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PROTECTION AGAINST SYSTEMIC POISONING BY MUSTARD GAS, DI(2-CHLOROETHYL) SULPHIDE, BY SODIUM THIOSULPHATE AND THIOCIT IN THE ALBINO RAT

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The lethal effects of mustard gas, di(2-chloroethyl) sulphide, in the albino rat have been counteracted by Thiocit, a mixture of sodium thiosulphate and trisodium citrate in the ratio 10 : 1, administered intraperitoneally in a dose of 2.75 g./kg. Thiocit afforded complete protection against greater than the median lethal dose of mustard gas whether given 10 min. before or 10 min. after mustard gas and raised the LD50 of mustard gas by approximately three times. The protection appeared whether the total dose of Thiocit was given in one injection or serially over 30 min. The effective doses of sodium thiosulphate and of Thiocit in rats were of the order of 3.0 g./kg. Sodium thiosulphate alone and Thiocit have been administered in single doses by slow infusion, by stomach tube and in drinking water. Both have shown activity by all routes of administration, but activity was greatest by intraperitoneal injection. The use of Thiocit in conjunction with mustard gas therapy is suggested.

Many attempts have been made to find a substance which offers effective protection against the systemic effects and death produced by poisoning with mustard gas, di(2-chloroethyl) sulphide. Numerous authors have demonstrated some alleviation of the local or systemic effects of mustard gas by cysteine and glutathione. Paulet and Chappet (1956) have discussed the more recent work and showed that phenylallylthiourea was as active as cysteine, but somewhat more effective than glutathione in reducing the severity of the leucopenia produced by sublethal injections of mustard gas into albino rats.

In spite of this extensive search, however, little success has been achieved in protecting against lethal doses of mustard gas. A large number of thio compounds has been tested on the basis of theoretical bonding with, and high competition factor for, mustard gas (Ogston, 1948). Sodium thiosulphate has a high competition factor (2.7×10^4), and has probably a relatively high permeability through the cell membrane ; it is non-toxic in very high doses and theoretically could combine with mustard gas. Citrates also have a high competition factor (1.7×10^2) (Callaway and Pearce, unpublished observations) for mustard gas ; they are natural constituents of body fluids and probably possess a fairly high permeability at the cellular level.

These two compounds were therefore investigated for possible prophylactic or therapeutic effects against poisoning by mustard gas. Each was tried separately and also in combination as Thiocit by several routes against lethal doses of the mustard gas.

METHODS

Sodium thiosulphate 500 mg./ml. and sodium citrate 100 mg./ml. were used in aqueous solution both for injection and for drinking purposes. Thiocit consisted of $Na_2S_2O_3,5H_2O$ (50 g.) and $Na_3C_6H_5O_7,5H_2O$ (5 g.) made up to 100 ml. with distilled water to give a total concentration of 550 mg./ml.

Male albino rats of the Wistar strain weighing 190 to 215 g. were used. Mustard gas solution in propylene glycol was freshly prepared for each experiment, such that the volume injected in ml. was equal to the body weight in kg. The agents were usually administered either 10 min. before (−10′) or 10 min. after (+10′) mustard gas.

After injection, the rats were returned to their cages and their general condition noted and survival times observed. No attempt was made to observe the blood picture, since the main objective was to find the best combination of time of injection and dose for protection against death from mustard gas poisoning.

Mustard gas was always administered subcutaneously into the loose skin of the right groin, and materials used in prophylaxis or therapy were

administered subcutaneously, intraperitoneally, intravenously using the lateral marginal vein (Pearce, 1957), or orally, as indicated in the results.

The Determination of Competition Factor. —The rate of liberation of acid from mustard gas in a solution of a non-acid-forming competitor under investigation was determined in the presence of a known concentration of a suitable acid-forming competitor of known competition factor in the manner described by Ogston (1948). The competition can then be calculated from the expression given by Ogston (1948), namely, molar concentration × competition factor.

RESULTS

Control Injections of Mustard Gas

The LD50 of mustard gas when administered subcutaneously to rats was 3.8 mg./kg. From the dosage/mortality curve the LD99 was predicted to be 6.27 mg./kg., and, in fact, no survivors occurred at this dose, almost all the rats dying between the third and fifth day. The animals showed severe emaciation, increasing leucopenia (Callaway and Pearce, unpublished observations), diarrhoea, loss of weight and marked deterioration in the condition of the fur. On the third or fourth day they usually became progressively weaker and died.

Sodium Thiosulphate by Intraperitoneal Injection.—These results are summarized in Table I. The best protection was obtained from a single intraperitoneal injection of sodium thiosulphate (2.75 to 3 g./kg.) given 10 min. before 6.27 mg./kg. mustard gas. Protection also occurred when the dose of mustard gas was increased to 8.15 mg./kg. and only 2/10 animals died. In nearly all instances the survivors remained actively healthy, though some were in poor condition for a day or two.

TABLE I

THE EFFECT OF INJECTING SODIUM THIOSULPHATE INTRAPERITONEALLY INTO MALE ALBINO RATS 10 MIN. BEFORE THE SUBCUTANEOUS INJECTION OF MUSTARD GAS

Treatment with sodium thiosulphate was given 10 min. before mustard gas injection.

Dose of $Na_2S_2O_3.5H_2O$ (g./kg.)	Dose of Mustard Gas (mg./kg.)	No. of Deaths: Day of Death 1	2	3	4	5	6	11	Total Mortality
	6·27				5				5/5
	3·8				2				2/5
3·0	6·27				1				1/5
3·0	3·8								0/5
	6·27				10				10/10
3·0	6·27				1				1/10
	6·27				9	1			10/10
2·75	6·27				1				1/10
	6·27			1	4				5/5
2·75	6·27							1	1/5
1·5	6·27							1	1/5
	10·6			1	4				5/5
2·75	10·6			2	5	1	2		10/10
2·75	8·15				2				2/10
2·75	6·27				1				1/10
	6·27				9				10/10

Sodium Thiosulphate by Intraperitoneal and Subcutaneous Infusion.—To decide whether prolonged administration would be as effective as a massive initial dose in giving protection, peritoneal and subcutaneous infusion experiments were undertaken. Sodium thiosulphate solution was injected gradually over a period of 33 min. immediately following the subcutaneous injection of mustard gas until the total amount received by each rat was 3 g./kg. in a total volume of about 1.2 ml. A control group received 0.85% sodium chloride solution instead of sodium thiosulphate under exactly the same conditions. In the group treated intraperitoneally, the mortality was 4/5, whilst in the group treated subcutaneously and in the control group the mortality was 5/5.

Oral Administration of Sodium Thiosulphate

In Drinking Water.—Previous work has indicated that oral therapy would be unlikely to be successful, but, in view of the success with intraperitoneal injections, 20 rats were divided into 4 groups of 5, which were treated in the following manner.

Group A was given normal drinking water daily for 7 days, then injected with a dose of mustard gas subcutaneously (4.0 mg./kg., being very slightly above the LD50), and continued with their normal drinking water. The mortality was 4/5.

Group B were offered a 0.5% aqueous solution of sodium thiosulphate in place of their normal drinking water daily for 7 days, then injected with a dose of mustard gas subcutaneously (4 mg./kg.) and continued drinking 0.5% thiosulphate for a further 7 days. The mortality was 2/5.

Group C were treated like those in Group B except that they received no mustard gas injections. The mortality was 0/5.

Group D received normal drinking water for 7 days, then were injected with a dose of mustard gas (4 mg./kg.) and given 0.5% sodium thiosulphate solution instead of drinking water for 7 days. The mortality was 1/5.

The drinking fluid was replaced daily and the intake observed, there being no difference between test and control. The weather was warm and approximately 30 to 50 ml. was drunk by each rat daily. The results suggested that there may have been a small measure of protection, though this was not statistically significant ($P>0.1$).

TABLE II

THE EFFECT OF GIVING SODIUM THIOSULPHATE BY STOMACH TUBE ON THE TOXICITY OF MUSTARD GAS TO RATS

Sodium thiosulphate was given 10 min. before (−10′); at (0′); 10, 20 or 30 min. after (+10′, +20′ or +30′) the mustard gas. In Expt. 5, sodium thiosulphate was given dissolved in 25% ethanol to find whether absorption was more rapid.

Expt. No.	No. of Animals	$Na_2S_2O_3,5H_2O$ (g./kg.)		Mustard Gas (mg./kg.)	$Na_2S_2O_3,5H_2O$ (g./kg.)			Final Mortality	Remarks
		−10′	0′	0′	+10′	+20′	+30′		Condition of Rats on Days Following Treatment (for example +3=3rd day after treatment)
1	10	2·0	2·0	6·27	2 0			5/10	9/10 poor on +3, 5/10 recovered. Deaths +4, +5
	10	—	—	6·27	—			10/10	Poor +3, dead +4
2	10	2·0	1·0	6·27	1·0			6/10	On +3 4 poor, 5 good, 1 dead. Deaths +3, +4
	10	—	—	6·27	—			10/10	Poor +3, dead +4
3	5	5·0		6·27				5/5	
	5		5 0	6·27				5/5	
	5			6·27	5·0			4/5	Nearly all poor +3, had diarrhoea, dead +4
	5			6·27		5 0		5/5	
	5			6·27			5·0	4/5	
	5			—			5.0	0/5	All quite fit throughout
	5			6·27				5/5	All poor, diarrhoea +3, dead +3, +4
4	5	1·5	1·5	6·27	1·5			4/5	+14 day terminated. 1 fit
	5	1·0	1·0	6·27	1·0			5/5	
	5		1·0	6·27	1·0	1·0		4/5	1 fit
	5			6·27	1·0	1·0	1·0	4/5	1 fit
	5			—	1·0	1·0	1·0	0/5	5 fit
	5			6·27				5/5	
5	5			6·27	0·6	0·6	0·6	3/5	2 fit
	5			6·27	0·9	0·9	0·9	5/5	
	5			6·27	1·2	1·2	1·2	3/5	2 fit
	5			6·27	1·5	1·5	1·5	4/5	1 fit
	5			—	1·5	1·5	1·5	0/5	5 fit
	5			6·27				5/5	

By Stomach Tube.—The results of administering sodium thiosulphate by stomach tube are given in Table II. The protection against the LD99 dose of mustard gas appeared to be better if the total dose was given in several instalments, close to the time of the mustard gas injection. Although nearly 50% survival was obtained against an LD99 dose of mustard gas, the animals were sometimes in poor condition on the third day but recovered later.

Sodium Thiosulphate by Intravenous Injection

A certain amount of protection against the LD99 of mustard gas was shown when 1.5 g./kg. sodium thiosulphate was injected intravenously at the same time as the subcutaneous injection of mustard gas (1/5 died) or 10 min. afterwards (3/5 died). All the control rats died (5/5).

Thiocit by Intraperitoneal Injection

The preceding experiments had shown that a reasonable protection against the lethal effects of mustard gas could be achieved with sodium thiosulphate, though in no instance was it complete. It seemed likely that the addition of a second competing ion, namely citrate administered simultaneously with the thiosulphate, might produce an even better protection. As we found that sodium citrate alone showed no promise against mustard gas poisoning, a solution of Thiocit was prepared as previously described. Table III gives the results of 5 separate experiments using Thiocit injected intraperitoneally 10 min. before or after the injection of mustard gas subcutaneously. All the controls died (25/25) after receiving 6.27 mg./kg. mustard gas (LD99) whereas none (0/25) died among those treated with 2.75 g./kg. of Thiocit. When the dose of Thiocit was reduced to 1.65 g./kg., 1 out of 5 died at the LD99 of mustard gas. Effective protection occurred even when the dose of mustard gas was increased to 8.15 mg./kg. and 10.6 mg./kg., the mortality being only 2 out of 10 at each dose, and the survivors remained in good condition, whereas 8 out of 10 died with 13.8 mg./kg. of mustard gas.

The results of injecting Thiocit (2.75 g./kg.) 10 min. after mustard gas (6.27 mg./kg.) showed that a completely successful treatment could be achieved against the lethal effects of this dose of mustard gas (Table III, Expt. 5). All the treated rats survived (0/10 died) compared with complete mortality among the controls (10/10 died). The survivors were in good condition and have been kept for 6 months during which time they have shown good general progress and health.

TABLE III

THE EFFECTS OF THIOCIT INJECTED INTRAPERITONEALLY BEFORE OR AFTER THE SUBCUTANEOUS INJECTION OF MUSTARD GAS IN RATS

Thiocit was injected 10 min. before (in Expts. No. 1 to 4) and 10 min. after (in Expt. No. 5) mustard gas. All the survivors were healthy 6 months later.

Expt. No.	Thiocit (g./kg.)	Mustard Gas (mg./kg.)	No. of Deaths: Day of Death 1	2	3	4	5	6	7	8	Total Mortality
1	2·75	Control									0/5
	2·75	6·27									0/5
	1·65	6·27				1					1/5
		6·27			1	4					5/5
2	2·75	6·27									0/10
		6·27			3	6		1			10/10
3		10·6			1	4					5/5
	2·75	10·6			1		1				2/10
	2·75	8·15				1	1				2/10
	2·75	6·27									0/10
		6·27				9	1				10/10
4	2·75	17·9			1	8	1				10/10
		13·8			5	5					10/10
	2·75	13·8			1	6	1				8/10
5		6·27				8	2				10/10
	2·75	6·27									0/10

Thiocit by Mouth

A dose of 3.3 g./kg. of Thiocit was administered by stomach tube at various times before and after 6.27 mg./kg. of mustard gas. Some protection was shown (Table IV), but this was not very marked, the total combined mortality in the treated groups being 15/20 compared with 5/5 in the untreated controls.

TABLE IV

THE EFFECT OF ADMINISTERING THIOCIT ORALLY ON THE TOXICITY OF MUSTARD GAS TO RATS

For explanation of −10′, +10′ and +20′, see Table II.

Thiocit (g./kg.) −10′	Mustard Gas (mg./kg.)	Thiocit (g./kg.) +10′	Thiocit (g./kg.) +20′	No. of Deaths: Day of Death 1	2	3	4	5	6	12	Total Mortality
3·30											0/5
3·30		6·27					1	1	1	1	4/5
	3·30	6·27					4				4/5
		6·27	3·30			1	1				2/5
		6·27	3·30			2	3				5/5
		6·27				3	1	1			5/5

DISCUSSION

Our results showed that sodium thiosulphate alone, but more particularly when combined with sodium citrate as Thiocit, was capable of protecting rats completely from a dose of mustard gas which would otherwise kill 99% of rats. Although a certain amount of protection occurred following the oral administration of both preparations, the most effective route was intraperitoneal. The results from four experiments in which 2.75 g./kg. of Thiocit was injected intraperitoneally either 10 min. before or 10 min. after 6.27 mg./kg. of mustard gas showed no deaths (0/35) among the treated rats compared with 35/35 deaths among the controls. In Table V, the mortality resulting from thiosulphate and Thiocit injected either 10 min. before or 10 min. after various doses of mustard gas is compared. It will be seen that the LD50 of mustard gas when Thiocit was used was raised approximately 3 times.

TABLE V

THE SUMMARIZED MORTALITIES FOLLOWING THE INTRAPERITONEAL INJECTION OF THIOSULPHATE OR THIOCIT WITH VARIOUS DOSES OF MUSTARD GAS

Dose of Mustard Gas (mg./kg.)	Thiosulphate (2·75 g./kg.)	Thiocit (2·75 g./kg.)	Mustard Gas Alone
17·90	—	10/10	10/10
13·78	—	8/10	10/10
10·60	10/10	2/10	5/5
8·15	2/10	2/10	—
6·27	1/10	0/10	10/10

Holiday, Philpot and Stocken (1950) have shown that sodium monothiophosphate is a weak antidote to mustard gas. They treated rats with 5 mg./kg. of mustard gas dissolved in ethanol. The 10-day mortality was reduced from 10/20 to 2/10 by sodium monothiophosphate.

Of the many substances which we have tested against the systemic effects and death from mustard gas poisoning, only hydroxylamine (Callaway and Pearce, unpublished observations) apart from Thiocit had hitherto shown any promise. Hydroxylamine, however, was unstable and caused occasional deaths from pneumonia. It was only effective under restricted conditions of *p*H, and then only for a short period in relation to the time of mustard gas injection, and was therefore not investigated further.

This work has shown that sodium thiosulphate and Thiocit are effective antidotes to mustard gas. In view of the potential value of mustard gas and its analogues in certain clinical conditions, the existence of the means of counteracting their effects may be of value. Thiocit may also provide a means of elucidating the mechanism by which systemic damage and death is caused in poisoning by mustard gas.

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