17106. Effect of BAL on Experimental Polyarthritis of Rats.*

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BAL (2:3-dimercaptopropanol) is used in counteracting gold toxicity which appears occasionally during chrysotherapy of rheumatoid arthritis in man. Experimental polyarthritis of rats produced by the L-4 strain of a pleuropneumonia-like organism likewise responds to gold therapy. Although the experimental disease in rats is pathologically not the same as rheumatoid arthritis in man it has been used as a means of making chemotherapeutic trials.[1]

The use of BAL in arthritic patients who are being treated at the same time with gold raises the possibility that the arthritis may be aggravated because the BAL would increase the excretion of gold and so decrease the amount of gold available for the disease process.[2,3] Since gold has a beneficial action in arthritis the use of a substance capable of facile combining with it might *per se* aggravate the arthritis. To examine this possibility the effect of the administration of BAL on the experimental arthritis of rats, not treated with gold, was studied.

Procedure. A total of 41 male and 49 female rats were given a total cumulative dose of 90 to 100 mg per kg body weight of BAL intramuscularly during a period of 6 weeks. For the first 5 days of the experiment 5 mg per kg of BAL (diluted in olive oil) was administered twice daily. Thereafter, the same dose was given twice weekly for 5 weeks.

On the second day of BAL injection the rats received 2.0 cc of a broth culture of the L-4 strain of pleuropneumonia-like organism intraperitoneally. The organisms were cultured according to a method described by Tripi and Kuzell.[1] Evaluation of the arthritic involvement was made by using a modification of the arthrogram described by Sabin and Warren.[4] This consists of a scoring square divided into 4 quarters. Each quarter represents one leg of the rat. Arthritis is noted by blacking out various portions of the square. A means of scoring these arthrograms according to the degree and extent of rat polyarthritis has been described elsewhere.[5] The arthrogram score is based on a possible score of 4 for a front leg and 5 for a hind leg, giving a maximum score of 18 for the animal.

A total of 37 male and 43 female rats was used as controls and received only the pleuropneumonia-like organism intraperitoneally. Ten rats injected with olive oil alone in comparable dosage showed no difference from these controls. There was no demonstrable change in growth of pleuropneumonia-like organisms in broth cultures *in vitro* to which pure BAL† had been added in concentrations of from 0.0007 to 0.2 mg per cc. Infected broth cultures containing BAL in a concentration of 0.2 mg per cc were injected intraperitoneally into rats and the typical arthritis was produced. Increasing the concentration of BAL in the broth cultures beyond 0.2 mg per cc inhibited the growth of the microorganism. Five rats (average weight 60 g) were given 5 mg per kg of BAL intramuscularly twice daily for 5 days and thereafter twice weekly for 5 weeks. These rats were compared with similar untreated controls regarding weight gain. There was no demonstrable dif-

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1 Tripi, H. B., and Kuzell, W. C., *Stanford Med. Bull.*, 1947, **5**, 98.

2 Macleod, J. G., *Ann. Rheum. Dis.*, 1947, **7**, 143.

3 Kuzell, W. C., Pillsbury, P. L., and Gellert, S. A., *Stanford Med. Bull.*, 1947, **5**, 197.

4 Sabin, A. B., and Warren, J., *J. Bact.*, 1940, **40**, 823.

5 Tripi, H. B., Gardner, G. M., and Kuzell, W. C., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 45.

† Supplied by Hynson, Westcott and Dunning, Inc., Baltimore, Md.

TABLE I.
Effect of BAL on Incidence and Extent of Arthritis and on Survival Rate of Infected Animals.

	Arthritis		
	Incidence, %	Extent (Arthrogram Avg)	Survival, %
BAL + pleuropneumonia infection	73	2.86	89
Pleuropneumonia infection only	55	1.83	95

The standard error of difference in incidence of arthritis is 7.3% while the observed difference is 18% so that the chance occurrence of the observed difference is 1:80.

ference in weight between these groups, thus indicating that the BAL had little general toxic effect on the rats.

Results. The arthrogram scores given in Table I compare the average arthritic involvement in the group receiving both BAL and the infection with that receiving the infection only. The extent of the average arthritic involvement in the individual animals receiving BAL is greater than in the infected controls. The survival rate is slightly less in the group receiving BAL. The incidence of arthritis is greater in the BAL medicated group.

Discussion. These results indicate that the administration of BAL increases the severity of an experimental arthritic process. Such a difference might be attributable to the general toxic effects of BAL rather than to direct enhancement of the growth of the infecting organism, but our failure to show a difference in weight between animals given BAL alone and untreated controls argues against this possibility. Peters *et al.* found that LD_{50} from the subcutaneous administration of BAL in oil to rats was 110 mg per kg body weight.[6] Waters and Stock[7] reported the American Reference Standard LD_{50} to be 105 mg per kg intramuscularly in rats. It would, therefore, appear that single doses of 5 mg per kg would not be expected to produce appreciable toxicity. The question then arises as to whether the slight toxic effects produced by low doses could increase the severity of the arthritis. This does not seem likely, since it has been found that other toxic substances such as 0.1% sodium phenobarbital, 0.01% arsenic trioxide, or 0.1% isopropyl alcohol in the drinking water for prolonged periods caused no increase in the severity of this experimental polyarthritis of rats.[8]

It is possible that BAL in low concentration might stimulate growth of the pleuropneumonia-like microorganisms and thus increase the severity of the arthritis. Fildes and Richardson[9,10] have shown that *Staphylococcus aureus* is able to utilize some mercapto compounds as a source of sulphur and, in fact, that sulfhydryl compounds are essential metabolites for many cells. Thus, if lack of sulfhydryl groups prevents the metabolism of bacterial cells it is possible that an abundance of these *SH* groups as furnished by BAL might actually stimulate the microorganisms *in vivo*.

Conclusions. BAL(2:3-dimercaptopropanol), administered to rats with polyarthritis produced by infection with L-4 strain of pleuropneumonia-like microorganism, causes a significant increase in incidence of arthritis, a more extensive type of joint involvement, and a slightly decreased rate of survival. Concentrations of BAL up to 0.2 mg per cc of broth failed to inhibit or to increase the growth of pleuropneumonia-like organisms *in vitro*. It is suggested that BAL in the low concentrations used *in vivo* may act by furnishing sulfhydryl groups needed in the metabolism of the microorganisms, and thus aggravate the polyarthritis, rather than by poisoning the animals and thus reducing their resistance to the infection.

6 Peters, R. A., Stocken, L. A., and Thompson, R. H. S., *Nature*, 1945, **156**, 616.

7 Waters, L. L., and Stock, C., *Science*, 1945, **102**, 601.

8 Tripi, H. B., Kuzell, W. C., and Gardner, G. M., *Ann. Rheum. Dis.*, in press, June 1949.

9 Fildes, P., and Richardson, G. M., *Brit. J. Exp. Path.*, 1937, **18**, 292.

10 Fildes, P., *Brit. J. Exp. Path.*, 1940, **21**, 67.

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