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ACTIONS OF BRITISH ANTI-LEWISITE (2 : 3-DIMERCAPTOPROPANOL)

BY

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Peters, Stocken, and Thompson (1945) and Waters and Stock (1945) described the effectiveness of 2:3-dimercaptopropanol (named British Anti-Lewisite or BAL by American workers) in countering the effects of arsenical poisoning. Apart from the importance of having an efficient antidote to arsenical agents in chemical warfare, there arose the possibility of using BAL for the treatment of the toxic manifestations of arsenical drugs in therapeutics. After investigations of the protective action of BAL against arsenical poisons carried out in this country and at the same time those on lewisite by Harrison, Durlacher, Albrink, Ordway, and Bunting (1946) the substance was used successfully by Carleton, Peters, Stocken, Thompson, and Williams (1946) and Eagle (1946) as an antidote in clinical cases of poisoning by such therapeutic agents as mapharside.

The protective action of BAL stimulated research into the mode of action of dithiols in detoxicating arsenic. Peters, Sinclair, and Thompson (1946) and Stocken and Thompson (1946) have shown that arsenic combines with the available sulphydryl groups in proteins and in this way interferes with the enzymes vital to cellular metabolism which depend for their activity on the presence of SH-groups; one such enzyme is pyruvate oxidase. A molecule of BAL contains two SH-groups and can thus re-activate enzymes which have been inhibited by arsenic (Stocken, Thompson, and Whittaker, 1947).

The nature of the reaction between arsenic and BAL made it seem probable that BAL would react similarly with other metals, and Gilman, Philips, Allen, and Koelle (1946) and Ginzler, Gilman, Philips, Allen, and Koelle (1946) showed that this was so with cadmium. Braun, Lusky, and Calvery (1946) showed the same with antimony, bismuth, chromium, mercury, and nickel. Lead and selenium were made more toxic by BAL, while thallium was not affected.

The toxic properties of BAL were investigated by Durlacher, Bunting, Harrison, Ordway, and Albrink (1946) and Modell, Chenoweth, and Krop (1946) who stated that the LD50 in rabbits was 99 mg./kg. and in cats 0.032 ml./kg. The chief symptoms of toxicity found were conjunctivitis, gastro-enteritis, tremors, and ataxia. Fatal doses caused convulsions and circulatory and respiratory failure. According to Modell, Gold, and Catell (1946) doses in excess of 3-5 mg./kg. in man cause blepharospasm, flushes, and unpleasant sensations.

It was decided to investigate the action of BAL on the toxicity of several metallic compounds deemed to be of clinical importance, viz.—mapharside, mercuric chloride, mersalyl, potassium antimony tartrate, lead acetate, sodium bismuth tartrate, sodium auro-thiomalate and chromium trioxide. In addition the effect of BAL on the physiological action of insulin was examined. As a preliminary step the toxicity of BAL itself was investigated.

EXPERIMENTAL

Methods.—The effects of BAL were investigated by injection of freshly prepared watery solutions of the compound by various routes into mice, rats, guinea-pigs, and rabbits. Rabbits anaesthetized with 25 per cent (w/v) urethane solution and cats anaesthetized with ether and chloralose (80 mg./kg. i.v.) were used to determine the actions of BAL on the blood pressure, heart (myograph), spleen volume (plethysmograph and piston recorder), leg volume (plethysmograph and tambour), and respiration (stethographic lever). Isolated rabbit auricles, isolated perfused cat hearts, and isolated strips of gut and uterus from rabbit, cat, and guinea-pig were examined in the usual way. The vessels of the rabbit ear were perfused with saline according to the method of Gaddum and Kwiatkowski (1938), but as no recording apparatus was available the outflow was measured in drops per 15 sec. using larger doses of drugs than would otherwise be necessary. The method of investigating the effect of BAL on the diuretic activity of mersalyl was that

described by Burn (1937) for the assay of the anti-diuretic potency of extracts of the posterior pituitary body.

The LD50 of the metallic compounds was roughly determined by intra-peritoneal injection in small groups of mice; 200 male mice of 20–30 g. weight were then taken in four groups of 50 and given graded doses of the metallic compound so as to cover an adequate range of toxicity. To half of each group of 50 BAL was then given in a standard dose of 40 mg./kg. i.p. and the mortalities noted after 24 nours. Where the detoxifying action of BAL was such as to cause all the animals so protected to survive, the experiment was repeated with higher doses of metallic compound in the animals receiving protection. All injections with one metal were done at a single session. The LD50 was read from the plot of log dose and probit of lethality.

Results

Toxicity of BAL

The LD50 of BAL dissolved in water was found to be 100 mg./kg. for white mice injected intraperitoneally, which agrees well with the 0.8 mM./kg. of Durlacher *et al.* (1946). Three guinea-pigs given 150 mg./kg. died, and three given 50 mg./kg. survived; 12 rats survived 40 mg./kg. i.p., and of six rabbits given 100 mg./kg. i.v. two died.

In mice a lethal dose given intraperitoneally caused immediate weakness of the legs; analgesia was marked at this early stage, severe nipping of the tail being ignored; respiration was slow and laboured, but no tremors were seen; clonic and tonic convulsions followed, interrupted by periods of coma; death was accompanied by signs of asphyxia. Sublethal doses produced marked ataxia and weakness but not convulsions. In rabbits and guinea-pigs small doses (20 mg./kg. i.p.) produced blepharospasm and sneezing in 15 min., followed by tremor, weakness of the legs, and ataxia in 30 min. to one hour. Salivation was notable, urine and faeces were passed frequently, and the respiration was deep and hurried. If the dose was lethal the tremor increased to generalized convulsions, respiration was markedly impaired, and death occurred in tonic convulsion. *Post mortem* the liver, spleen, kidneys, and gut were congested and the lungs covered with small haemorrhages. Section of the lungs revealed haemorrhagic exudates.

Circulatory and respiratory effects

In rabbits anaesthetized with 25 per cent (w/v) urethane, BAL in a dosage of 0.5–1.0 mg./kg. in saline had no appreciable effect on the blood pressure or respiration, whereas 4.0 mg./kg. caused a sharp rise in blood pressure of some 20–30 mm.Hg and a stimulation of respiration which was maintained for several minutes; this effect of small doses of BAL on the circulation and breathing in the rabbit is illustrated in Fig. 1. Larger doses (20–40 mg./kg.) caused a transient rise in blood pressure and stimulation of respiration which was followed by a steady decline in pressure and failure of breathing. If the lethal dose was approached this course might be interrupted by convulsions, though usually the blood pressure declined to zero and the animal died

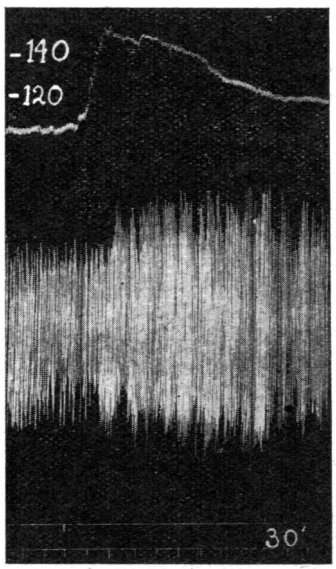

FIG. 1.—Rabbit ♂ 2.4 kg. Urethane 25 per cent i.v. and ether. Upper record carotid blood pressure, lower record respiration (stethograph, inspiration down). Time in 30 secs. BAL (4.0 mg./kg. i.v.) causes a rise in blood pressure and stimulation of respiration.

quietly after a few final gasps. This was in contrast to the mode of death of the unanaesthetized animal which convulsed violently, but the lethal dose was no smaller in the unanaesthetized animal, nor was the toxic effect modified by atropine sulphate, vagotomy, or artificial respiration. The isolated auricles of the rabbit continued to beat well in the presence of BAL in high concentration (1 in 10,000). The perfused vessels of the rabbit ear were constricted by BAL. Single injections of 3.0 mg. BAL in saline caused sharp but very transient vaso-constriction; constant perfusion with BAL (1 in 10,000) produced a reduction in flow.

In cats anaesthetized with ether, chloralose or nembutal, intravenous BAL invariably caused an abrupt fall in blood pressure accompanied by transient shrinkage of the spleen. If 20 mg./kg. was given the blood pressure fell abruptly and was quickly but usually only partially restored,

though sometimes it rose again above the initial level for a few minutes. The abrupt fall in pressure was accompanied by shrinkage of the spleen, or sometimes by dilatation ; the phase of recovery, partial or complete, was accompanied by active splenic dilatation. The leg was constricted and the heart unchanged in vigour and amplitude of beat so that splanchnic dilatation would appear to account for the early stages of the fall in pressure. The relative degree of alteration in flow in the splanchnic and limb circulations accounts for the varying responses of the blood pressure. Respiration was stimulated. Within a few minutes a progressive fall in blood pressure and inhibition of respiration ensued. Neither the abrupt nor the progressive fall in blood pressure was prevented by atropine sulphate (1.5 mg./kg.), by bilateral vagotomy or by artificial respiration, so that there is no question of vagal inhibition playing a part in the initial fall in pressure nor of failure of oxygenation of the blood playing a part in the later fall in pressure. After a few minutes, while the blood pressure was steadily falling, the spleen contracted maximally. The blood pressure at this stage could be temporarily restored by injection of adrenaline or infusion of saline. The restorative effect of saline soon passed off, blood pressure fell progressively, the spleen dilated again, respiration became slow and gasping, the leg volume remained reduced, and if the dose was large enough death occurred despite artificial respiration. These changes are illustrated in Fig. 2. When oxygenation was maintained by artificial respiration, the heart continued to act well and only failed 5–10 minutes after blood pressure had reached zero. The isolated perfused heart of the cat (Langendorff) showed no ill-effects from the injection of up to 5.0 mg. BAL and the rate of coronary flow was unchanged. The progressive fall in blood pressure with an active heart, in the presence of peripheral vasoconstriction, suggests a steady leakage of fluid from the circulation. The petechial haemorrhages seen on the lungs and liver indicate damage to capillaries and small vessels. The mean packed cell volume in five anaesthetized cats was 36.6 per cent (the blood having been spun for 25 min. at 5,000 r.p.m.); three hours after the administration of 100 mg. BAL/kg. and shortly before death took place it was 48.3 per cent, an increase of 32 per cent. This indicates a severe degree of haemoconcentration, despite the fact that an average of 25 ml. of saline and other solutions had been given intravenously in the course of the experiments, and it would appear to be the primary cause of death in anaesthetized animals, as Chenoweth (1946) indicated.

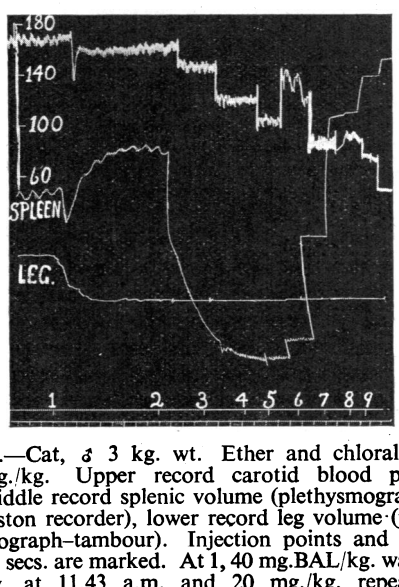

FIG. 2.—Cat, ♂ 3 kg. wt. Ether and chloralose, 80 mg./kg. Upper record carotid blood pressure, middle record splenic volume (plethysmograph and piston recorder), lower record leg volume (plethysmograph–tambour). Injection points and time in 30 secs. are marked. At 1, 40 mg.BAL/kg. was given i.v. at 11.43 a.m. and 20 mg./kg. repeated at 12.0 a.m., 1.56 p.m., and 2.10 p.m. (total 100 mg./kg.); 10.0 ml. saline at 12.21 temporarily restored the pressure. The time relationships are as follows: 1 = 11.43 a.m.; 2 = 11.50 a.m.; 3 = 11.54 a.m.; 4 = 12.06 p.m.; 5 = 12.24 p.m.; 6 = 1.45 p.m.; 7 = 1.53 p.m.; 8 = 2.10 p.m.; 9 = 2.20 p.m.

The odour of BAL could be detected in blood, urine, tears, and expired air, and in the freshly cut organs after death.

Action of BAL on the toxicity of metallic compounds. Arsenic

The arsenical preparation used was mapharside, freshly made up and injected in 0.25 ml. saline intraperitoneally into groups of white mice. The LD50 for mapharside alone was found to be 34.4 mg./kg. ; BAL had a strongly protective action, raising the LD50 to 350 mg./kg. or tenfold. This action is illustrated in Fig. 3 and is in agreement with the findings of Stocken, Thompson, and Whittaker (1947) with rats.

Twenty-four guinea-pigs of 350–400 g. weight were given a daily injection of mapharside (15 mg./kg.) subcutaneously ; twelve were given in addition a daily injection of BAL (40 mg./kg.) intraperitoneally. Eight of the guinea-pigs receiving arsenic alone had died with symptoms of restlessness, twitching, diarrhoea, wasting, and weakness between the fifth and tenth day of injection, when the remainder were killed. The guinea-pigs receiving BAL remained well and were sacrificed on the tenth day. Fresh specimens of liver,

kidney, spleen and gut were sectioned, stained, and examined. The chief lesions caused by arsenic poisoning were necrosis of the liver lobules with much debris and exudate, severe disintegration of the glomeruli and tubules of the kidney, catarrhal changes in the mucous membrane of the gut and congestion of the spleen with necrotic changes in the pulp and nodules. These changes were prevented by BAL therapy, the most striking differences being in the kidney. It is evident that the combination of arsenic and BAL causes little tissue damage during its excretion.

Antimony

The preparation of antimony used was tartar emetic, which proved to have an LD50 of 56.2 mg./kg.; 40 mg. BAL /kg. i.p. had a protective action on mice poisoned with this compound, raising the LD50 to 71.6 mg./kg. The results are illustrated in Fig. 3.

Chromium

The preparation used was chromium trioxide, which had an LD50 of 66.3 mg./kg. BAL (40 mg./kg. i.p.) had a protective action against chromium poisoning, raising the LD50 to 85.8 mg./kg.; this effect is illustrated in Fig. 3.

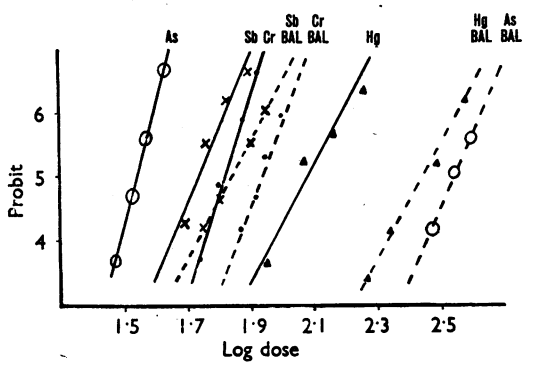

FIG. 3.—The protective action of BAL (40 mg./kg. i.p.) on the toxicity of arsenic (mapharside) antimony (tartar emetic), chromium (chromic acid) and mercury (corrosive sublimate) in groups of white mice. The ordinates are probits of lethality, the abscissae logs. of the dosage. BAL decreased the toxicity of each metal, an effect illustrated by the shift of the lines to the right.

Twelve guinea-pigs were shaved and given 0.25 ml. of 5 per cent (w/v) chromic acid solution intracutaneously as a wheal. After five days there were well-established circular ulcers at each site of injection with a black sloughing eschar about half an inch across and a raised red areola for a quarter-inch round the ulcer. The animals had recovered from the constitutional upset resulting from chromium absorption. At this point they were divided into two groups and half the animals treated with a daily application of acriflavine ointment to the ulcers; the other half received the same ointment made up to contain 10 per cent of BAL. The BAL ointment was kept cool between applications and retained its pungent odour to the end of the experiment. The ulcers treated with BAL became soft, filled in rapidly and had healed in 30 days; the ulcers treated with acriflavine alone were still indurated and approximately one quarter their original size after 40 days, when the animals were sacrificed. It is suggested that treatment with BAL ointment, and, if necessary, systematically administered BAL, would be of value in industrial chrome ulceration.

Mercury

The preparation tested was mercuric chloride, the LD50 of which was 120 mg./kg.; BAL had a protective effect on animals poisoned with this salt, raising the LD50 to 281 mg./kg. (see Fig. 3). According to Long and Farah (1946) intravenous injection of BAL reduces the lethality of intravenous 'salyrgan' in mice, and protects the cardiovascular system of anaesthetized dogs from the toxic effects of this mercurial compound. In the present work the LD50 of mersalyl B.P. solution was found to be 169 mg./kg. i.p. in mice. BAL (40 mg./kg. i.p.) immediately after the diuretic lowered the LD50 to 100 mg./kg. For mersalyl given intravenously the LD50 was 120 mg./kg. and BAL (20 mg./kg.) also given intravenously protected many of the mice from the violent convulsions caused by intravenous mersalyl and raised the LD50 to 165 mg./kg.

The effects of BAL are discussed later, but some clarification was provided by the results of tests of the effect of mersalyl on water diuresis in groups of rats. BAL (40 mg./kg. i.p.) acted as an antidiuretic, suppressing urine for 3–4 hours. Mersalyl (100 mg./kg. i.p.) caused immediate anuria and death followed after 48 hours, but if the two were given intraperitoneally within a few minutes of one another suppression was much less than with either alone, and the rats survived. In a double experiment using 16 rats the animals when watered and given subcutaneous saline had a diuresis of which the peak occurred after 65 min.; after subcutaneous mersalyl (1.0 mg./kg.) and saline the peak occurred at 72 min. Repetition of mersalyl (1.0 mg./kg.) and saline after one week gave a figure of 73 min., whereas mersalyl s.c. and BAL (4.0 mg./kg.) i.p. gave a figure of 90 min. It follows that mersalyl and BAL in large doses each have a delaying effect on the excretion of urine from normal rats and that given together they tend to cancel one another; with small doses this effect is not seen.

Chronic mercurial poisoning was induced in 12 rabbits by giving them mercury perchloride (1.0 mg./kg.) for ten days, and in 16 guinea-pigs by giving

5.0 mg./kg. i.p. daily. The animals so treated lost weight and developed diarrhoea and tremors and died, the guinea-pigs living for only 4 days, the rabbits for 12–16 days. Half the rabbits were also given 2.5 mg. BAL/kg. s.c. daily, and half the guinea-pigs 40 mg./kg. The animals given BAL survived in good health until sacrificed. Microscopy revealed marked necrosis of liver, kidney, and gut in the animals poisoned with mercury, and an absence of these lesions in the animals protected with BAL.

Lead

The salt used was plumbi acetatis B.P., the LD50 of which was 461 mg./kg. i.p.; BAL (40 mg./kg. i.p.) had an additive effect on the toxicity of this substance, the LD50 falling to 416 mg./kg.; this effect is shown in Fig. 4.

Gold

The salt used was sodium aurothiomalate and the LD50 found was 1.096 g./kg. i.p.; BAL had an additive effect on the toxicity of this compound, reducing the LD50 to 812 mg./kg., an effect shown in Fig. 4. Successful clinical use of BAL in cases of gold poisoning is reported by Cohen, Goldman, and Dubbs (1947).

Bismuth

The salt tested was sodium and potassium bismuth tartrate, the LD50 of which was found to be 676 mg./kg. i.p. BAL had an additive effect on the toxicity of this compound, reducing the LD50 to 288 mg./kg.; this effect is shown in Fig. 4.

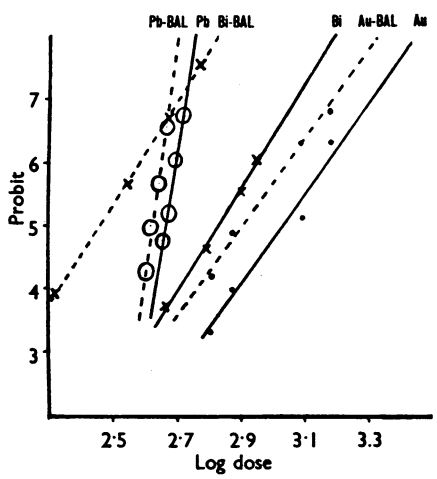

FIG. 4.—The action of BAL (40 mg./kg. i.p.) on the toxicity of lead (lead acetate), bismuth (sodium and potassium bismuth tartrate) and gold (sodium auro-thiomalate) in groups of white mice. The ordinates are probits of lethality and the abscissae logs. of dosage. BAL increased the toxicity of each metal, an effect illustrated by the shift of the lines to the left.

Effect of BAL on the action of insulin

Barron, Miller, and Meyer (1947) state that the action of insulin (0.8 U./kg.) is inhibited by BAL in doses of 0.1 g./kg. i.v. Larger doses of BAL reduce the blood sugar of rabbits and prove fatal.

In the present experiments a group of five rabbits about 2.0 kg. weight were starved overnight and a blood sample of 0.5 ml. drawn from the marginal ear vein of each rabbit and pooled in a heparinized tube. The animals were given 0.5 U./kg. soluble insulin subcutaneously and pooled blood samples collected every hour. After three days the experiment was repeated with the addition of 25 mg. BAL/kg. i.p. The mean blood sugar level after three hours had fallen by 75 per cent with the animals given insulin alone, whereas with insulin and BAL the level at three hours had fallen by 21 per cent. The insulin by itself gave a more precipitate and a more prolonged fall in blood sugar level than did the insulin in the same animals treated with BAL. This inhibitor effect of BAL on the action of insulin is illustrated in Fig. 5.

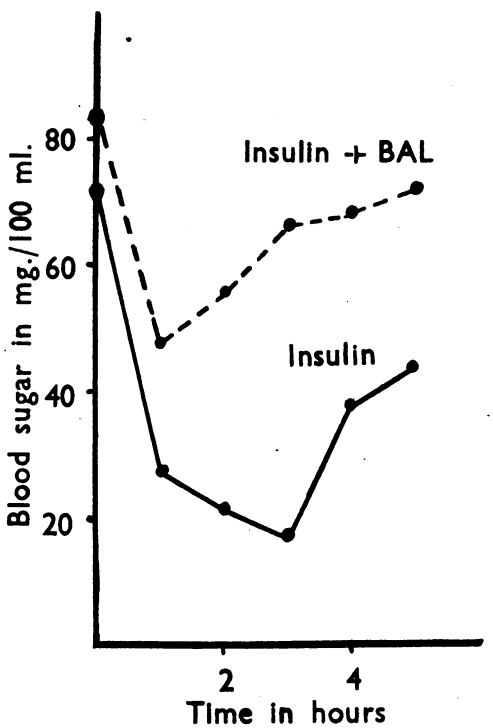

FIG. 5.—The mean blood sugar curve in five rabbits after 0.5 U./kg. soluble insulin s.c., and the effect of BAL (25 mg./kg. i.p.) in reducing the effect of the same dose of insulin in the same animals.

Discussion

It has been shown in the present work and in the work of others quoted that BAL is an effective antidote to poisoning by arsenic, mercury, antimony, chromium, nickel, and cadmium. McCance and Widdowson (1946) found that the closely related BAL-glucoside promotes excretion of copper and zinc salts, and it has further been shown that iron and thallium are unaffected by thiol compounds whereas lead and selenium are rendered more toxic. Bismuth and gold were found to be made more toxic to mice by BAL in the present work but according to Braun *et al.* (1946) BAL rendered bismuth less toxic to rabbits and according to Ragan and Boots (1947) gold less toxic to man. These differing results together with the apparently contradictory results obtained in the present work with mersalyl, and also the complex manifestations of the toxicity of BAL itself are attributable to the presence of sulphydryl groups in its molecule. Webb and van Heyningen (1947) point out that BAL inhibits the activity of any enzyme which contains a heavy metal, capable of being linked with available SH, as prosthetic group to the protein moiety of the enzyme. Such an activity would account for the widely differing phenomena of BAL poisoning—convulsive action on the C.N.S., drop in blood sugar, interference with respiration, and presumably also the damage to the small vessels which produces such marked alterations in the circulation. The smooth muscle of peripheral vessels appears to be much more sensitive to BAL than the muscle of gut, uterus, the heart, or coronary arteries, and the differing initial response of the spleen and limb volumes to intravenous injection of BAL needs further examination.

Gilman *et al.* (1946) point out that with cadmium the compound formed *in vivo* by BAL is a soluble substance which proves on isolation to have a greater toxicity than cadmium chloride itself. This increased toxicity is caused by increased pathogenicity to the kidney. The arsenic, mercury, chromium, and antimony preparations used to determine the LD50 in mice are highly irritant to the peritoneum, quickly absorbed and cause gross renal damage on excretion; intraperitoneal treatment with BAL reduced the toxicity of these compounds and prevented visible damage to the kidney. The products of linkages between BAL and these metals must be of such a nature that they are not toxic to the kidney on excretion. The reduction of acute toxicity of the metallic compound may be due to production of a non-irritant compound by reaction with BAL in the peritoneal cavity, and this compound may be absorbed more slowly and excreted in a non-toxic form. The salts of gold, bismuth, and lead which were used have a much higher LD50 than the group of metals discussed above, when given intraperitoneally. They are not so irritant as the former; they tend to be precipitated and to be absorbed slowly, and thus the cause of their acute toxicity is probably different from that of the others. BAL-metal complexes with these metals may be absorbed more quickly or be more toxic than the metallic salts themselves.

A point of importance is suggested by the finding of Gilman *et al.* (1946) that the BAL-cadmium complex formed *in vitro* differs markedly from the complex formed *in vivo*. In the present work with mice the metallic salts were given intraperitoneally followed by the BAL at the same site. If an *in vitro* type of precipitate were formed in the peritoneal cavity, this might well differ in stability, absorption rate, and toxicity from the type of compound formed *in vivo* in rabbits which were injected intramuscularly at different sites with bismuth and BAL by Braun *et al.* (1946) or from that in man given gold and BAL at separate sites and times by Ragan and Boots (1947). The same phenomenon of formation of different mercaptides with different toxicities according to the conditions of reaction of the metal and the thiol compound may account for the opposite effects of BAL on the toxicity of mersalyl given intraperitoneally and intravenously.

The many toxic properties of BAL and the variability of its effects indicate that some other related compound would be preferable for therapeutic use. Danielli, Danielli, Mitchell, Owen, and Shaw (1946) give evidence that BAL-glucoside may be suitable.

Summary

British Anti-Lewisite (BAL) has an LD50 of 100 mg./kg. i.p. in white mice. It causes conjunctivitis, ataxia, rapid and then impaired respiration and convulsions in small mammals. In anaesthetized rabbits small doses (4 mg./kg.) cause a temporary rise in blood pressure, but in cats only a fall in pressure is seen; this is considered to be due initially to splanchnic dilatation, but the main effect on the circulation of anaesthetized cats is a progressive fall in blood pressure despite constriction of the leg and spleen and an active heart. Loss of fluid from the capillaries leading to haemo-concentration and a state of shock is held to be the cause of death in anaes-

thetized animals, whereas convulsion is the cause of death in intact animals.

BAL has a protective action on white mice poisoned with arsenic, mercury, antimony, and chromium and a deleterious effect on mice poisoned with lead, gold, and bismuth. It prevents or relieves tissue damage caused by chronic poisoning with arsenic, mercury and chromium. BAL inhibits the action of insulin. Some apparent anomalies in the detoxifying action of BAL are discussed.

A pure sample of BAL was obtained from the Ministry of Supply. The expenses of the investigation were in part defrayed by a grant from the Rankin Research Fund, University of Glasgow, to one of us (J. H.), and in part by a grant from the Medical Research Council. One of us (J. D. P. G.) holds an I.C.I. Fellowship in Pharmacology, University of Glasgow. We desire to express our grateful appreciation of the interest taken in the work by the late Prof. N. Morris.

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