15683

Effect of 2,3-Dimercaptopropanol (BAL) on Acute Poisoning by Tervalent and Quinquevalent Antimonial Drugs.*

JAMES F. GAMMILL, CHESTER M. SOUTHAM, AND H. B. VAN DYKE.
From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York.

Among the numerous reports on the experimental use of BAL to combat poisoning by inorganic and organic metallic compounds, the investigation of Braun, Lusky and Calvery[1] is the only one describing work with antimonial drugs. These authors concluded from their work with rabbits that "With the administration of BAL, the tolerance of the rabbits to lethal doses of compounds of antimony was increased more than 50%." Our experiments were all performed with young rats in which we were able to demonstrate a protective action of BAL against only one antimonial, tartar emetic. The lethal action of 4 other antimonial drugs, including Fuadin and Neostam which were also used by Braun, Lusky and Calvery, either was not affected or was increased by the administration of BAL.

Methods. All the experiments were performed with young albino rats (Sherman strain) usually weighing about 75 g (range 55-140 g). A total of 210 female and 885 male rats was used. Each group, usually of 10 animals, if treated with BAL, had a companion group of 10 of the same weight range and sex receiving only the antimonial drug. The dose of antimonial chosen was sufficient to kill 60-100% of animals in the preliminary experiments with single doses. If the BAL had no clearly favorable influence on the lethal action of the drug, the dose of the latter was reduced to determine whether there was an appreciable beneficial or deleterious action of BAL. In some experiments, smaller doses of an antimonial drug and of BAL were administered repeatedly.

In every experiment the antimonial and the BAL solutions were administered by different routes. When an aqueous solution of the antimonial drug was injected intraperitoneally, BAL in oil, according to the formula of Eagle,[2] was given subcutaneously after dilution of the solution by peanut oil. Solutions of BAL in oil were unsatisfactory for repeated injection over a period of days owing to the accumulation of unabsorbed oil. In later experiments aqueous solutions of drug and of BAL were injected by the same routes as those used by Stocken and Thompson[3] in experiments with sodium arsenite; the antimonial drug was injected intramuscularly and freshly-prepared aqueous BAL solution was given intraperitoneally. The antimonial drugs tested were tartar emetic (antimony potassium tartrate). Fuadin† (NaSb-bis-pyrocatechindisulfonate of Na), Neostibosan† (Sb complex of *p*-aminophenylstibonic acid, *p*-acetylaminophenylstibonic acid, antimonic acid and diethylamine), Neostam‡ (nitrogen-glucoside of sodium *p*-aminophenylstibonate), and Stibanose† (Solustibosan or monohydroxytriethylammonium salt of 2,3,antimonic ester of sodium hexonate). The antimony of tartar emetic and of Fuadin is tervalent, whereas it is quinquevalent in the other 3 compounds. Stibanose is of such low toxicity that the large volume of concentrated solution required for a lethal effect could not be injected intramuscularly. The aqueous solutions of Neostam and Neostibosan for intramuscular administration contained respectively 20 g and 30 g of drug per 100 ml of solution.

* This investigation was aided by a grant from the Sterling-Winthrop Research Institute.

1 Braun, H. A., Lusky, L. M., and Calvery, H. O., *J. Pharm. and Exp. Therap., Suppl.*, 1946, **87**, 119.

2 Eagle, H., *J. Ven. Dis. Inf.*, 1946, **27**, 114.

3 Stocken, L. A., and Thompson, R. H. S., *Biochem. J.*, 1946, **40**, 535.

† Kindly supplied by the Sterling-Winthrop Research Institute.

‡ Kindly supplied by the Burroughs-Wellcome Company.

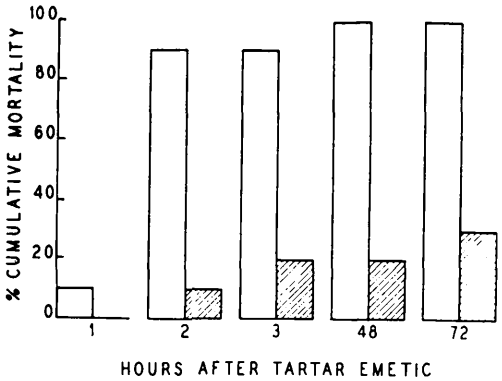

FIG. 1.

Effect of BAL-treatment in the rat on the lethal action of one intramuscular injection of 60 mg of tartar emetic per kg. A saturated aqueous solution of BAL (about 6%) was injected intraperitoneally 5 minutes after tartar emetic in a dose of 0.1 ml per 100 g (about 60 mg per kg). Half of this dose of BAL was repeated 3, 6, and 24 hours after the tartar emetic. One drug-control rat died between the 3rd and the 11th hours of the experiment; no BAL-treated rats died after 72 hours. Each group consisted of 10 rats.

White columns: drug alone.

Shaded columns: drug followed by BAL-treatment.

Results. Ten experiments were performed with tartar emetic administered either intraperitoneally or intramuscularly. In every experiment except one, treatment with BAL either increased the survival period or prevented death, in comparison with groups of rats simultaneously receiving only the solution of tartar emetic. Illustrative experiments are shown in Fig. 1 and 2. A control experiment with very large doses of $HgCl_2$ as the lethal agent is shown in Fig. 3; an expected protective action of BAL was demonstrated although more survivals after the last dose probably would have been observed had BAL treatment been continued after the last injection of $HgCl_2$ solution. In the one inconsistent experiment, tartar emetic in increasing doses was injected once daily intraperitoneally into each rat of 3 groups of 10 each. The initial dose was 30 mg per kg and the last dose, on the 11th day, was 60 mg per kg. However, only 20% of the control rats died whereas of those receiving BAL in oil in addition to tartar emetic, 80% of the second group (20 mg of BAL per kg immediately after tartar emetic) and 30% of the third group (20 mg of BAL per kg immediately after tartar emetic and repeated 3 hours later) did not survive. The subcutaneous tissues of the animals treated with BAL contained several milliliters of oil at the end of the experiment. No satisfactory explanation of the deleterious effect of BAL-treatment or of the remarkable tolerance of the control rats to intraperitoneally-administered tartar emetic in this experiment has been found.

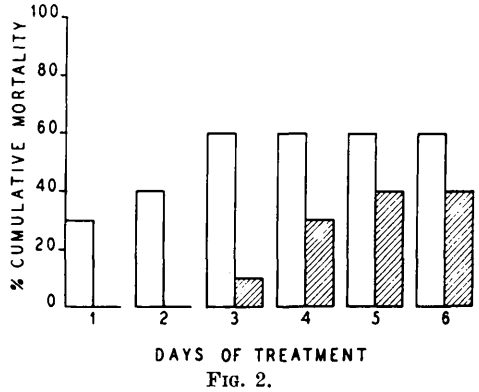

FIG. 2.

Effect of BAL in oil subcutaneously on survival after intraperitoneal injection once daily of 25 mg of tartar emetic per kg in aqueous solution. One dose of BAL (20 mg per kg) was given immediately after each of the 6 injections of tartar emetic. There were no deaths after the 6th injection. Each group consisted of 10 rats.

White Columns: drug alone.

Shaded columns: drug followed by BAL-treatment.

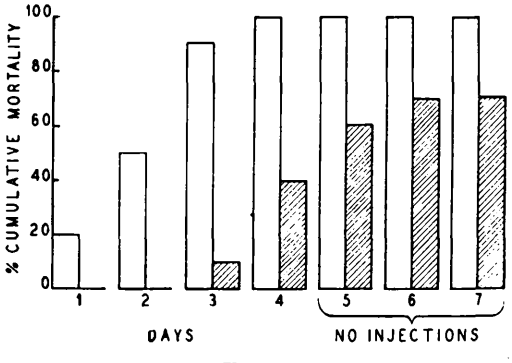

FIG. 3.

Effect of BAL in oil subcutaneously on survival after intraperitoneal injection of 5 mg of $HgCl_2$ per kg once daily at beginning of each of first 4 days. One dose of BAL (20 mg per kg) was given immediately after each injection of $HgCl_2$ solution. The 3 surviving rats were sacrificed. Each group consisted of 10 rats.

White columns: $HgCl_2$ alone.

Shaded columns: $HgCl_2$ and BAL.

Six experiments were performed with the other tervalent antimonial, Fuadin. In one experiment with BAL in oil the mortality in the drug control group was identical with that in the BAL-treated group. In all the other experiments, whether aqueous BAL or BAL in oil was administered, BAL shortened greatly the period of survival and the rate of recovery from toxic or LD_{40-60} doses of Fuadin. This is clearly shown in the experiment of Fig. 4A. A control experiment demonstrating the efficacy of aqueous BAL in arsenite poisoning is illustrated in Fig. 4B, confirming the report of Stocken and Thompson.[3]

Two experiments with aqueous solutions of Neostam and BAL demonstrated that treatment with BAL causes an earlier death (at a dosage of 600 mg of Neostam per kg) or increases the mortality rate (at a dosage of 450 mg of Neostam per kg). The latter experiment is summarized graphically in Fig. 5. Neostibosan was the quinquevalent

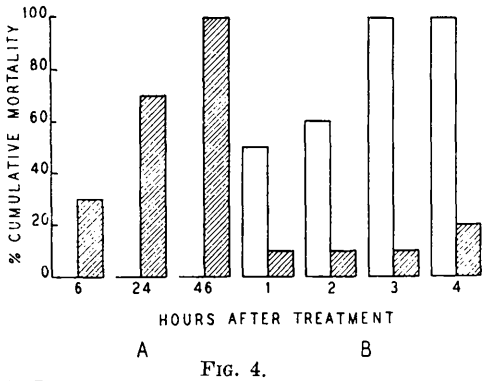

FIG. 4.

A. Lethal effect of treatment with BAL in rats receiving a single intramuscular dose of 250 mg of Fuadin per kg. Without BAL-treatment no rats died within 96 hours. In the group of rats receiving 0.1 ml of saturated aqueous solution of BAL per 100 g (about 60 mg per kg), 70% died within 24 hours and all were dead within 46 hours. Each group consisted of 10 rats.

B. Protective action of BAL in arsenite poisoning. Each rat received, per kilogram, an intramuscular injection of 18 mg of As_2O_3 neutralized by NaOH. The intraperitoneal injection of saturated aqueous solution of BAL was given 10 minutes after the arsenite (0.1 ml per 100 g); a second dose of BAL (0.05 ml per 100 g) was injected 4 hours later. There were no deaths after 4 hours. Each group consisted of 10 rats.

White columns: arsenite alone.

Shaded columns: Fuadin and BAL (A) or arsenite and BAL (B).

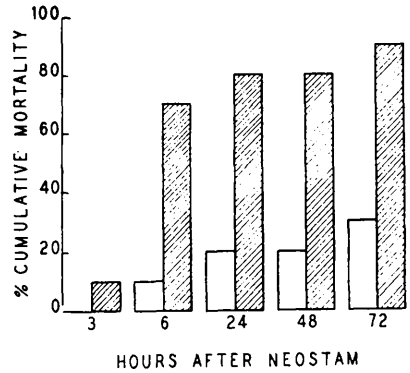

FIG. 5.

Lethal action of BAL in rats receiving a single intramuscular injection of 450 mg of Neostam per kg. BAL, as a single intraperitoneal injection of 0.1 ml of a saturated aqueous solution per 100 g (about 60 mg per kg), was given 5 minutes after the injection of Neostam. No further deaths occurred between the 72nd and the 240th hours. Each group consisted of 10 rats.

White columns: Neostam alone.

Shaded columns: Neostam and BAL.

antimonial used in 6 satisfactory experiments in which single or repeated daily injections were made. Subcutaneous BAL in oil or intraperitoneal aqueous BAL increased the mortality rate. For example, after 1500 mg of Neostibosan per kg intramuscularly, 50% of the BAL-treated animals and none of the drug controls were dead after 3 hours; at the end of the experiment, 40% of the drug control group were dead and 70% of the group receiving intraperitoneal BAL as well as Neostibosan had succumbed. The low toxicity of Stibanose required intraperitoneal injections (*e.g.*, a single dose of 7000 mg per kg caused only 20% mortality) and the BAL was administered in oil subcutaneously. The effect of BAL (20 mg per kg as a single dose or 2 doses 4 hours apart) apparently was to increase the mortality rate, but this effect was not shown to be significant.

Discussion. The findings here reported, in which rats were the experimental subjects, demonstrate that BAL is of real value in lessening the mortality rate of animals receiving single or repeated doses of tartar emetic. This conclusion is in agreement with that reached by Braun, Lusky and Calvery, whose experiments were all performed with rabbits receiving intramuscular injections of

aqueous solutions of drug or of drug and of BAL. The lethal effects of the other 2 antimonials used by these authors, Fuadin and Neostam, contrary to their results, were found clearly to be enhanced by the administration of BAL. Similarly, BAL increased the lethal action of poisonous doses of Neostibosan. The dimercaptan did not alter the death rate from large intraperitoneal doses of Stibanose. The doses of BAL, whether dissolved in peanut oil or in water, were sufficient to save the lives of animals receiving lethal doses of either mercuric chloride or sodium arsenite. BAL in oil was administered in doses well below levels which by themselves are followed by any manifestation of poisoning. The largest intraperitoneal dose of aqueous solution of BAL used (about 60 mg per kg) can cause central stimulation in some rats, as shown by generalized muscular hyperactivity; however, this dose, followed by 30 mg per kg 4 hours later, can be given daily for at least 3 days without killing any animals.

Other experiments will have to be performed to determine, if possible, what is the explanation of the increased toxicity of Fuadin, Neostam and Neostibosan, if BAL be administered a short time later. The increased mortality associated with BAL-treatment occurred mainly within 24 hours; a significant difference in mortality was evident after 6 hours. It is possible that an acutely toxic complex substance is formed *in vivo* by the interaction of the dimercaptan and any of the 3 antimonials which themselves are not well-defined compounds; however, it is more acceptable to interpret the increased mortality as a summation of toxic effects. It seems reasonable to conclude that the favorable action of BAL in rats acutely poisoned by tartar emetic is the result of the formation of one or more mercaptides of antimony much less toxic than tartar emetic, just as is the case when an arsenical or a mercuric salt is rendered innocuous by the administration of BAL.

Summary. In rats, the acute lethal action of tartar emetic is significantly reduced by the administration of BAL. BAL increases the mortality rate in rats receiving Fuadin, Neostam or Neostibosan. The mortality rate in rats given large doses of Stibanose is not reduced by BAL.

15684 P

Electron Micrographs of Bacterial Cultures Infected with Bacteriophage.

O. F. EDWARDS AND RALPH W. G. WYCKOFF.

From the National Institute of Health, Bethesda, Md.

Now that we have learned to recognize individual virus particles through the electron microscopy of purified suspensions it has become practical to approach the more important question of how these particles are produced within the cells they infect. Probably bacteria with their bacteriophage provide the simplest virus system immediately accessible to existing technics. This is partly because sperm-like bacteriophage particles[1,2] can easily be recognized even in the presence of other particles of similar submicroscopic size and partly because bacteria are hosts small enough to allow an electron microscopic examination of both their surface and their internal structures.

To gain a satisfactory understanding of the steps involved in bacteriophage-production it is essential to be able to photograph sensitive bacteria under the conditions in which they grow and become infected. We have found that this can be done by studying replicas made of the surfaces of agar

[1] Luria, S. E., Delbrück, M., and Anderson, T. F., *J. Bact.*, 1943, **46**, 57.

[2] Sharp, D. G., Taylor, A. R., Hook, A. E., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 259.