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REDUCTION OF THE TOXICITY OF "RADIOMIMETIC" ALKYLATING AGENTS IN RATS BY THIOL PRETREATMENT

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Abstract—A number of thiols have been tested for their ability to protect rats against lethal doses of Merophan, an aromatic nitrogen mustard; and other biological alkylating agents. Cysteine, thiourea, AET and a thiazolidine have been shown to reduce the toxicity of the more chemically reactive alkylating agents but to have no effect against the less reactive ones.

INITIATED by the early work of Patt *et al.* in 1949,[1] a large number of compounds have been tested for their ability to protect animals against the damaging effects of ionizing radiations. The most effective group of compounds so far found are derivatives containing, or capable of forming *in vivo* the basic structure $HS.(CH_2)_n.NR_2$ (where n = 1 to 3). Included in this group are the alkyl*iso*thiouronium halides, dithiocarbamates, thiazolidines, cysteamine and cysteine.[2, 3]

Extending this work to the so-called radiomimetic agents, it was soon shown that cysteine could give good protection against the toxicity and leucopenia induced by methyl-di-2-chloroethylamine (HN2).[4, 5] Whereas in the radioprotection experiments, β-S-2-aminoethyl*iso*thiouronium bromide (AET) has been shown to be the most effective protecting agent (giving the largest dose reduction factor), in the experiments with HN2 it appears that the magnitude of protection is directly proportional to the molar dosage of thiol.[6] Cysteine gives the best protection against the toxicity of HN2 since, on account of its low toxicity, it can be given at a molar dosage of up to three times that of most other thiols.

The object of this present work has been to determine whether the toxicity of other alkylating agents of clinical interest can be reduced by thiol pretreatment without adversely affecting their tumour inhibitory properties. Assuming that the mechanism of protection depends on the concentration of thiol in the protected tissue, then thiol pretreatment would only present an advantage in cancer chemotherapy over treatment with the alkylating agent alone, if the protecting thiol were to be concentrated to a less extent in tumour tissue than in other tissues sensitive to the alkylating agent. Reduction in toxicity would otherwise be paralleled with reduction in tumour inhibitory properties.

Both cysteamine and AET have been shown to be highly concentrated in the sites of the body sensitive to HN2 namely, spleen, bone marrow and intestine.[7, 8] Since

Peczenik[9] found that a better inhibition of the Walker tumour in rats could be obtained when HN2 was given after cysteamine than when HN2 was given alone, then cysteamine presumably is concentrated to a lesser extent by this tumour than by the other HN2 sensitive sites. Similarly AET pretreatment reduced the toxicity of HN2 without a marked effect on its anti tumour action.[7] Another thiol, 2-amino-1-mercaptobutane, has also been shown to affect the toxicity of HN2 more than its anti-leukaemic action.[10]

One way in which a thiol could attain a high concentration in the normal cell but a low concentration in tumour cells has been suggested by Ross.[11] He has pointed out that basic compounds will concentrate in cells with a lower internal pH as a consequence of the greater ability of the non-ionized form to diffuse through cellular membranes. Conversely, acidic compounds will concentrate in cells with a higher internal pH. By treating tumour bearing animals with massive doses of glucose, the cellular pH of many tumours falls to about 6·0 while other cells are unaffected, remaining at pH 7·0.[11] Using a thiol containing an acidic group, it should be possible, by treating the animal with glucose to discourage the concentration of the thiol in the tumour. For a thiol containing an acidic group of p*K*a 4, the theoretical concentration ratio would be 10:1 between two cells at pH 7·0 and 6·0, respectively.

Initially a number of acidic thiols were tested, but their toxicity was such that they could only be given in low doses and no protection was observed. More thiols containing a carboxyl or other acid group are now being synthesized in the hope of finding less toxic compounds. Also tested have been a number of thiols previously shown to protect against the toxicity of radiation or HN2. Even with neutral thiols, by treating the tumour bearing animal with glucose, it may be possible to reduce the protective effect of the thiol in the tumour. This is because thiols react more readily with the alkylating agents in the RS^- form. The amount of thiol in this more reactive RS^- form will be dependent on the pH of the cell, the lower the pH the less of the RS^- form. It follows that in the tumour bearing animal pretreated with glucose, even with uniform distribution of the thiol throughout the body tissues, there will be a smaller proportion of the thiol in the reactive form in the tumour cell at pH 6·0 than in the normal cell at pH 7·0. Glucose treatment would offer no advantage when used in combination with the basic thiols like cysteamine and mercaptoethylguanidine (formed from AET), since in this case the thiol would be highly concentrated inside the tumour cells.

Previous attempts to reduce the toxicity of alkylating agents other than HN2 have not given encouraging results. Cysteine has been reported to be ineffective in protecting against the toxicity of triethylene melamine (TEM), a polyethylene-imine,[12] while AET and cysteamine did not reduce the toxicity of Endoxan—a compound capable of releasing nor-HN2 *in vivo*.[13] Cysteine and cysteamine were also unable to prevent the leucopenia induced by another ethylene-imine, N-benzoyl-N,N,N′,N′-diethylene-triphosphoramide,[14] and Melphalan, an aromatic nitrogen mustard, did not show a reduced toxicity in the presence of 1-amino-2-mercaptobutane, a thiol giving good protection against the toxicity of HN2.[10]

The present paper reports the results of some experiments designed to assess the effect of various thiols in reducing the toxicity of an aromatic nitrogen mustard Merophan (*o*-di-2-chloroethylamino-DL-phenylalanine).[15] Some preliminary results are also presented on the ability of cysteine to reduce the toxicity of other types of alkylating agent.

Experiments are at present in progress to determine whether Merophan gives a more effective inhibition of the established Walker tumour after thiol pretreatment. The effect of glucose treatment in selectively reducing the protective action of the thiol in the tumour is also being studied. The results of these experiments will be presented at a later date.

MATERIALS AND METHODS

Female rats from an inbred Wistar colony were used of from 200 to 250 g in weight, and 8 weeks old. They were maintained on a standard 'rat cake' diet and water *ad lib.* All compounds were administered intraperitoneally, if possible in aqueous solution at pH 7·5, or alternatively as the sodium salt in buffer, also at pH 7·5. Merophan was dissolved in methanol and administered as a 5% aqueous methanol solution. Compounds insoluble in methanol or water were given in aqueous propylene glycol or in neat propylene glycol.

Toxicities were assessed in groups of five or ten animals at each dose level, and most of the thiols used were tested at 90 per cent of the maximum tolerated dose. The LD_{50} of Merophan was determined alone, and after thiol pretreatment by the method described by Weil[16] using groups of ten animals for each dose level and at four or more dose levels logarithmically spaced.

Since Merophan is readily hydrolysed in aqueous solution, the solutions were freshly prepared and used within 10 min. As a control ten animals were given the Merophan solution alone and unless the expected number of deaths occurred in this group the experiment was discounted. As both thiol and mustard were given intraperitoneally there was a possibility that any protection obtained was due to a reaction in the peritoneum between the mustard and the thiol. Samples of peritoneal fluid were taken from animals given 1000 mg/kg of cysteine at various time intervals and their cysteine content estimated. Only small quantities of cysteine were detected 15 min after the injection, and none at 30 min.

After treatment, the animals were observed for a period of at least 3 weeks. Usually animals dying from a lethal dose of Merophan die within 3 to 6 days and after the onset of diarrhoea. Such deaths are considered to arise from gastrointestinal damage. Occasionally death did not occur until from 12 to 16 days, and was preceded by a continual weight loss and persistent diarrhoea.

RESULTS

The optimum time for administration of the thiol in both radioprotection experiments and in experiments with HN2 appears to be from 15 to 30 min beforehand. This was confirmed in our experiments by giving cysteine at various time intervals before and after the injection of Merophan. From Table 1 it can be seen that the best protection was obtained when the cysteine was given from 15 to 30 min before the mustard.

The protective effect of the thiols was determined by estimation of the LD_{50} of Merophan alone, and after treatment with 90 per cent of the maximum tolerated dose of the thiol (given 30 min beforehand). The dose reduction factor which is given by the ratio:

$$\frac{LD_{50}\ \text{Merophan after thiol pretreatment}}{LD_{50}\ \text{Merophan alone}}$$

is a measure of the effectiveness of the compound in protecting against the toxicity of Merophan. Good protectors were considered to be the derivatives which gave a dose reduction factor of two or more. Table 2 illustrates the effectiveness of a range of sulphur containing compounds in reducing the LD_{50} of Merophan. L-Cysteine hydrochloride at 1000 and 500 mg/kg, thiourea, AET and 2:2-dimethylthiazolidine-4-carboxylic acid were the only compounds to give a dose reduction factor of two or

TABLE 1. REDUCTION OF THE TOXICITY OF MEROPHAN (9 MG/KG, I.P.) BY L-CYSTEINE HYDROCHLORIDE (1000 MG/KG, I.P.)

Pretreatment	Time of administration	Number of deaths	% of animals protected
Cysteine	2 hr before	10/10	0
Cysteine	1 hr before	4/10	60
Cysteine	30 min before	0/10	100
Cysteine	15 min before	0/10	100
None	—	10/10	0
Cysteine	30 min after	10/10	0

more. N-Acetyl-2-phenylthiazolidine-4-carboxylic acid, and 2-thiohydantoin appeared to increase the toxicity of Merophan.

Cysteine was also tested for its ability to prevent the toxicity of other alkylating agents, namely L-leucylmelphalan ethyl ester hydrochloride (CB 3262) an aromatic nitrogen mustard, N,N'-bis(2:3-epoxypropyl)-4:4'-dipiperidyl, (Eponate), an epoxide, triethylene-iminethiophosphoramide, (thio-TEPA) an ethyleneimine, and Myleran and Dimethylmyleran, two sulphonoxy alkanes. The results are summarized in Table 3. Cysteine gave good protection against the toxicity of leucylmelphalan, only slight protection against the toxicity of the epoxide and had no effect against thio-TEPA, Myleran and Dimethylmyleran. Dimethylmyleran was administered in 10% aqueous propylene glycol and in a control experiment it was shown that cysteine gave 100 per cent protection against the toxicity of Merophan (6 mg/kg) when the mustard was also administered in 10% aqueous propylene glycol. Myleran was given in neat propylene glycol or in arachis oil and no protection was obtained with cysteine pretreatment. However, cysteine was also ineffective in protecting against the toxicity of Merophan if the mustard was given in either neat propylene glycol or arachis oil. Probably the alkylating agent is absorbed at a much slower rate when given in these solvents.

The effect of Merophan on the blood count with and without thiol pretreatment was also investigated. Merophan at a dose of half the LD_{50} caused an 85% drop of neutrophils over the first 5 days and recovery was complete by the seventh day being followed by a neutrophilia (Fig. 1). Lymphocytes were less affected falling to about 60 per cent of the normal value and showing complete recovery within 7 days. Platelets fell to 50 per cent of normal and had recovered by the sixth day. The animals

TABLE 2

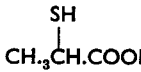
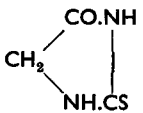
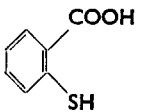
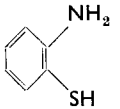
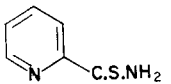
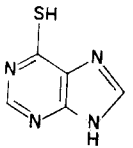
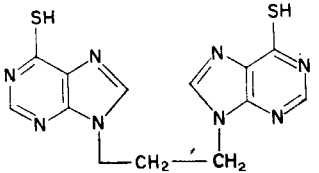
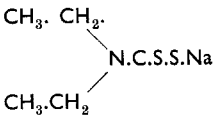
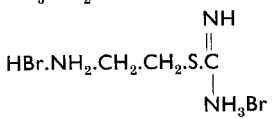
Pretreatment				Number of deaths from the following doses of Merophan (mg/kg)						LD_{50} Merophan (mg/kg)	95% Confidence limits	Dose reduction factor
Compound	Formula	Dose (mg/kg)	Solvent	2·66	4·0	6·0	9·0	13·5	20·25			
	No pretreatment			0/20	85/115	76/80	60/60	30/30	20/20	3·67	4·16 3·24	
Sodium thiosulphate	$Na_2S_2O_3$	2000	H_2O	0/10	7/10	10/10	10/10			3·67	4·16 3·24	1·0
Sodium thiocyanate	NaCNS	500	H_2O	0/10	6/10	10/10	10/10			3·82	4·36 3·35	1·0
α-Mercaptoacetic acid	$HS.CH_2.COOH$	75	NaOH buffer	0/10	2/10	5/10	10/10			5·52	6·58 4·67	1·5
α-Mercaptopropionic acid		25	NaOH buffer		3/10	10/10						
β-Mercaptopropionic acid	$HS.CH_2.CH_2.COOH$	25	NaOH buffer		4/10							
Thio-acetic acid	$CH_3.CO.SH$	75	NaOH buffer		3/10							
2-Thiohydantoin		125	Propylene glycol		10/10							
o-Thiobenzoic acid		100	NaOH buffer		9/10							

TABLE 2—*continued*

Compound	Formula	Pretreatment Dose (mg/kg)	Solvent	Number of deaths from the following doses of Merophan (mg/kg) 2·66	4·0	6·0	9·0	13·5	20·25	LD_{50} Merophan (mg/kg)	95% Confidence limits	Dose reduction factor
o-Aminobenzene thiol		50	50% Aq. propylene glycol		9/10							
Isonicotinic acid thio-amide		200	Propylene glycol		6/10							
6-Mercapto-purine		200	NaOH buffer		6/10							
Bis(6-mercapto-9-purinyl)ethane		300	NaOH buffer	0/10	0/10	10/10	10/10			4·89	4·89 4·89	1·3
Sodium diethyl dithiocarbamate		400	H_2O	0/10	8/10	10/10	10/10			3·53	3·93 3·17	1·0
β-S-2-Aminoethyl-isothiouronium bromide HBr		200	Buffer		0/10	0/10	10/10	10/10		7·35	7·35 7·35	2·0

4A TABLE 2—*continued*

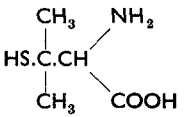
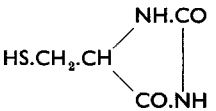
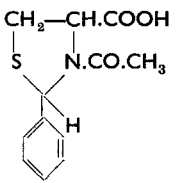
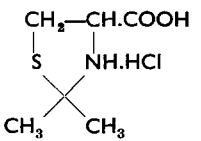
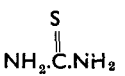
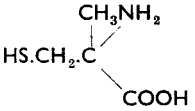
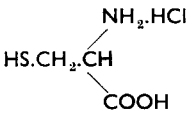
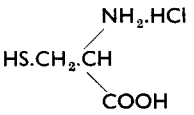
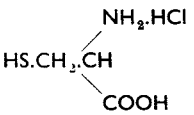
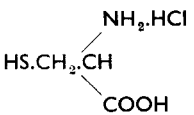
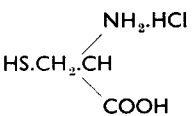
Pretreatment				Number of deaths from the following doses of Merophan (mg/kg)						LD_{50} Merophan (mg/kg)	95% Confidence limits	Dose reduction factor
Compound	Formula	Dose (mg/kg)	Solvent	2·66	4·0	6·0	9·0	13·5	20·25			
Cysteamine HCl	$HS.CH_2.CH_2.NH_2.HCl$	150	H_2O	0/10	4/10	7/10	10/10			4·69	5·62 3·92	1·3
DL-Penicillamine		150	H_2O	0/10	2/10	5/10	10/10			5·52	6·55 4·64	1·5
L-Cysteine hydantoin		400	H_2O	0/10	0/10	8/10	10/10			5·30	5·90 4·75	1·4
N-Acetyl-2-phenyl-thiazolidine-4-carboxylic acid		1000	NaOH / H_2O		10/10							
2:2-Dimethylthia-zolidine-4-carboxylic acid HCl		500	H_2O		0/10	0/10	10/10	10/10		7·35	7·35 7·35	2·0
Thiourea		1200	H_2O		0/10	0/10	5/10	9/10		9·0	10·50 8·86	2·5

TABLE 2—*continued*

Compound	Pretreatment: Formula	Dose (mg/kg)	Solvent	Number of deaths from the following doses of Merophan (mg/kg): 2·66	4·0	6·0	9·0	13·5	20·25	LD_{50} Merophan (mg/kg)	95% Confidence limits	Dose reduction factor
DL-α-Methyl cysteine HCl		500	$\frac{NaOH}{H_2O}$				9/10					
L-Cysteine-HCl		62·5	$\frac{NaOH}{H_2O}$	0/10	6/10	9/10	10/10			3·99	4·70 3·38	1·1
L-Cysteine-HCl		125	$\frac{NaOH}{H_2O}$	0/10	6/10	10/10	10/10			3·99	4·70 3·38	1·1
L-Cysteine-HCl		250	$\frac{NaOH}{H_2O}$		0/10	3/10	10/10	10/10		6·50	7·36 5·75	1·8
L-Cysteine-HCl		500	$\frac{NaOH}{H_2O}$		0/10	0/10	0/10	10/10		11·02	11·62 11·02	3·0
L-Cysteine-HCl		1000	$\frac{NaOH}{H_2O}$		0/10	0/10	0/10	2/10	10/10	15·24	16·71 13·65	4·2

also lost weight over the first 4 days the average loss being 20 g. In the animals pretreated with cysteamine (150 mg/kg) essentially the same blood picture was seen. When the animals were pretreated with cysteine (1000 mg/kg) however, the animals appeared to be almost completely protected showing no weight loss and only a slight fall in the blood elements.

TABLE 3. EFFECT OF CYSTEINE (1000 MG/KG) ON THE TOXICITY OF VARIOUS ALKYLATING AGENTS

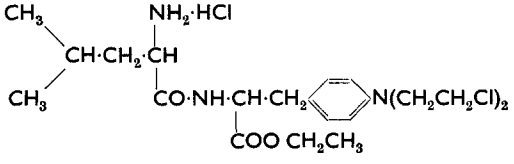
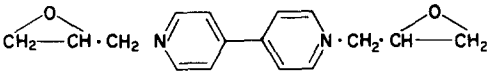
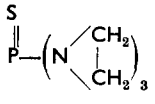
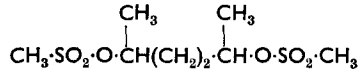
Compound	Dose (mg/kg)	Number of deaths
L-Leucylmelphalan ester HCl'd	12	3/5
	15	5/5
	15 + cysteine	0/5
	20	5/5
	20 + cysteine	0/5
	25	5/5
	25 + cysteine	0/5
	30	5/5
	30 + cysteine	3/5
	35	5/5
	35 + cysteine	5/5
N,N-bis(2:3 epoxypropyl)-4:4-dipiperidyl	50	8/15
	50 + cysteine	0/15
	75	15/15
	75 + cysteine	6/15
		
Thio-TEPA	30	4/5
	30 + cysteine	4/5
		
Dimethylmyleran	7	8/10
	7 + cysteine	9/10
		
Myleran	16	3/10
	16 + cysteine	2/10
$CH_3 \cdot SO_2O(CH_2)_4 \cdot O \cdot SO_2 \cdot CH_3$	17	10/10
	17 + cysteine	9/10

Fig. 2 shows the blood response pattern to L-leucylmelphalan ethyl ester hydrochloride (CB 3262) at 6·5 mg/kg. After cysteine pretreatment this mustard must be given at 16·0 mg/kg in order to obtain the same effect on the blood pattern. This is a dose reduction of 2·5. The LD_{50} of leucylmelphalan is approximately 12 mg/kg and after cysteine pretreatment this is reduced to about 30 mg/kg (Table 3). This also corresponds to a dose reduction of 2·5.

DISCUSSION

The thiols that protect against radiation toxicity are believed to act by either directly reacting with free radicals formed by the irradiation or by protecting radiosensitive

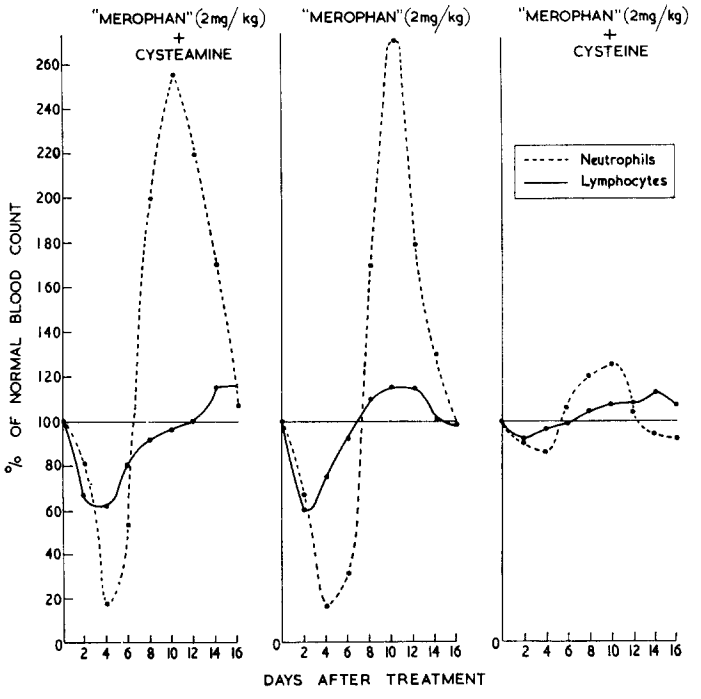


FIG. 1. Effect of pretreatment with cysteamine (150 mg/kg i.p.) or cysteine (1000 mg/kg i.p.) on the haematological response of the rat to Merophan.

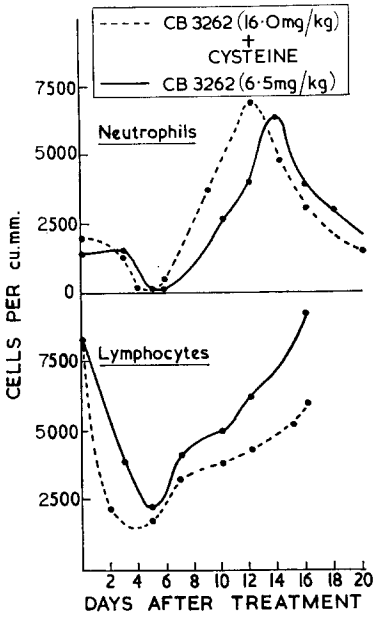


FIG. 2. Reduction in haematological response of the rat to leucylmelphalan (CB 3262) by pretreatment with cysteine (1000 mg/kg).

–SH containing molecules of the cell by the formation of reversible mixed disulphides.[17] Protection against the toxicity of the mustards might similarly be obtained by direct reaction of the thiols with the mustard or by the formation of mixed disulphides. However, if the latter mechanism is the one by which the thiols afford their protection, then it must be assumed that the toxicity of the mustards is due primarily to a reaction with —SH groups in the cell. There is at present no evidence which indicates that this is the case. Sulphur mustard certainly reacts with DNA *in vivo*[18] and disulphide formation would not presumably prevent this reaction.

Cysteine gave no protection against Merophan at doses below 250 mg/kg and even at this level the protection obtained was only slight. Good protection was obtained at 500 mg/kg. In this connection it is of interest that Eden *et al.*[19] showed that in rats, no free cysteine could be detected in liver until it was given by i.p. injection in amounts of 150 mg/kg or greater. If this is also the case in the other tissues protected by cysteine from damage by Merophan, then it would seem that protection against the mustards is only obtained when there is a significant level of free SH in the tissue. We are at present investigating the free SH levels, after cysteine pretreatment, in those tissues most affected by Merophan namely, spleen, bone-marrow and small intestine, in an attempt to correlate protective action with the level of free SH. It is also of interest that Eden *et al.* found that after i.p. administration of cysteine, the level of free cysteine in the liver was highest about 15 min after the injection. In our experiments cysteine gave its maximum protective effect when it was given 15–20 min before the mustard.

The thiols found to give the best protection against Merophan, namely thiourea and cysteine are not the best radiation protectors. AET which is probably the most effective in protecting against radiation gave only moderate protection against the toxicity of Merophan, and cysteamine which is as efficient as cysteine as a radiation protector gave no significant protection against Merophan. While deaths from Merophan are entirely "gastrointestinal" deaths occurring within 6 days, deaths after whole body irradiation fall into two classes.[20] Animals may die from a "gastrointestinal" death similar to the death from Merophan or from a "haematopoietic" death, i.e. one resulting from bone marrow damage, and occurring normally between 12 and 18 days after the irradiation. At the LD_{100} radiation dose rats normally die from bone marrow damage but at higher radiation doses the "gastrointestinal" death predominates. One might speculate that AET is strongly concentrated in the bone marrow and so protects more effectively against the "haematopoietic" death while cysteine being more strongly concentrated in the gastrointestinal mucosa protects more efficiently against the "gastrointestinal" death. It would seem of interest to compare the effects of cysteine and AET on the two types of radiation toxicity. Willoughby[21] has recently shown that di*iso*propylfluorophosphonate can protect against the "intestinal" but not against the "bone marrow" radiation death.

There also appears to be a correlation between the reactivity of the alkylating agent towards nucleophilic centres and the extent of protection obtained. The most reactive alkylating agents are the aliphatic nitrogen mustards, and Brandt and Griffin[4] found that cysteine pretreatment before HN2 administration gave a dose reduction factor of between 6 and 7. In our experiments the most chemically reactive compound used was the aromatic nitrogen mustard Merophan and with cysteine pretreatment the dose reduction factor was 4. L-Leucylmelphalan, another aromatic nitrogen mustard,

which is less reactive than Merophan, gave a dose reduction factor between 2 and 3. The toxicity of the epoxides, ethylene-imines and sulphonoxyalkanes which are considered to be less reactive *in vivo* was reduced only slightly or not at all by cysteine pretreatment. Cysteine is quite rapidly metabolized *in vivo*, and if protection depends on the reaction with free cysteine and the alkylating agent in the cell, then protection would be obtained only if a large portion of the alkylating agent reacted during the time free cysteine was present in the cell. One might obtain protection against the less reactive alkylating agents by giving the cysteine in such a way that it remained at a high level in the cell for a long period. Analogously, thiol protection against radiation is dependent on the dose rate. Leitch[22] has shown that while AET gives good protection against 460 r total body irradiation at a dose rate of 20 r/min, the protective effect diminished when the rate was decreased.

2:2-Dimethylthiazolidine-4-carboxylic acid which gave moderate protection against Merophan, probably acts by releasing cysteine *in vivo*. This compound readily forms cysteine *in vitro* on hydrolysis, while 2-phenyl-N-acetyl thiazolidine-4-carboxylic acid which gave no protection is quite stable *in vitro*.. Njaa[23] has similarly shown that those thiazolidines that break down readily *in vitro* to form cysteine can be used as a cysteine source by *L. Mesenteroides* while the more stable thiazolidines cannot.

Thiosulphate reacts very rapidly with Merophan *in vitro* but had no protective effect *in vivo*. Being completely ionized at physiological pH, thiosulphate does not enter cells. The lack of protection observed indicates that thiol protection occurs by an intracellular reaction.

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REFERENCES

1. H. M. PATT, E. B. TYREE, R. L. STRAUBE and D. E. SMITH, *Science* **110**, 213 (1949).
2. Z. M. BACQ and P. ALEXANDER, *Fundamentals of Radiobiology*. Butterworth, London (1961).
3. A. KALUSZYNER, P. CZERNIAK and E. BERGMANN, *Rad. Res.* **14**, 23 (1961).
4. E. L. BRANDT and A. C. GRIFFIN, *Cancer* **4**, 1030 (1951).
5. A. S. WEISBERGER, R. W. HEINLE and B. LEVINE, *J. Clin. Invest.* **31**, 217 (1952).
6. P. SALERNO and M. FRIEDEL, *U.S. Atomic Energy Comm. Nuclear Sci. Abs.* **9**, (1955).
7. D. P. RALL, M. G. KELLY, R. W. O'GARA, B. I. SHNIDER and C. G. ZUBROD, *J. Pharmacol.* **122**, 63A (1958).
8. L. ELDJARN and O. NYGAARD, *Arch. Intern. Physiol.* **62**, 476 (1954).
9. O. PECZENIC, *Nature, Lond.* **172**, 454 (1953).
10. R. J. RUTMAN, F. S. LEWIS and C. C. PRICE, *Proc. Amer. Ass. Cancer Res.* **3**, 264 (1961).
11. W. C. J. ROSS, *Biochem. Pharmacol.* **8**, 235 (1961).
12. E. I. GOLDENTHAL, M. V. NADKARNI and P. K. SMITH, *Rad. Res.* **10**, 571 (1959).
13. E. HAAS, *Strahlentherapie* **114**, 468 (1961).
14. I. PEISAKHOVICH, P. SOLDGUB and I. TELENGATOR, *Cancer Chemotherapy Abs.*, **1**, 1091 (1960).
15. T. A. CONNORS, W. C. J. ROSS and J. G. WILSON, *J. Chem. Soc.* **2994** (1960).
16. C. S. WEIL, *Biometrics* **8**, 249 (1952).
17. A. PIHL and L. ELDJARN, *Advances in Radiobiology* p. 147. Oliver and Boyd, Edinburgh (1957).
18. P. BROOKES and P. D. LAWLEY, *Biocham. J.* **77**, 478 (1960).
19. E. EDEN, H. B. GRANOWSKI, W. G. JONES and J. A. LINNANE, *Aust. J. Biol. Sci.* **10**, 217 (1957).
20. L. A. ELSON, *Symposium on Radiobiology* p. 235. Butterworth, London (1955).
21. D. A. WILLOUGHBY, *Brit. J. Radiol.* **34**, 807 (1961).
22. J. LEITCH, *Rad. Res.* **15**, 641 (1961).
23. A. B. NJAA, *Nature, Lond.* **192**, 463 (1961).