British Anti-Lewisite as an Antidote for Acute Mercury Poisoning

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In another paper of this series (Thompson & Whitaker, 1947) a review is given of the application of BAL to the treatment of intoxication by metals other than arsenic. Using the brain pyruvate enzyme as a test system these authors showed that *in vitro* BAL was able to protect against the toxic action of compounds of antimony, gold and mercury. Concurrently with this work experiments were carried out to determine whether BAL was also effective *in vivo* as an antidote to injected or orally administered mercuric chloride.

Many authors (Rosenthal, 1933, 1934; Wolpaw & Alpers, 1942; Rozhkov & Arkhipov, 1939; Barnes, 1939; Hug, 1938) have claimed that rongalite (sodium formaldehyde sulfoxylate) is an effective antidote for mercuric chloride poisoning. On the other hand, Modell, Gold, Winthrop & Foot (1937) reviewing the evidence in conjunction with their own experimental results came to the conclusion that 'while sulfoxylate may prove of some clinical value in reducing the mortality in bichloride of mercury poisoning, the problem of controlling the chief cause of the high mortality in human poisoning remains unsolved'. They found that when the mercury and the antidote were given orally to cats no appreciable protection was afforded when the delay in treatment was longer than 15 min. Munoz (1935) working with rats found very little protection after 5 min. In view of this doubtful value of rongalite an attempt was therefore made to assess the relative merits of this substance and BAL in acute oral poisoning.

EXPERIMENTAL AND RESULTS

BAL therapy in mercury poisoning

Acute mercury poisoning was produced in rats by intraperitoneal injection of an aqueous solution of mercuric chloride; half of the animals were used as controls and after an interval of 10 min. the other half were given a solution of BAL in benzyl benzoate-peanut oil mixture as made up for therapeutic use (Eagle, 1943). To avoid local detoxication, the antidote was injected intramuscularly into the hind leg.

It will be seen from Table 1 that the dosage-mortality curve for HgCl_2 is very steep and that a high degree of protection is afforded by BAL against an amount of mercuric chloride greater than

that required to produce 100% fatalities. These experiments clearly show that BAL is an effective antidote to mercury when introduced by injection

Table 1. *The effect of intramuscular-injected BAL against injected mercuric chloride*

No. rats	Dose HgCl_2 (mg./kg.)	Dose BAL (mg./kg.)	Survivors	
			No.	(%)
10	10	Nil	0	0
10	5.5-5.75	Nil	0	0
7	4	Nil	0	0
10	2	Nil	10	100
10	10	50	1	10
10	5.9-6.25	50	9	90
9	4.0-4.2	50	9	100
10	2	50	10	100

into the animal and it was considered desirable to see if a similar degree of protection could be obtained when the poison was given orally. In order to minimize variations in the results it was found necessary to employ the following conditions:

(1) The treated and untreated animals of a particular experiment were selected from rats with the same history.

(2) The rats were starved for 20-24 hr. before commencing the experiment.

(3) A post-mortem examination was carried out on all the animals which died and on those which survived the observation period of not less than one month. The post-mortem examination was necessary to ensure that no complicating factors were present; for example, it was noticed that in a batch of rats received on one occasion a few of the animals had lung abscesses; all such batches were excluded.

The mercuric chloride, usually a 1% solution, was given by stomach tube using a No. 3 gum elastic catheter. For oral administration a fresh aqueous solution of BAL was employed and for injection the standard 5% preparation in oil. Table 2 gives the toxicity figures for mercuric chloride, Table 3 the results with BAL given orally and Table 4 with BAL by intraperitoneal injection.

From these results it appears that absorption of the mercury is too rapid to allow local detoxication in the stomach (Table 3) and that it is essential to introduce the antidote more quickly into the circulation (Table 4). In this case significant protection can be obtained at 15 min., but it was felt that the therapy might be improved by a combination of the

two techniques especially since post-mortem examination revealed local damage in the stomachs of injected animals.

Table 2. *The toxicity of mercuric chloride given by mouth*

No. animals	Dose (mg./kg.)	Survivors	
		No.	(%)
8	6	8	100
7	12	7	100
8	24	8	100
12	50	4	33
12	75	0	0
40	100	1	2.5
6	150	1	17
31	200	0	0

Table 3. *The effect of BAL against mercuric chloride (both orally administered)*

No. animals	Dose HgCl ₂ (mg./kg.)	Dose BAL after 15 min. (mg./kg.)	Survivors	
			No.	(%)
6	50	75	0	0
12	75	75	2	17
6	100	75	0	0
6	150	75	0	0
6	200	75	0	0

Table 4. *The effect of BAL (intraperitoneal injection) against mercuric chloride (given orally)*

No. animals	Dose HgCl ₂ (mg./kg.)	Dose BAL (mg./kg.)	Time of BAL injection (min.)	Survivors	
				No.	(%)
6	50	75	15	6	100
12	100	75	15	8	75
12	100	75	30	3	25
12	100	75	60	5	41

Before commencing an extensive series of experiments on the combined therapy, a comparative test was carried out to see which of three injection routes gave the best results.

Three groups of rats were given 100 mg./kg. HgCl₂ by mouth and 30 min. later, BAL (50 mg./kg.) was injected either intraperitoneally, intramuscularly or subcutaneously. At 120 min. each of the groups received 25 mg./kg. oral BAL. All animals survived, but at the end of 3 weeks the subcutaneous group showed a loss in weight while the other two groups showed a gain. On the basis of the figures given in Table 5 it seems there is little to choose between intramuscular and intraperitoneal injection, but in view of the slight advantage shown by the latter this method was the one used in subsequent experiments. For technical reasons, intravenous injections were not attempted, but it is possible that the use of BAL-INTRAV may lead to an improved therapy.

Table 5. *A comparison of various injection techniques in the treatment of oral poisoning with mercuric chloride*

No. animals	Dose HgCl ₂ (mg./kg.)	Injection route	Initial gross wt. (g.)	Gross wt. change (g.)	
6	100	Intraperitoneal	1516	+ 48	(3%)
6	100	Intramuscular	2087	+ 12	(0.6%)
6	100	Subcutaneous	1460	- 158	(- 10.8%)

In order to allow some degree of comparison between the various methods of applying BAL as an antidote, the amount of BAL used was kept constant at 75 mg./kg.; thus, when two routes were employed 50 mg./kg. were injected and 25 mg./kg. were given orally. The results obtained with the combined therapy (Table 6) indicate an enhanced survival rate

Table 6. *The effect of BAL (oral and injected) against mercuric chloride (oral)*

No. animals	Dose HgCl ₂ (mg./kg.)	Time before injection of BAL (min.)	Time before oral dosage with BAL (min.)	Survivors	
				No.	(%)
12	50	15	30	12	100
12	100	15	30	12	100
9	100	30	120	7	78

as compared with the single injection method. It might be argued that this improvement was merely due to the division of the dose with the result that a concentration of the drug was maintained over a longer period of time; this was shown not to be the case by an experiment in which nine animals were injected at 15 min. with 50 mg./kg. and at 30 min. with 25 mg./kg.; of these animals, only four survived.

The effect of rongalite on oral mercuric chloride poisoning

Although in the past the best results appear to have been obtained by the intravenous injection of rongalite, it was considered worth while to compare

Table 7. *Rongalite in the treatment of mercuric chloride poisoning*

No. animals	Dose HgCl ₂ (mg./kg.)	Rongalite treatment		Survivors	
		Dose (mg./kg.)	Time before dosage (min.)	No.	(%)
12	50	400	15 (oral)	6	50
9	100	400	15 (oral)	0	0
12	50	100	15 (intrap.)	8	66
		100	30 (oral)		
		100	15 (intrap.)		
9	100	100	30 (oral)	0	0

rongalite with BAL when administered under the same conditions. The rongalite was made up in bicarbonate solution as recommended by Modell *et al.* (1937) and the results are given in Table 7.

DISCUSSION

The evidence presented in the previous paper by Thompson & Whittaker (1947) demonstrates that BAL is able to protect the pyruvate oxidase system of brain from the toxic action of mercury. Since at the time it was not possible to carry out *in vitro* experiments designed to show whether BAL was also able to bring about a reversal of the inhibition it was decided to expedite the practical application of BAL by direct trial *in vivo*. It has been found that intramuscular injection of BAL is an effective antidote to acute systemic poisoning caused by injection of mercuric chloride. Similar work (quoted by Waters & Stock, 1946) has also been carried out in the U.S.A. Although acute mercury poisoning in man can be produced by contact, it usually occurs by ingestion, and it was considered of interest, therefore, to investigate in rats the effect of BAL and also of rongalite against orally administered mercury. When the compounds* were given by mouth, no significant change was observed in the survival rate (Tables 2, 3 and 7). When, however, the antidotes were given by the combined injection and oral technique the difference in the results was striking. Thus, after dosing with 100 mg./kg. HgCl_2 , 39 of a group of 40 rats died, whilst complete survival

was obtained in the BAL group of 12; of nine rongalite-treated rats all died. These figures do not necessarily prove that the BAL has brought about a complete reversal of the toxicity, but taken in conjunction with the evidence of Barron & Kalnitsky (1944), who found that BAL was able to reactivate succinoxidase poisoned with 4-chloromercuribenzoic acid, and of Bloom (1943), who used BAL to prevent the development of vesication caused by 2-thienylmercuric chloride, they do suggest that partial reactivation at least has taken place. From this work it appears that the conclusions reached by Modell *et al.* in respect of the therapeutic value of rongalite are substantiated and that BAL is a more effective antidote for the treatment of acute systemic mercuric chloride poisoning.

SUMMARY

1. Acute mercury poisoning in HgCl_2 -injected rats is prevented by injection of BAL.
2. Rats are also protected from a lethal dose of HgCl_2 given by mouth if BAL is administered by injection and supplemented by oral dosage. This treatment is superior to rongalite given under the same conditions.

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REFERENCES

- Barnes, J. (1939). *Lancet*, no. 236, p. 89.
 Barron, E. S. G. & Kalnitsky, G. (1944). By communication.*
 Bloom, W. (1943). By communication.*
 Eagle, H. (1943). By communication.*
 Hug, E. (1938). *Chem. Abstr.* (1939), 5069.
 Modell, W., Gold, H., Winthrop, G. J. & Foot, E. B. (1937). *J. Pharmacol.* **61**, 66.
 Munoz, J. M. (1935). *C.R. Soc. Biol. Paris*, **120**, 500.
- Rosenthal, S. M. (1933). *U.S. Pub. Hlth Rep.* **48**, 1543.
 Rosenthal, S. M. (1934). *J. Amer. med. Ass.* **102**, 1273.
 Rozhkov, W. M. & Arkhipov, W. A. (1939). *Chem. Zbl.* **1939**, 1, 4648.
 Thompson, R. H. S. & Whittaker, V. P. (1947). *Biochem. J.* **41**, 342.
 Waters, L. L. & Stock, C. (1946). *Science*, **102**, 601.
 Wolpaw, R. & Alpers, N. (1942). *J. Lab. clin. Med.* **27**, 1387.

* Information available on application to Ministry of Supply, London.

A Note on the Microdetermination of British Anti-Lewisite

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The introduction of 2:3-dimercaptopropanol (BAL) for the treatment of systemic arsenical intoxication in man made it desirable to study the distribution of BAL in the body after injection, and also the rate and mode of its excretion. A method for the microdetermination of BAL was therefore required, and the present note describes an improvement of a procedure suggested by Calvery (1943), and its application to plasma, blood and urine.

EXPERIMENTAL

Microdetermination in simple aqueous solution

In 1943, Calvery reported the successful determination of small amounts of BAL using the colour formed by BAL with cobalt nitrate, but he gave no details of the method, and had applied it only to aqueous solution and to the aqueous humour of the eye.