excitation action on either the neurones or on the muscle preparations we tested. One explanation of the results of Auditore and Hendrickson which occurs to us is that they may have tested their isolated peptide without neutralising it. The synthetic compounds were very acidic and, when tested on the rectus abdominus without first being neutralised, were found to cause contractions in the same manner as other acidic solutions will do. The neutralised peptides, however, has no activity.

Agricultural Research Council,
Institute of Animal Physiology,
Brabaham, Cambridge.

D. MORRIS
D. W. STRAUGHAN*

* Present address:
Department of Psychological Medicine, University of Edinburgh, Scotland.

REFERENCES

1. J. V. AUDITORE and H. HENDRICKSON, *Int. J. Neuropharmac.* **3**, 1 (1964).
2. A. CURATOLO, P. D'ARCANGELO, A. LINO and A. BRANCATI, J. *Neurochem.* **12**, 339 (1965).
3. D. R. CURTIS, *Physical techniques in Biological Research*, Vol. V, Part A, 144. Ed. W. L. NASTUK Academic Press, London and New York, (1964).
4. K. KRNJEVIC and J. W. PHILLIS, *J. Physiol. (Lond.)* **165**, 274 (1963).
5. D. R. CURTIS and J. C. WATKINS, J. *Neurochem.* **6**, 117 (1960).

Biochemical Pharmacology, 1965, Vol. 14, pp. 1681–1683. Pergamon Press Ltd., Printed in Great Britain.

Reduction of the toxicity of radiomimetic alkylating agents in rats by thiol pretreatment—V. The effect of thiol pretreatment on the anti-tumour action of Merophan

(*Received* 4 *March* 1965; *accepted* 13 *July* 1965)

IT IS well established that many thiols can protect animals against the toxic effects of certain tumour inhibitory nitrogen mustards[1–4] Combined treatment of thiol and nitrogen mustard would only be of advantage in cancer chemotherapy over treatment with nitrogen mustard alone if the protection given to the animal by the thiol were greater than the protection given to the tumour. The selectivity of anti-tumour action of the nitrogen mustard as judged by therapeutic index would remain unaltered if host protection was paralleled by protection to the tumour. Peczenic[5] and Therkelsen,[6] and recently Rutman,[7] have shown that for certain tumours the therapeutic effectiveness of HN2 can be increased by pretreating the animals with either cysteamine or 3 amino mercaptobutane.

The effect of either A.E.T. or cysteine pretreatment on the therapeutic index of the aromatic nitrogen mustard Merophan (*o*-di-2-chloroethylamino-DL-phenylalanine) measured in rats bearing the Walker carcinoma and mice bearing the ADJ/PC5 plasma cell tumour has been investigated.

MATERIALS AND METHODS

The plasma cell tumour was implanted into BALB/C$^-$ mice as previously described.[8] Merophan was given as a single i.p. dose ten days after transplantation, when the tumours measured between 4 × 4 and 8 × 8 mm. Ten days after the injection the animals were killed and the tumours weighed. Treated tumour weights were expressed as a percentage of control tumour weights and these percentages plotted against log dose. The dose to inhibit tumour growth to 10 per cent of the control value (90 per cent inhibitory dose, $I.D._{90}$) was obtained by interpolation on the curve. The LD_{50} was similarly obtained by plotting the percentage survivors against log dose. The therapeutic index, which is a measure of the selectivity of the drug or drug combination, was the ratio LD_{50}/ID_{90}. The dose ratio was 1·5 for mice and 2 for rats. The dose range was sufficient to include the LD_{100} and ID_{90} in the one experiment.

The Walker tests in Chester Beatty male albino rats* were carried out in the same manner except that Merophan was injected the day after transplantation. Each point on the survival and tumour inhibition curves was obtained by treatment of groups of 6–10 animals. Thiols were given intraperitoneally 30 min before the nitrogen mustard except where otherwise stated. All compounds were given in aqueous solution adjusted to pH 7·0. For measurement of non-protein SH levels a sample

* Carried out under the supervision of F. J. C. Roe and B. C. Mitchley.

of tissue was placed in a weighed bottle containing 2 ml of 5% (w/v) sulphosalicylic acid and re-weighed to obtain the wet wt of the tissue. After homogenisation with an ultraturrax homogeniser the homogenate was centrifuged and an aliquot of the supernatant used for estimation of free SH, essentially by the method of Ellman, as previously described.[9]

Pretreatment with either cysteine or AET leaves unaltered the therapeutic index of merophan

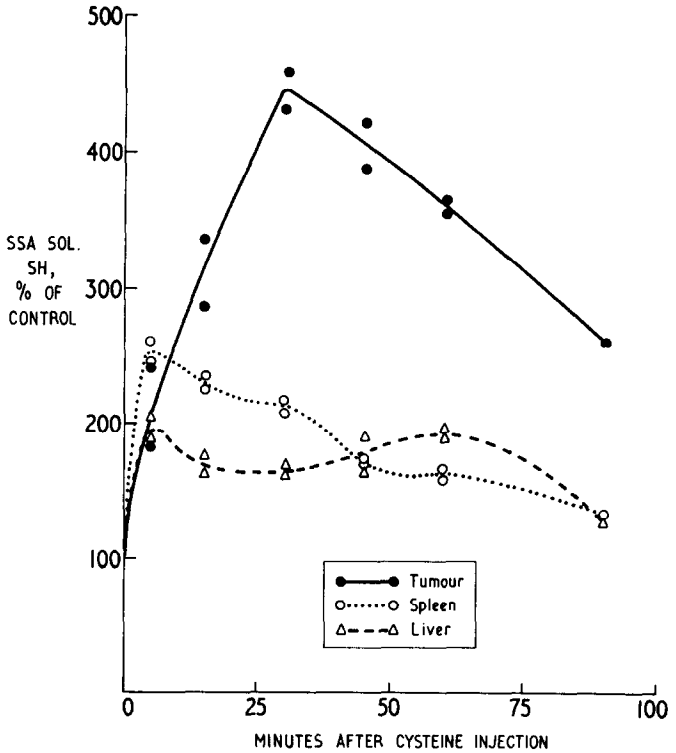

FIG. 1. Rise in acid soluble SH in tissues of the C⁻ mouse following l.p. injection of 1 g/kg cysteine hydrochloride.
Control values (μg SH per 100 mg. wet wt.) Spleen: 12
Liver: 22
plasma cell Tumour: 6

TABLE 1. EFFECT OF THIOL PRETREATMENT ON TOXICITY AND ANTI-TUMOUR ACTION OF MEROPHAN

Pretreatment	ID_{90} (mg/kg)	LD_{50} (mg/kg)	Therapeutic Index
Walker Carcinoma			
None	0·24	3·4	14·2
AET 200 mg/kg (30 min before)	0·31	4·7	15·0
Cysteine 1g/kg (30 min before)	1·6	19·0	11·9
ADJ/PCS *plasma cell tumor*			
None	2·3	4·2	1·9
AET 200 mg/kg (30 min before)	3·8	10·6	2·8
Cysteine 1g/kg (30 min before)	8·5	10·6	1·25
Cysteine (same time as Merophan)	7·1	11·3	1·60

measured in rats bearing the Walker carcinoma. Although cysteine reduced the lethal effect of merophan by a factor of five, the anti-tumour effect was reduced by a similar amount. AET had only a small effect in protecting host and tumour.

AET, however, afforded a significant increase in the therapeutic index of merophan measured in mice bearing the ADJ/PC5 plasma cell tumour, while, conversely, cysteine given 30 min beforehand reduced the therapeutic index. The mechanism by which cysteine protects against merophan toxicity is not established, but in previous work it has been demonstrated that there is a direct relationship between the extent of protection and the rise in SH concentration of tissue occurring after injection of cysteine.[10] From Fig. 1 it can be seen that, if merophan is given 30 min after the cysteine, the alkylating agent is reaching tissues when the increased SH concentration induced by cysteine is more pronounced in the tumour than in the liver or spleen. If this increased SH level bears a relationship to the degree to which the tissue is protected, then the tumour might be expected to be protected more than the host, with a subsequent reduction in the therapeutic index of merophan. In order to confirm this finding, merophan and cysteine were given together. From Fig. 1 it can be seen that for the first 10 min after injection of cysteine the increased SH concentration of the three tissues is about the same order, and one might expect less effect on the therapeutic index of merophan. The therapeutic index of merophan is in fact less altered if cysteine and merophan are given together.

These findings, in agreement with previous work, show that a moderate increase in therapeutic index may be achieved with some tumours by thiol pretreatment, but not with others. The time of thiol pretreatment may well be a critical factor. It is doubtful whether these results offer any clinical advantage, since the gain in selectivity, when it occurs, is small, and the thiols must be employed at high dose levels to ensure a significant protection of the host.

SUMMARY

Neither cysteine nor AET increased the selectivity of action of merophan in rats bearing the Walker tumour, while in mice bearing a plasma cell tumour, AET enhanced the selectivity of action as measured by therapeutic index. Time of administration of the thiols was important in determining the relative protective action on the host and tumour, and was well correlated with time curves of SH concentration in the tissues.

Chester Beatty Research Institute,
Institute of Cancer Research,
Royal Cancer Hospital,
Fulham Rd.,
London SW3.

T. A. CONNORS
A. JENNEY JR.
M. E. WHISSON

Acknowledgements—The authors wish to thank Miss G. Howard and Mrs. B. Tan for technical assistance. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council and the British Empire Cancer Campaign for Research, by the Public Health Service Research Grant No. CA-03188-08 from the National Cancer Institute, U.S. Public Health Service and by the award of a Wellcome Fellowship (A.J.) and an Anti-Cancer Council of Victoria Fellowship (M.E.W.).

REFERENCES

1. E. L. BRANDT and A. C. GRIFFIN, *Cancer* **4**, 1030 (1951).
2. A. S. WEISBERGER, R. W, HEINLE and B. LEVINE, *J. Clin. Invest.* **31**, 217 (1952).
3. E. GOLDENTHAL, M. NADKARNI and P. SMITH, *Radiat. Res.* **10**, 571 (1959).
4. T. A. CONNORS and L. A. ELSON, *Biochem. Pharmac.*, **11**, 1221 (1962).
5. O. PECZENIC, *Nature, Lond.*, **172**, 454 (1953).
6. A. J. THERKELSEN, *Biochem. Pharmac.*, **1**, 258 (1958).
7. R. J. RUTMAN, F. S. LEWIS and C.C. PRICE, *Cancer Res.*, **24**, 626 (1964).
8. V. M. ROSENOER and M. E. WHISSON, *Biochem. Pharmac.*, **13**, 589 (1964).
9. T. A. CONNORS, A. JENEY (Jnr)., J. A. DOUBLE and L. A. ELSON, *Biochem. Pharmac.*, **14**, 569 (1965).
10. G. CALCUTT, T. A. CONNORS, L. A. ELSON and W. C. J. ROSS, *Biochem. Pharmac.*, **12**, 833 (1963).