed by the successive weekly fertility of eight treated males. A single dose (15 mg/kg) induced sterility in the first week after treatment whilst a short course (five daily injections, 5 mg/kg) produced a biphasic response due to action on late and early stages of spermatogenesis. The spermatogenic stages present at the time of treatment are shown above the curves and enable the fertility data to to correlated with the cell types affected.

Action on spermatogenesis

After a single dose (15 mg/kg), sterility followed in the first week followed by a gradual return to normal fertility by the 4th week (Fig. 1). This represents a maximal action on spermatozoa within the distal regions of the epididymis, which has not been encountered with any other substance examined (Jackson, Fox & Craig, 1961). No notable change in litter sizes occurred during the 9th and 10th weeks, suggesting that, in contrast to the action of busulphan (Jackson *et al.*, 1961), no effect had occurred on the spermatogonial stages. When five daily doses of 5 mg/kg were administered, however, an extension of the sterile phase occurred in the early weeks followed by recovery of fertility and later development of sterility from week 10 onwards due to an action on spermatogonia (Fig. 1). The striking cumulative effect of this compound on the spermatogonial stage demonstrates the importance of an appropriate dose regime in order to reveal a positive biological effect. Daily oral administration of 2 mg/kg rapidly induces sterility by the antispermatozoal effect, which is later superseded by sterility associated with aspermia as a result of the

spermatogonial action. A lower dose (0.5 mg/kg daily, by mouth) produces and maintains a very low fertility in which the antispermatozoal effect is the operative mechanism.

Action on the peripheral blood count

In the rabbit, a single dose (15 mg/kg) caused a marked depletion of neutrophils from the peripheral circulation on the third and fourth days after treatment followed by a return to normal levels within a week. In the rat, the same dose produced a leucopenia (Fig. 2),

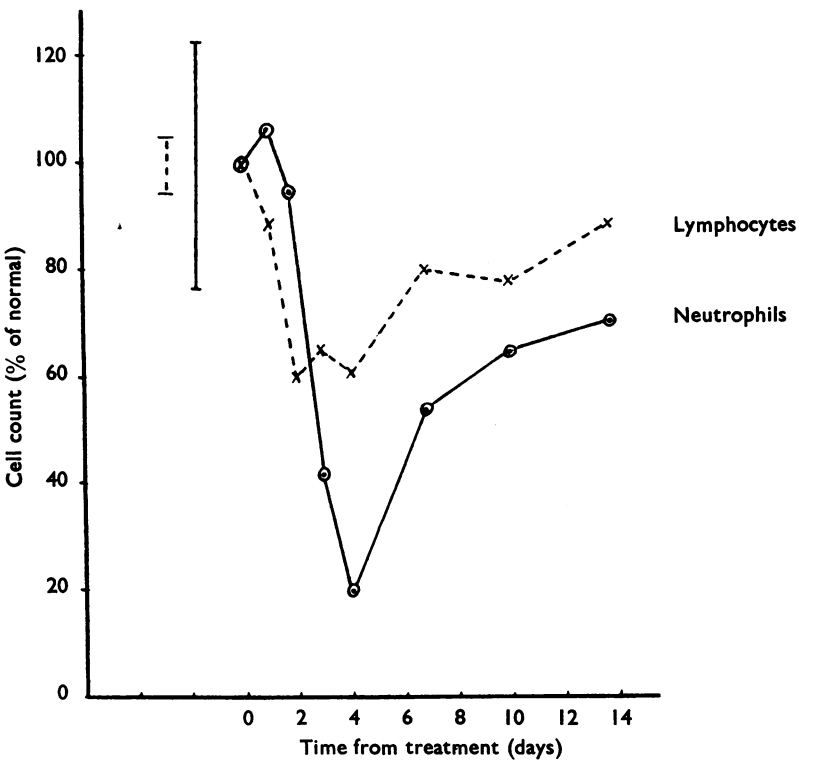

Fig. 2. Transient leucopenia followed intraperitoneal injection into rats of methylene dimethanesulphonate (15 mg/kg). The minimal neutrophil count occurred after about 4 days. The mean normal counts in these rats are neutrophils, 4,000 cells/mm^3, and lymphocytes, 14,000 cells/mm^3, and the normal ranges are shown by the vertical lines on the left.

with a minimal neutrophil count after 3 to 4 days. A comparable effect upon neutrophils also occurred after a short course of treatment by mouth (five daily doses of 5 mg/kg). In this experiment, however, the count fell gradually to its minimal value on day 9 from the first dose, and had returned to normal by day 20 (Fig. 3). The overall action on leucopoiesis resembles that caused by irradiation rather than by busulphan (Elson, 1963).

In spite of its instability, this simple disulphonic ester is thus highly active by mouth against certain stages of development of the seminiferous epithelium and of haemopoietic cells. The tumour inhibitory actions and spermatogonial effects resemble those of the higher homologue, busulphan. Its ability to reach and render spermatozoa nonfunctional

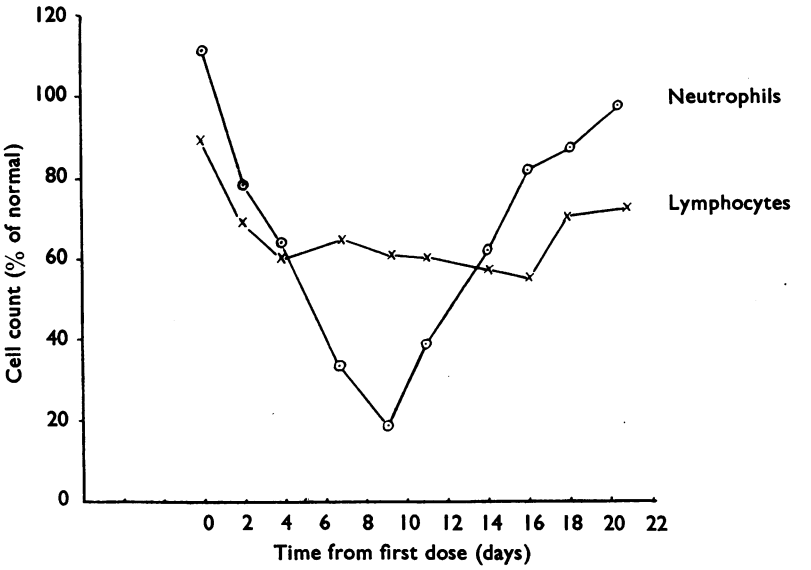

Fig. 3. Methylene dimethanesulphonate by mouth (five daily doses, 5 mg/kg) produced depression of circulating lymphocytes and neutrophils. The neutropenia was severe and transient, with a minimum value about day 9.

is remarkable and represents a further change in focus of action of alkane sulphonic esters upon the spermatogenic system. The timing of the maximum depressant action upon circulating granulocytes suggests greater susceptibility of later stages of development of these cells than occurs with busulphan.

SUMMARY

1. In the rat, methylene dimethanesulphonate produces inhibition of the Walker carcinoma, depression of haemopoiesis and antifertility effects in the male animal referable to interference with proliferating cells.

2. An additional unique feature of its action is the rapid production of sterility by an action upon morphologically mature spermatozoa in the terminal region of the epididymis.

3. Although readily hydrolysed in aqueous solution, the compound is effective by mouth in small dose and is highly cumulative.

REFERENCES

ELSON, L. A. (1963). *Radiation and Radiomimetic Chemicals*, p. 41. London: Butterworths.

EMMONS, W. D. & FERRIS, A. F. (1953). Metathetical reactions of silver salts in solution. II. The synthesis of alkyl sulfonates. *J. Amer. chem. Soc.*, **75**, 2257.

GALTON, D. A. G. (1956). The use of myleran and similar agents in chronic leukemias. *Advanc. Cancer Res.*, **4**, 73–112.

HAUT, A., ABBOTT, W. S., WINTROBE, M. M. & CARTWRIGHT, G. E. (1961). Busulfan in the treatment of chronic myelocytic leukemia. The effect of long term intermittent therapy. *Blood*, **17**, 1–19.

JACKSON, H. (1954). The development of resistance in the Walker carcinosarcoma to the action of triethylene melamine. *Brit. J. Cancer*, **8**, 336–345.

JACKSON, H., FOX, B. W. & CRAIG, A. W. (1961). Antifertility substances and their assessment in the male rodent. *J. Reprod. Fertil.*, **2**, 447–465.

MILLER, E. (1961). The effects of Nonane on malignant disease. *Cancer Chemother. Reps.*, **14**, 11.

ROBERTS, J. J. & WARWICK, G. P. (1961). The mode of action of alkylating agents. II. Studies of the metabolism of Myleran. The reaction of Myleran with some naturally occurring thiols *in vitro*. *Biochem. Pharmacol.*, **6**, 205–216.

ROSS, W. C. J. & DAVIS, W. (1957). Some potentially cytotoxic alkyl sulphonates. *J. chem. Soc.*, 2420–2422.

TIMMIS, G. M. & HUDSON, R. F. (1958). Comparisons of the biological effects of ionizing radiation and certain alkylating agents in cell systems. (Discussion, part II.) *Ann. N.Y. Acad. Sci.*, **68**, 727–730.

TRAMS, E. G., NADKARNI, M. V. & SMITH, P. K. (1961). On the mechanism of action of the alkylating agents. II. Effects of nitrogen mustard, myleran and X-radiation on nucleic acid biosynthesis. *Cancer Res.*, **21**, 567–570.