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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* 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.

In a preliminary study of the reaction carried out in this laboratory it was found that the intensity of the colour formed with cobalt nitrate by a given amount of BAL was increased by making the solution more alkaline before addition of the BAL. Warming the solution before adding the BAL had a similar effect. It was also found necessary to add a stabilizing colloid such as gum arabic to prevent the solutions turning cloudy on standing. Thus the pH and the temperature of the solutions before addition of the BAL had to be controlled, and the following final method was therefore adopted.

Cobalt nitrate solution (1 ml. of 0.5 %) was mixed with 0.5 ml. 2 % gum arabic solution and sufficient 0.1 N-borate buffer (pH 9) to bring the final volume, including the BAL solution, to 10 ml. This reagent solution was heated to 45° in a water-bath, the BAL solution was added, and the mixture was allowed to stand at 45° for 10 min. The colour intensity was then measured with a Pulfrich photometer using light of wave-length 4700 Å., this being the wave-length of maximal light absorption of the colour.

Linear proportionality between colour intensity and BAL concentration was obtained under these conditions in the range 10–200 µg. of BAL. The solutions were heated only to 45° because a precipitate, which would have interfered with the colour estimations, was formed at higher temperatures.

In the later work with biological fluids, acetic acid was added to stabilize the BAL, and the presence of this acid in aqueous BAL solutions caused a slight fall in colour intensity. This fall was not due to a shift in pH caused by the acid, the buffering capacity of the solution being sufficient to maintain the pH at 9.0. Physiological concentrations of NaCl did not interfere with colour development. The method gave satisfactory recoveries (96–102 %) using aqueous solutions of BAL of known strength.

Specificity of the method. Other thiols give colours with cobalt nitrate under the conditions described, and although in many cases the colours are indistinguishable by the naked eye from the colour given by BAL, the points of maximum light absorption are usually slightly different from that of the BAL colour. The intensities of the colours, calculated on a basis of SH content, vary widely.

*Applications of the method to plasma,
blood and urine*

Added BAL could not be recovered quantitatively from plasma, blood or urine, but in view of the highly reactive nature of 1:2-dithiols, it seems likely

that the losses represent actual destruction of part of the BAL, rather than any failure in the method of estimation.

If BAL is added to plasma (containing NaF to prevent clotting), and the mixture is acidified and ultrafiltered, 65–70 % of the BAL can be recovered. The losses are probably due partly to oxidation by air, a process which occurs in simple aqueous solution at physiological pH, and which would be catalyzed by traces of iron porphyrins and metals in the plasma (Barron, 1943). In addition, some BAL apparently reacts with proteins, since only 70 % of added BAL could be recovered from an artificial 7 % protein solution.

To estimate recoveries from blood, BAL was mixed with the blood, the red cells were removed by centrifugation and the plasma acidified and ultrafiltered. This method gave average recoveries of between 30 and 69 %, depending on the initial concentration of BAL. In blood, the main cause of destruction is probably oxidation by haemoglobin within the red cells (Barron, 1943).

If normal urine containing BAL is added directly to the cobalt solution, average recoveries of between 56 and 88 % are obtained. Urine containing albumin yielded recoveries of 60–81 %, the measurements being carried out with the extracts after precipitation of the proteins with trichloroacetic acid. In every case, an increase in the initial concentration of BAL produced higher recoveries. In urine the losses seem to be due to reaction of BAL with some oxidizing agent, an equivalent amount of a reducing substance being liberated, since while the recoveries by the cobalt method are low, practically quantitative recoveries are obtained by iodine titration.

SUMMARY

1. A method is described for the micro determination of BAL, using the colour formed by BAL with cobalt nitrate.

2. The method has been applied to the measurement of the recoveries of added BAL from plasma, blood and urine. Although it has not been found possible to obtain a quantitative recovery from any of these fluids, the losses appear to be due to destruction of BAL by chemical reaction rather than to any defect in the method of estimation.

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