sidered to have had typical scrub typhus although neither presented all the classical signs of the disease. Thus, Patient 46 failed to show a rash or eschar while Patient 47 failed to develop a positive Weil-Felix reaction. Both persons received the drug orally over a period of 16 hours. Patient 46 was given an initial dose of 3.0 g followed by 4 doses of 0.25 g at 3-hour intervals. The regime for Patient 47 was similar except that the initial amount was 4.0 g. The response of these 2 patients following therapy was entirely comparable to that observed in other cases of scrub typhus treated with fermentation chloromycetin. It will be recalled that the febrile period of scrub typhus, which usually lasts for 14 days in untreated cases, was terminated in 31 hours (average for 25 patients) after treatment with chloromycetin was instituted.[10]

Our experience, although limited, indicates that synthetic chloromycetin possesses the same low level of toxicity for embryonated eggs, mice and men that has characterized the fermentation type[3,5,10] of drug.

Conclusion. Chloromycetin prepared by chemical synthesis appears to possess the same rickettsiostatic and virustatic properties in experimental infections and the same usefulness in treating patients with scrub typhus that have been demonstrated for chloromycetin produced by *Streptomyces venezuelae,* N. Sp.

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Condensation of 2, 3-Dimercaptopropanol (BAL) with Oxophenarsine Hydrochloride: Toxicity and Chemotherapeutic Effect.

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(Introduced by E. K. Marshall, Jr.)

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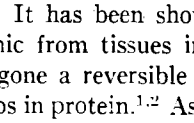
The use of 2,3-dimercaptopropanol (BAL) in the treatment of arsenical poisoning has been well established, and the pharmacological and biochemical basis for its action has been thoroughly explored. It has been shown that BAL mobilizes arsenic from tissues in which the metal has undergone a reversible binding with sulfhydryl groups in protein.[1,2] As a result BAL increases the excretion of administered arsenic from the body[3,4] and is a useful antidote in poisoning from arsenical gases or complications following administration of oxophenarsine hydrochloride.[2-7]

It has been shown that when arsenic reacts with keratin the ratio of combination with sulfur of the protein is 1 As : 2 S, and this suggested that a relatively stable ring of the type



is formed.[1] It is therefore postulated that BAL detoxifies by the removal of arsenic from the protein system and incorporation *in vivo* into a more stable cyclic

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1 Stocken, L. A., and Thompson, R. H. S., *Biochem. J.*, 1946, **40**, 529.

2 *ibid.*, p. 535.

3 *ibid.*, p. 548.

4 Eagle, H., Magnuson, H. J., and Fleischman, R., *J. Clin. Invest.*, 1946, **25**, 451.

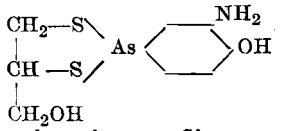
5 Longcope, W. T., Luetscher, J. A., Jr., Wintrobe, M. M., and Jagen, V., *J. Clin. Invest.*, 1946, **25**, 528.

6 Carleton, A. B., Peters, R. A., Stocken, L. A., Thompson, R. H. S., and Williams, D. I., *J. Clin. Invest.*, 1946, **25**, 497.

7 Durlacker, S. H., Bunting, H., Harrison, H. E., Ordway, N. K., and Albrink, W. S., *J. Pharmacol. and Exp. Therap.*, 1946, **87**, Supplement, 28.

thioarsenite with BAL itself.[2,8]

Three papers[9-11] have appeared dealing with some of the pharmacological properties of the thioarsenite which is formed *in vitro* by condensation of oxophenarsine hydrochloride and BAL. For convenience, the compound will be called BAL-OXO. Its structure is as follows:



However, there is a conflict concerning the toxicity of this compound relative to the parent oxophenarsine hydrochloride. Peters and Stocken,[9] using a propylene glycol solution of the hydrochloride of BAL-OXO, found that in rats it was approximately four times as toxic as the parent arsenoxide. Excess BAL reduced this toxicity markedly. On the other hand, Riker[11] found in cats that BAL-OXO was at least 4 times less toxic than the parent compound. Friedheim and Vogel[10] gave toxicity figures of BAL-OXO for the mouse and the rabbit which are considerably lower than those published elsewhere for oxophenarsine hydrochloride.[12] No direct comparison was made however. These workers also found that BAL-OXO was therapeutically active in mouse trypanosomiasis and rabbit syphilis. Although no quantitative evaluation of the drug was made, they suggested that it might be a useful chemotherapeutic agent.

The present experiments were designed to explore the relative toxicity, and therapeutic value in *T. equiperdum* in mice, of BAL-OXO and the parent oxophenarsine hydrochloride.

Materials and methods. A single strain of mice weighing 20-25 g was used throughout. Rats used for toxicity weighed 120-160 g. Animals were from Carworth Farms, Inc.

Drugs were administered in a single dose intraperitoneally. In the therapeutic experiments they were given 24 hours after a suspension of approximately 100,000 organisms of *T. equiperdum* was injected into the peritoneal cavity of the mice.

The oxophenarsine hydrochloride was a commercial preparation containing 31.8% arsenic.†

BAL-OXO was prepared as follows: To 1.88 g (.008 mols) of oxophenarsine hydrochloride in 100 ml N HCl was added 1 g (.008 mols) of 2,3-dimercaptopropanol in 20 ml methanol. Sodium hydroxide (3N) was added until neutrality was reached. A copious light pink precipitate was formed and filtered under suction. The precipitate was washed with water, methanol and ether, and dried in air. The compound was a fine light pink powder, with a melting point of 122-124°C. Analysis showed an arsenic content of 24.5% and 24.6%. The theoretical value is 24.6%. This compound was used within 3 months after it was made. There was no darkening or observable change in solubility characteristics. For solution and use in animals the following procedure was followed: 100 mg drug was dissolved in 12 ml water and 3 ml N HCl, with gentle heating. Normal NaOH was then added until the solution was only slightly acid (approx. 2.5 ml used), but no precipitate had formed. It was made up to appropriate volume and used in this form, which is the hydrochloride of BAL-OXO.

Doses of both compounds are expressed in terms of mg of arsenic per kg body weight.

In toxicity experiments all animals were observed for 14 days. Almost all deaths occurred in the first 48 hours, after oxophenarsine hydrochloride. Deaths from BAL-OXO were often delayed for 96 hours. Mice in therapeutic tests were observed for 30 days. Most deaths were in the first two weeks and none occurred beyond the twentieth day. Ten untreated infected controls died within 5 days.

Results were plotted as per cent survival against the logarithm of the dose for both toxicity and therapeutic experiments. In Fig.

8 Whittaker, V. P., *Biochem. J.*, 1947, **41**, 56.

9 Peters, R., and Stocken, L. A., *Biochem. J.*, 1947, **41**, 53.

10 Friedheim, E. A. H., and Vogel, H. J., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 418.

11 Riker, W. F., *J. Pharmacol. and Exp. Therap.*, 1946, **87**, Supplement, 66.

12 Eagle, H., Hogan, R., Doak, G. O., and Steinman, H. G., *Public Health Rep.*, 1944, **59**, 765.

† We wish to thank Dr. A. C. Bratton of Parke, Davis Company for supplying this compound in a chemically pure state.

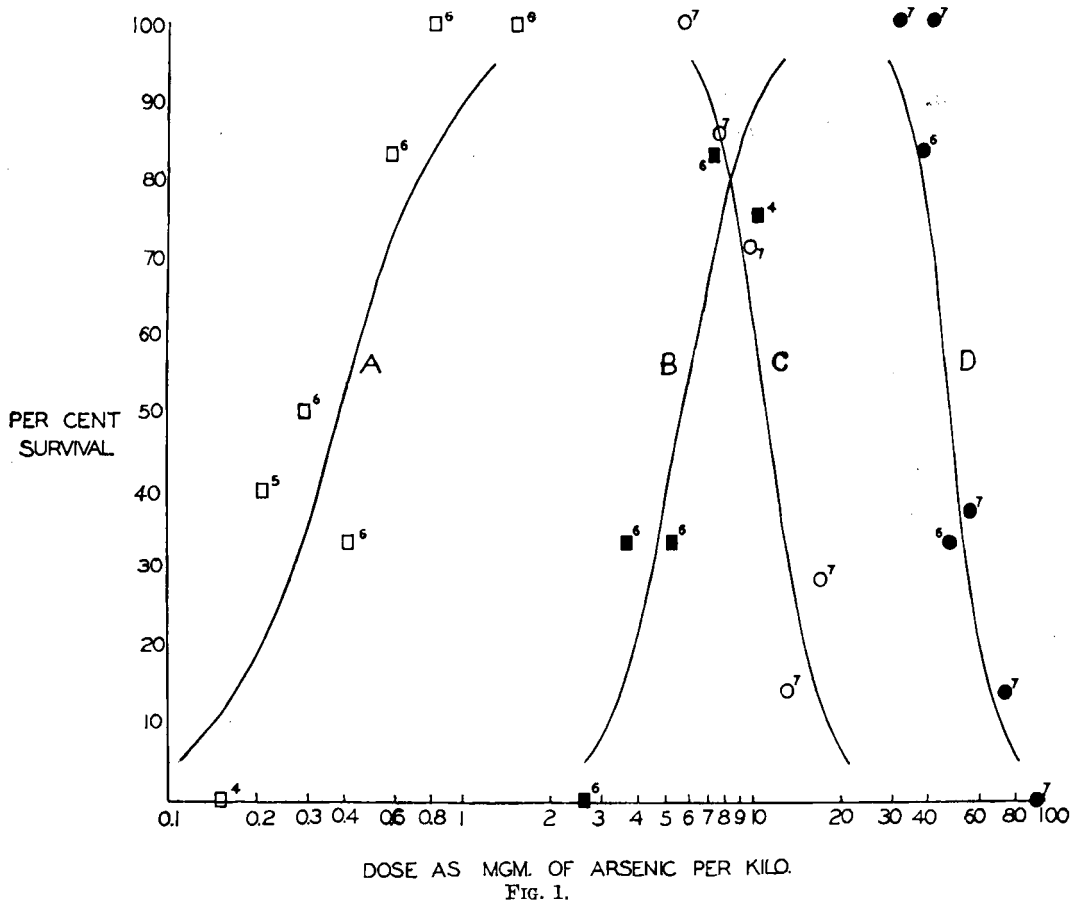


FIG. 1.

The mouse toxicity, and therapeutic effect of oxophenarsine HCl and BAL-oxo in *T. equiperdum* in mice.

Key: □ Oxophenarsine HCl therapy, curve A.
■ BAL-oxo therapy, curve B.
○ Oxophenarsine HCl toxicity, curve C.
● BAL-oxo toxicity, curve D.

The small numbers on the figure represent the number of animals used at each dose.

1, integrated normal frequency curves have been fitted to the observed data. "Chemotherapeutic index" is defined as the dose lethal to 50% of animals/dose curing 50% = LD_{50}/CD_{50}.

Results. Fig. 1 shows the data obtained for 4 experiments on mice. Smooth curves to approximate these points were constructed as described above. The acute toxicity (curves C and D) experiments were run simultaneously for the two drugs. Approximately one month later the treatment of *T. equiperdum* was run simultaneously for both drugs (curves A and B). The approximate 50% survival point may be read from Fig. 1, and will give the LD_{50} (curves C and D) and the CD_{50} (curves A and B) for both compounds. Because of the comparatively small number of animals used no attempt was made to attach a standard error to these measurements. For oxophenarsine hydrochloride the ratio LD_{50}/CD_{50} is approximately 30. This is in agreement with Eagle, Hogan, Doak and Steinman whose figure is 26.6.[12] The corresponding ratio for BAL-OXO is approximately 10.

It is evident therefore that the condensation of BAL with oxophenarsine hydrochloride yields a compound of lower "chemotherapeutic index" than the parent arsenoxide

The toxicity curves (C and D) show that

conversion of the arsenoxide to the thioarsenite has the effect of *decreasing* the acute toxicity in mice by a factor of 4. This agrees with Riker's data on cats.[11] Peters and Stocken,[9] who reported that this conversion *increased* the toxicity by approximately 4, worked entirely with rats. It was essential, therefore, to know if the disagreement was due to a species difference, and we set up several acute toxicity experiments on rats. For oxophenarsine hydrochloride, we were in fair agreement with Peters and Stocken who reported the LD_{50} as 5 mg As/kg. Our animals survived 6.5 mg As/kg and died at 10 mg As/kg. For BAL-OXO, however, there was an enormous disparity; our rats survived 32 mg As/kg and died at 64 mg As/kg, whereas Peters and Stocken reported that the LD_{50} was between 1-2 mg As/kg. It will be observed that our figures for rats agree well with our mouse data (curves C and D). Species difference alone, therefore, would clearly not account for the results of Peters and Stocken, which disagree with those of other workers, including ourselves.

Discussion. These findings suggest that in the combination of oxophenarsine hydrochloride with BAL the net toxicity for the host and for the parasite are not affected to the same degree. This agrees with Ercoli and Wilson[12] who found that relatively more BAL was needed to interfere with the toxicity of oxophenarsine hydrochloride than was needed to interfere with its trypanocidal and therapeutic effect. Both our findings and theirs, therefore, show that BAL lowers the therapeutic index of the arsenical.

The precise reason for this effect is not completely evident, but at least two hypotheses would explain the facts. First, the dithioarsenite may act as a whole and have a different relative toxicity for host and parasite than does oxophenarsine hydrochloride. On the other hand, BAL may simply alter the distribution of active arsenical between trypanosome and host. We believe that the latter view is more in keeping with all that is known of the action of arsenicals. If the dithioarsenite acts as a whole, it must act by a mechanism not explainable by the -SH-arsenoreceptor theory which is now generally accepted. For this reason, we believe that the oxophenarsine-BAL compound acts through dissociation into a compound whose arsenic is available to sulfhydryl groups in protein.

In any case, it has been confirmed that BAL-OXO does possess significant therapeutic activity, although its therapeutic index is only about 1/3 that of oxophenarsine hydrochloride. It is entirely possible that other arsenic-sulfhydryl compounds might show a greater therapeutic efficacy than the one we have studied, but from this single example it appears that combination of arsenical with a detoxifying dithiol reduces its therapeutic effect to a greater degree than its toxicity.

Regarding toxicity alone, the major discrepancy between our results and those of Riker[11] and Friedheim[10] on the one hand, and those of Peters and Stocken[9] on the other, are quite inexplicable. It is possible that differences in the preparation or stability of the drug are responsible. Secondly, they gave their compound in propylene glycol. A circumstance which suggests that Peters and Stocken were not dealing solely with the effect of the BAL-OXO is that on this drug their animals died faster than with oxophenarsine hydrochloride alone, and usually succumbed within 2 hours. In our experiments quite the reverse was noticed: even on relatively high doses, death following the thioarsenite was delayed over that of the arsenoxide.

Summary. 1. The condensation product of BAL and oxophenarsine hydrochloride (BAL-OXO) was studied. Its toxicity in mice and chemotherapeutic activity in *T. equiperdum* is reported, in comparison with similar data, for oxophenarsine hydrochloride.

2. The acute toxicity of the thioarsenite, BAL-OXO is approximately ¼ that of the arsenoxide (oxophenarsine hydrochloride) from which it was prepared.

3. The BAL-OXO has less therapeutic advantage, *i.e.*, lower LD_{50}/CD_{50}, in mouse trypanosomiasis than oxophenarsine hydrochloride itself.