ON THE PROPERTIES

OF

BRITISH ANTI-LEWISITE.

THESIS for the DEGREE of M.D.

by

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A C K N O W L E D G M E N T S.

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SECTION ONE.

I.

HISTORICAL INTRODUCTION.

Inorganic arsenic was used as a therapeutic agent by the ancient Greeks and Romans and played a conspicuous rôle as the favourite drug of the professional poisoners of the Middle Ages. Had it not been for the efforts of chemists, arsenic might have remained somewhat limited in its therapeutic uses. Chemical research from the beginning of this century showed that both trivalent and pentavalent inorganic arsenic could be readily introduced into a large number of organic molecules. These organic arsenicals were found to be much less toxic to higher animals than to trypanosomes, spirochaetes, amoebae and other micro-organisms.

Ehrlich, in his researches at the beginning of this century, used the term chemotherapy to denote the treatment of parasitic infections by chemical agents. His search for substances, which would be highly toxic to parasites, with a low toxicity to man culminated in the discovery of trypanicidal dyes and organic

arsenical compounds. Of these arsenical compounds the well-known arsphenamine or "606" has achieved a place in medical history.

It was soon realised that the toxicity and therapeutic efficiency of the organic arsenicals depended on many complex factors, such as, the inter-relation of rate of absorption, rate of distribution, storage and rate of decomposition in the human tissues and on many other factors imperfectly known. As a result of the introduction of the organic arsenicals into medical therapeutics, much research has been carried out to elucidate the basic mechanism or mechanisms by means of which arsenic injures living cells and exerts its lethal effects on protozoal and metazoal organisms.

The application of science to modern warfare has clearly indicated the tremendous destructive capabilities of modern science. Fortunately in an attempt to combat these destructive powers an antidote to arsenical poison gases has been discovered. This antidote, called B.A.L. (British Anti-Lewisite), was the result of research by Peters, Stocken and Thompson (1939-1942) working in the Biochemistry Department, Oxford.

With the advent of arsenical smokes and blistering gases into modern chemical warfare, attention was again given to the problem of the mode of action of arsenic and to the prevention and treatment of its toxic and lethal effects. The finding of an effective antidote to these toxic agents of warfare became a most urgent problem.

It is pleasing to record, that B.A.L. has been found to be an effective antidote to the local and systemic effect of arsenical warfare agents. In addition, the compound appears to have important clinical applications in the treatment of toxic manifestations of arsenical therapy and poisoning with many other metals. Perhaps more important still, it has advanced the understanding of fundamental biochemical mechanisms.

II.

EXPERIMENTAL BACKGROUND.

1. The Discovery of B.A.L.

At the beginning of World War II research work was initiated in the Biochemistry Department, Oxford University, by Peters, for the Chemical Defence Research Department, Ministry of Supply. The aim of the research was to discover an antidote to arsenical vesicants such as lewisite (CH.CL : $AS.CL_2$) and arsenical smokes used in warfare.

This research resulted in the discovery of an antidote to lewisite. This antidote, now familiarly known as B.A.L. the name given to it by the Americans, is also known as British Anti-Lewisite. Chemically it is 2,3, dimercapto-propanol

$$\begin{array}{l} CH_2 - SH \\ | \\ CH - SH \\ | \\ CH_2 - OH \end{array}$$

It is a colourless liquid of specific gravity 1.21. It is soluble in water to the extent of some six per cent and it possesses a strong sulphurous odour like other mercaptans.

The discovery of B.A.L. was the result of planning and assessing the results of much previous work done at Oxford and other research centres before World War I and between it and World War II.

Biochemical research conducted by Peters (1936a), on the brain lesion produced in pigeons by the lack of aneurin, showed that the pathological lesion was produced by the partial failure of an enzyme system - the pyruvate oxidase enzyme system - due to the lack of aneurin pyrophosphate (co-carboxylase). In this year Peters (1936b) showed also that the vesicant, dichlordiethyl sulphone, poisoned the pyruvate enzyme system, which oxidises lactates in brain tissue in a similar manner to that of the enzyme inhibitor, iodoacetic acid. As iodoacetic acid was a weak vesicant, Peters suggested that the similar vesicant properties of dichlordiethyl sulphone and iodoacetic acid might possibly be due to their action on the pyruvate enzyme system. He also found that sodium arsenite poisoned the enzyme system in a similar manner. These experimental results agreed with the observations of Onaka (1911), Szent Gyorgyi (1930) and Voegtlin, Rosenthal and Johnson (1931), that enzyme system oxidations and particularly keto acid oxidation were

poisoned by the action of arsenites. These researches drew attention to the possibility, that pathological changes in the living tissues might be due to the partial or complete failure of an enzyme or enzymes, responsible for the metabolism in the tissues concerned and more important still suggested the enzyme at fault.

Another interesting line of approach was suggested by the work of Hopkins and Dixon (1922) who discovered glutathione, a protein containing a thiol (-SH) group, and showed that arsenical compounds, such as diphenyl-chlorarsine, were capable of combining with and thus removing the fixed thiol groups in muscle and skin, and thus interfering with the thiol or sulphydryl groups necessary for the catalysis of glutathione.

Walker (1925) showed that diphenyl-chlorarsine was capable of abolishing the fixed thiol (-SH) group in washed muscle tissue and skin and that the thiol (-SH) group could not be liberated by treatment with cyanide. From this it was postulated by Walker (1928) that chemical combination between the arsenical and the thiol group had occurred, rather than oxidation of the thiol group.

The discovery of glutathione and the observations of Walker, working under the direction of Peters, linked up with the work carried out independently by Voegtlin, Dyer and Leonard (1923, 1925), who suggested that the toxic action of arsenic on living cells was due to the combination of arsenic with certain essential compounds in protoplasm containing the thiol (-SH) group.

This research work by Voegtlin et al and Walker focussed Peter's attention on the -SH group as a possible receptor for the arsenic. This idea was strengthened in later years by the work of Rosenthal and Voegtlin (1930), Quastel and Wheatley (1932), and Dickens (1933), who showed that iodoacetic acid readily combined with compounds containing the -SH group, and postulated that the arsenic combined with the -SH group of enzyme systems. Cohen, King and Strangeways (1931) showed that the thioarsenites of the type $R.AS(SR')_2$, (where R and R' represent organic radicles), were dissociable in alkaline solution and this important observation, taken in conjunction with the above experimental results, gave grounds for the supposition that the toxic effects produced by the combination of arsenic with the -SH group might be a reversible process.

Hence the postulate, that the poisoning activity of arsenicals was due to a selective action upon the pyruvate system by the combination of the arsenical with some essential -SH group, necessary for the normal activity of the enzyme system, formed the starting point of the research work by Peters et al for an antidote to the arsenical chemical warfare agent, lewisite. Since substituted chlorarsines, e.g. lewisite, were known to be rapidly hydrolysed to the oxide, arsenoxide, it was considered that the toxic effects of arsenical vesicants on the skin were examples of the toxic action of arsenoxides upon living cells and that the vesicant action would depend upon the lipoid solubility of the arsenical vesicant in the skin, thus allowing the vesicant to penetrate the keratin layer of the skin and reach the capillaries, epidermal and dermal cells.

Further work by Peters, Stocken and Thompson (1940) showed that sodium arsenite and lewisite could produce marked inhibition of the pyruvate enzyme system in brain tissue. Some other enzymes, such as succino-hydrogenase, were affected much less if at all. It was proved, in vivo, that poisoning with arsenite led to an increased level of pyruvate in the blood,

thus confirming that arsenic was capable of interfering with the oxidation of carbohydrate at the pyruvate stage. This work linked up with the earlier work of Thompson and Johnson (1935) who showed that there was a raised blood pyruvate level in aneurin (thiamin) deficiency. The above results supported the idea of a selective action by sodium arsenite and lewisite on enzymes, and particularly of their powerful action on the pyruvate enzyme system, and suggested that the pyruvate enzyme system would be suitable for use as an *in vitro* test for the further exploration of the mechanism of the production of the toxic reactions of arsenic on living tissues.

However, in spite of promising indications that thiol compounds would be effective antidotes to arsenical poisoning, Sinclair (1940) showed that no monothiol compound or dithiol compound, e.g. diethyldithiocarbamate, was effective against lewisite or arsenite in enzyme and animal tests. This also applied to cysteine and glutathione, which in excess had been shown by Voegtlin, Dyer and Leonard (1925) and Eagle (1939) to have some protective activity against the toxic effects of therapeutic arsenoxides. The

difficulty was further increased by the observations of Strangeways (1937), who had shown that compounds of arsenicals with thiols could be as toxic as the original arsenical. This important observation was verified by fresh evidence produced by Ing and Robertson (1940). As Peters emphasised, the behaviour of new thioarsenites *in vivo* is not necessarily the same as their behaviour *in vitro*.

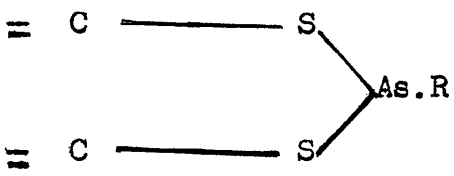
At this stage in the elucidation of the problem, it seemed that the possible lines of further progress would be limited either to more systematic trial of other thiol compounds without further biochemical guidance or a study of the relative dissociation of the various thioarsenites. At this juncture, Stocken and Thompson (1946) began to collect further information upon the interaction of thiol groups in proteins, by analysis of compounds of arsenic with a protein containing -SH groups and these experiments finally led to the solution of the problem.

Goddard and Michaelis (1934) showed that kerateine, the reduced product of keratin, contained many -SH groups. Stocken and Thompson (1946) treated aqueous solutions of kerateine with lewisite under physiological conditions of temperature and H'ion concentration and

found that the arsenic content of the lewisite - kerateine compound could be closely correlated with the thiol content of the parent protein. Lewisite and arsenite were found to combine with kerateine, producing water - soluble protein derivatives and the arsenicals were found to be combined solely with the thiol groups of the kerateine. The conclusion that the arsenic in these derivatives was present in true chemical combination with the thiol groups and not merely present in a non-specific physical association with the proteins was based on three main facts. Firstly, when kerateine was treated with lewisite or arsenite, compounds were formed of which the arsenic content was quite independent of the amount of arsenic present. Secondly, the arsenic content of the derivatives of arsenic with kerateine were found to bear a direct relationship to the thiol content of the kerateine. Thirdly, the thiol content of kerateine was found to be sufficient to account for all the arsenic found in the derivatives. It was also seen that the lewisite - kerateine compound was stable on dialysis and that oxidation of the sulphydryl (-SH) groups of the kerateine to the disulphide form (-S-S-) (meta-keratin)

before treatment with lewisite led to a large reduction in the amount of arsenic combined. An interesting fact emerged from these experiments, namely, that 75% of the arsenic was in combination with two thiol groups in the kerateine molecule. This observation, in conjunction with the ease of re-oxidation of the kerateine to the disulphide form, led Stocken and Thompson to postulate that the high toxicity of lewisite and trivalent arsenic organic compounds was due to their ability to combine with the essential thiol (-SH) groups in certain tissue proteins, particularly the enzyme concerned, to form stable ring compounds. On chemical grounds it was supposed that compounds of a dithiol with arsenic of Type I would be expected to be more stable than compounds of an organic arsenical with two molecules of a monothiol compound of Type II.

Type I.



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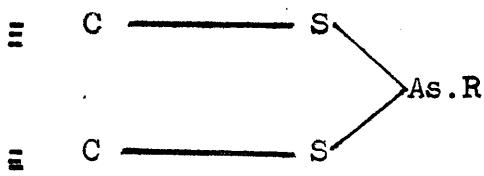
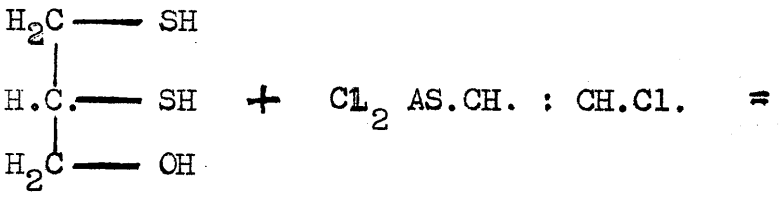


Thus simple dithiol compounds might form relatively stable ring compounds with lewisite or other trivalent organic arsenicals and so compete successfully for the arsenic with the 'dithiol' proteins in the tissues. If this supposition was true thioarsenites formed with

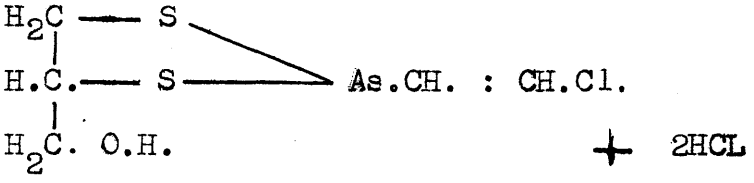
monothiols would dissociate so as to allow their arsenic to combine in ring formation with the tissue thiols and this in turn might be more dissociable than the more stable ring compound formed with the simple dithiols. Experimental proof of this was provided by Stocken and Thompson (1946) in 1942, by comparing the relative rates of hydrolysis of different types of thioarsenites and lewisite-kerateine. The discovery, that the arsenical protein, formed by the interaction of lewisite and kerateine, contained over 70% of its arsenic in combination with sulphur, suggested that the vesicant arsenicals could act by reacting with two neighbouring essential thiol groups in enzyme proteins, for example, those of the pyruvate enzyme system forming a relatively stable 5-membered ring compound - a cyclic thioarsenite.

As a result of the above work, the study of the therapeutic possibilities of simple dithiols was begun in 1940 by Stocken and Thompson (1946). The simple dithiols were chosen because Philpot (1940) had found, in a related research, that short chain fatty acids penetrated the skin more readily than long chain compounds. Toluene dithiol was first tested on the

pyruvate enzyme system <u>in vitro</u> with promising results, followed by ethane dithiol. Neither of these compounds, however, proved suitable for the skin treatment of arsenical vesicants in animals and the new compound 2,3 dimercapto-propanol (111) was prepared. It was found to penetrate the skin and proved on trial to be very satisfactory. The reaction of this compound with lewisite is:-



111.



This dithiol, to which the name B.A.L. (British Anti-lewisite) was given by the Americans, proved highly effective in preventing the toxic action of lewisite on the pyruvate oxidase enzyme system of brain tissue. On trial under experimental conditions, B.A.L. was found to be very much more powerful in inhibiting the action of lewisite than any other dithiol compound prepared and investigated. Further investigations showed that B.A.L. was not only capable of preventing the toxic action of lewisite but, in addition, could reverse the

toxic action, provided that the toxicity had not been too long established and was able to prevent the inhibition of skin respiration by lewisite. By comparison, it was found that monothiols, such as cysteine and monothio-ethylene glycol could not produce these detoxicating effects in equivalent concentrations to B.A.L.

The next step in the problem was to prove that these *in vitro* biochemical findings could be sustained in experiments *in vivo*. The *in vivo* experiments were carried out in rats and rabbits. It was found that animals contaminated with lewisite could be saved by B.A.L. applied in drops to the skin or by the injection of aqueous solutions, up to two hours after contamination, when the animals were seriously ill. It was observed that the treatment by B.A.L. of lewisite burns in rats was followed by a marked increase in the urinary excretion of arsenic during the ensuing 24 hours, amounts up to 33% of the arsenic applied to the skin as lewisite was recovered in the 24 hr. urine sample. This increase of arsenic in the urine was observed even when treatment with B.A.L. was delayed until 1 hour after contamination with the lewisite. The control animals developed diarrhoea and passed much mucus, while the animals receiving B.A.L.

were not so affected. The subcutaneous and intraperitoneal injection of B.A.L. into rabbits and rats was rapidly followed by the excretion of an unidentified thiol or thiols in the urine. As a result of these successful experiments Stocken, Thompson and Vey (1941) showed that vesication produced in human volunteers by lewisite and phenyldichlorarsine could be prevented by the local application of B.A.L. up to one hour after injury. At this time, well marked signs of injury to the skin, such as oedema and erythema, had developed. In many cases it was observed that the residual erythema on the contaminated skin area, twenty-four hours after treatment with B.A.L. was much less both in intensity and size than that already present at the time of treatment; and in some of the experiments the rapid subsidence of the skin oedema was so marked that to the observers it suggested an actual reversal of the underlying pathological changes brought about by the arsenic. In comparison with B.A.L., it was found both in man and animals, that the monothiol, monothioethylene glycol, did not influence the severity of the vesications produced by lewisite. Subsequently the above results were confirmed by Mann and Pirie in Oxford (1941), and by Leishman, Uhde, Dawson, and Dunphy

at Porton in 1941. These workers showed that B.A.L. prevented the destruction of the eyes of rabbits contaminated with lewisite up to periods of 20 minutes after contamination.

Fell and Allsopp (1946) in 1941 carried out a series of tissue culture experiments, to find out whether the toxic action of lewisite on cells, cultivated in vitro, could be prevented or cured by suitable treatment with B.A.L. It was found that cultures of the sclerotic from 11-day chick embryos poisoned with lewisite were restored by treatment with B.A.L. The drug exerted a curative action on the poisoned cells of the tissue culture. Further enzyme studies at Cambridge, in the United States, and at Oxford by Stocken and Thompson (1944) and Whittaker (1945) showed that isolated enzymes and enzymes in tissues were protected by B.A.L. from the toxic effects of lewisite and other arsenicals.

Webb and Heymingen (1947) in 1943 studied the action of B.A.L. on enzyme systems. Of a large number studied, seven were strongly inhibited by B.A.L. polyphenol oxidase, carbonic anhydrase, catalase, peroxidase, aldehyde mutase, phosphorylase and glyoxalase. Four of the seven enzymes inhibited by B.A.L. were known to be metallo-proteins. Keilin and Mann (1940) showed that

polyphenol oxidase contained copper, and carbonic anhydrase contained zinc. Elliott (1932), Keilin and Hartree (1936), and Keilin and Mann (1937) proved that catalase and peroxidase possessed an iron-containing prosthetic group. Webb and Heyningen from these results suggested that B.A.L. specifically inhibits metal enzymes by combining with the metals, due to the high affinity of B.A.L. for them. So far aldehyde mutase and phosphorylase have not been shown to be enzymes containing metals but Webb and Heyningen consider that this possibility is not perhaps excluded. Lohman (1932) showed glyoxalase needed glutathione as co-enzyme for reaction and Webb and Heyningen suggested that the inhibition of this enzyme by B.A.L. may be competitive, the dithiol groups in B.A.L. having a greater affinity for the enzyme than the monothiol group in glutathione. As a result of their research, B.A.L. may be considered as a potent inhibitor of metal-containing enzymes, with the exception of the cytochrome system.

2. American Work on B.A.L.

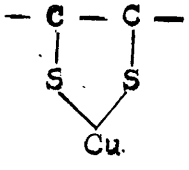
Full details regarding B.A.L. and experimental findings were sent to the United States by Peters and his associates through official channels and B.A.L. itself was received there in 1941. Thereafter an intensive research programme was undertaken, including the investigation of the biochemistry, toxicology, pharmacology and clinical applications of the substance.

Barron, Miller, Bartlett, Meyer and Singer (1947) in 1942 investigated the effect of lewisite on the various types of enzyme reactions concerned with cellular metabolism and concluded that trivalent arsenicals exerted their toxic action by combining with the thiol (-SH) groups of the activating protein of enzyme systems. Tissue respiration was interfered with, due to the combination of arsenicals with the large group of enzymes containing the sulphydryl (-SH) groups. These are essential for carbohydrate and fat metabolism. It was found that lewisite had little effect on the enzyme systems concerned with protein metabolism. The inhibition of enzymes, produced by lewisite and other arsenicals, could be prevented by B.A.L. and, even when established, B.A.L. could reverse the process of inhibition.

McLeod (1942) and Eagle (1942-1943) showed that B.A.L. in reversing enzyme inhibitions produced by arsenic, acted by virtue of the fact that B.A.L. had greater affinity for the arsenic than the attacked tissue thiols. When trypanosomes or spermatozoa were subjected to lethal concentrations of arsenic, they lost their motility and showed degenerative changes. When B.A.L. was added, motility and normal cell structure were regained. It would appear that B.A.L. actually removes the arsenic from the cells because the supernatant fluid contains an increased amount of arsenic. Eagle, Magnuson and Fleischman (1946) in 1948 verified the observation of the English workers that, when B.A.L. was administered to animals previously poisoned with arsenicals, it caused a marked increase of arsenic to appear in the urine.

Barron, Miller and Kalnitsky (1947) working on the oxidation of the dithiols in 1943, showed that B.A.L. behaved like the monothiols cysteine and glutathione on oxidation. B.A.L. behaved as a sluggish non-autolizable oxidation - reduction system, easily oxidised by catalytic amounts of iron porphyrine and copper. Iron porphyrin and copper were found to be the most powerful oxidation catalysts while ferric chloride, potassium ferricyanide and cytochrome oxidase (in presence of

cytochrome c) were also capable of oxidizing B.A.L. British Anti-Lewisite combines with a number of heavy metals, such as iron, lead, antimony, bismuth, copper, cobalt, nickel, selenium and antimony to form coloured metal-B.A.L. complexes, mostly insoluble. With mercury, zinc and cadmium, white precipitates with B.A.L. were formed while it was found that magnesium combines with B.A.L. to form colourless soluble compounds. In the presence of oxygen, B.A.L. destroyed iron porphyrin compounds such as haemin and oxyhaemoglobin by opening the porphyrin ring. B.A.L. reacted instantaneously with methaemoglobin and reduced it to haemoglobin. These workers found that the rate of oxidation of B.A.L. using copper as a catalyst, seemed to depend on the distance of the -SH groups from each other. When dithiols were tested, with the -SH groups separated from each other by four carbon atoms, there was very little oxidation. This effect was explained by assuming that the oxidation of B.A.L., in the presence of copper, was preceded by the intermediate formation of an unstable copper cyclic compound.



which depends on the -SH groups being close together.

Barron, Miller, Bartlett, Meyer and Singer (1947) experimented in 1944 with the inhibition of enzymes containing -SH groups by lewisite and their reactivation by dithiols. Lewisite was found to inhibit all the enzyme systems containing -SH groups except d-amino-acid oxidase, yeast carboxylase and transaminase. In every case B.A.L. reversed the enzyme inhibition produced by lewisite and was found to be much more powerful than the monothiol glutathione. The respiration of rat brain slices was inhibited by lewisite and reactivated by B.A.L. The anaerobic glycolysis of rat brain slices was inhibited by the addition of lewisite but, on addition of B.A.L. the inhibition was prevented and the anaerobic glycolysis reactivated. Lewisite also inhibited the synthesis of carbohydrate from pyruvate by kidney slices. B.A.L. reactivated this synthesis.

Barron, Miller and Meyer (1947) studied the effect of B.A.L. on the activity of enzyme system and on the metabolism of tissues, in order to understand the toxic effects observed, when large quantities were injected into animals. They verified the findings of Webb and

Heyningen (1947), that B.A.L. produced inhibition of some enzyme systems by uniting with the heavy metals which form the prosthetic group of the protein part of the enzyme. Barron et al considered, that the reduced respiration of tissue slices, produced by B.A.L. and other dithiols, was probably due to combination of the following factors, namely the reduced rate of oxidation of cytochrome c, the inhibition of metallo-protein enzymes and the inhibition of enzymes containing -SH groups by the oxidation product of dithiols. Since B.A.L. is a reducing agent, it was considered that it might reduce the -S-S- groups of insulin and thus destroy its physiological effect. It was found that B.A.L. alone had no effect on the blood sugar level in rabbits but that B.A.L. with insulin prevented the normal hypoglycaemic action of insulin. In this connection Durlacher, Bunting, Harrison, Ordway and Albrink (1947) found an initial increase in the blood sugar level after the injection of B.A.L.

Experimental therapeutics were begun in the spring of 1942 when a large number of animal experiments were carried out, testing the effect of B.A.L. against the arsenicals used in chemical warfare. The results fully confirmed British reports. It was as a result of

American work that B.A.L. was prepared in a suitable medium for intramuscular injection. These animal experiments led to further studies to determine the therapeutic effectiveness of B.A.L. in various vehicles, the best routes of administration and the best dosage schedule. Eagle, Magnuson and Fleischman (1946) showed that a stable, sterile, preparation of B.A.L. in benzyl-benzoate-peanut oil solution was most suitable for intramuscular injection. The most effective dosage schedule in animals was shown to be four injections of B.A.L., at two to four hourly intervals in the first twenty-four hours, followed by six daily injections. Eagle et al (1946), Modell, Gold and Cattell (1946), and Sulzberger, Baer, and Kanof (1946) carefully investigated the toxicity of this preparation of B.A.L. in oil by intramuscular injection in man. This work showed that B.A.L.-in-oil, injected intramuscularly, was tolerated best at a dosage level of 2.5mg. to 3.0mg/kg. body weight in human beings. It only resulted in very mild reactions in a percentage of volunteers tested. It was found that if the dose was increased to 5mg/kg. body weight, more than half the subjects experienced reactions. The chief reactions to B.A.L. in human subjects, reported by Eagle et al (1946), Sulzberger et al (1946), Modell

et al (1946), Carleton, Peters, Stocken, Thompson and Williams (1946), and Longcope, Luetscher, Wintrobe and Jager (1946) were some or all of the following symptoms; nausea, vomiting, headache, burning sensation in the mouth, nose and eyes, sweating, restlessness, weakness, limb pains, trunk pains, jaw pains and generalised aches and pains. The heart rate was often increased with sometimes a rise in both diastolic and systolic blood pressure. However, these various reactions were found to be transient and had subsided within four hours from the time of injection. The discovery that B.A.L.-in-oil could be safely injected in man led to B.A.L.-in-oil being given a trial in cases of arsenical poisoning.

The medical applications of B.A.L. were now investigated by many American workers. Eagle (1946), Eagle and Magnuson (1946), and Luetscher, Eagle and Longcope (1946), treated toxic reactions due to intensive schedules of anti-syphilitic arsenical treatment. At this time widespread use of intensive schedules of arsenic therapy was being made in the American armed forces, and in the civilian population with a resulting great increase in arsenical toxic reactions.
Eagle and Magnuson were therefore able to initiate a carefully supervised therapeutic trial of B.A.L. in

clinics, where intensified arsenic therapy was being carried out. Longcope et al (1946) treated patients from industrial plants who had been accidentally exposed to arsenicals. The majority of toxic arsenical reactions were cases of arsenical dermatitis and haemorrhagic encephalitis following intensive mapharside therapy. The resulting data strongly suggested that B.A.L., properly administered, was effective in the treatment of arsenical dermatitis, arsenical encephalopathy and accidental massive overdosage with mapharside. Eagle (1946) suggested that B.A.L. was probably of value in some cases of blood dyscrasias due to arsenotherapy but considered it to be of no value in the majority of cases of so-called arsenical jaundice.

Longcope and Luetscher (1946) gave B.A.L. a trial in twenty-three patients, suffering from acute mercury poisoning by corrosive sublimate, and found the drug to be most successful thus bearing out the findings of Gilman, Allen, Philips and St. John (1946) that B.A.L. was successful in preventing acute mercury poisoning by corrosive sublimate in rabbits and dogs. Thus animal experiments and clinical trials demonstrated that B.A.L. was effective in mercury poisoning.

3. Canadian Researches on B.A.L.

Canadian research on the properties of B.A.L. began in the latter part of 1941. The first investigation undertaken by Young (1946) was a comparison of the antidotal activity and relative toxicity of B.A.L. with a series of synthesised monothiols, dithiols and trithiols, when employed to counteract the effect of lewisite. The lewisite was applied to the skin of rats and it was found that, almost invariably, the lives of the animals were saved if B.A.L. was applied to the dosed area of the skin not later than two hours after the lethal amounts of lewisite had been applied. Simpson and Young (1946) showed that, even when treatment with B.A.L. was delayed for a longer period, the animals sometimes survived. Zbarsky, Simpson and Young (1946) showed that protection against the systemic effects of lewisite in rats was afforded by B.A.L. when applied to a skin site, other than that contaminated with lewisite or when B.A.L., dissolved in propylene glycol, was given by intramuscular injection to the rats.

Manson, Zbarsky and Young (1946) found that B.A.L.

when applied to the skin of the rats, exerted an antidotal action to sodium arsenite when given by intraperitoneal injection. Berenbom (1946) observed that, when rats were given lethal amounts of cadmium chloride by intraperitoneal injection, they sometimes survived if they were treated percutaneously with B.A.L.

Zbarsky (1946) confirmed and extended the findings of Peters, Stocken and Thompson (1945) that there was an increased urinary excretion of arsenic following the administration of B.A.L. to rats dosed with lewisite. Zbarsky, Manson and Young (1946) showed that, whether B.A.L. was applied to the skin site contaminated with lewisite, or to a separate site, or given by intramuscular injection in propylene glycol, the arsenic content of the urine excreted during the ensuing twenty-four hours after dosage with lewisite was increased, when B.A.L. was given immediately or one or six hours after the lewisite. B.A.L., given twenty-four or forty-eight hours after the administration of lewisite to the skin, had less effect on the urinary excretion of arsenic in the ensuing twenty-four hours. The faecal excretion of arsenic was not influenced significantly by treatment with B.A.L. Although B.A.L.exerted an antidotal effect

in sodium arsenite poisoning in rats, it was found that the urinary excretion of arsenic was only slightly increased. In contrast to the effect of B.A.L. on sodium arsenite excretion in urine, Berenbom (1946) showed that, when rats were given half the L.D.50 dose of cadmium chloride by intraperitoneal injections, there was very little urinary excretion of cadmium but, when B.A.L. was given in addition to cadmium chloride, large amounts of cadmium were excreted in the urine. This urinary excretion of cadmium produced by B.A.L. tended to prevent the accumulation of cadmium in the liver, which occurred when B.A.L. was not administered.

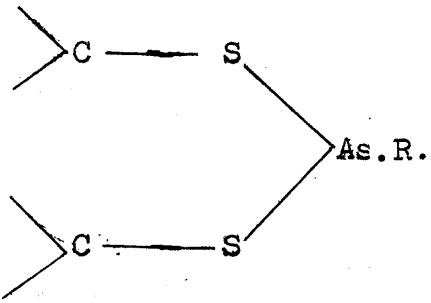
Young and Simpson (1946) prepared radioactive B.A.L. by incorporating radioactive sulphur isotope S^{35} in the B.A.L. molecule and this compound was used in studies of the absorption and metabolism of B.A.L. When radioactive B.A.L. dissolved in propylene glycol, was injected into rats by the intramuscular route, it was found to pass rapidly from the site of injection, for very little S^{35} was found to be present in the dosed muscle six hours after injection. Percutaneous and intramuscular administration of radioactive B.A.L. to rats, showed that it was distributed throughout the organism. Apart from somewhat higher concentrations

in the kidney and intestines, radioactive B.A.L. did not appear to accumulate in any one of the main organs. The most characteristic feature of the experiments was the rapidity with which radioactive B.A.L. was excreted in the urine. These workers considered that little of the radioactive B.A.L., excreted in the urine of the rats given the compound, was in the form of unchanged B.A.L.

Summary.

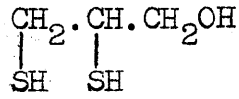
The toxicity of arsenicals is due to the fact that they combine with and interfere with the function of essential physiological groupings in the cells of living tissues and especially of the cellular thiol or sulphydryl (-SH) groups. The -SH groups are associated with the enzyme proteins.

When lewisite reacts with kerateine, a protein containing -SH groups, approximately 75% of the bound arsenic is in combination with two thiol groups in a stable 5-membered ring structure.



This stable combination of arsenic with tissue proteins is only to a slight degree reversible by monothiols such as cysteine and glutathione.

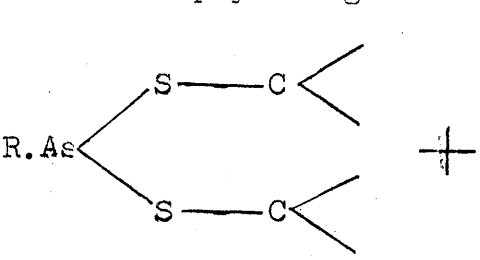
2, 3, dimercaptopropanol, B.A.L. a dithiol,



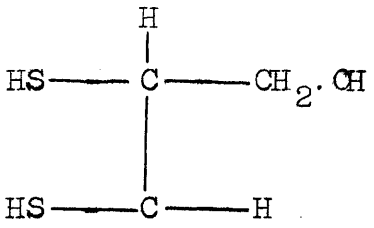
supplying a readily mobilisable supply of -SH groups,

is found to be far more effective than the monothiols and protects tissue enyzymes from the toxic effect of arsenicals.

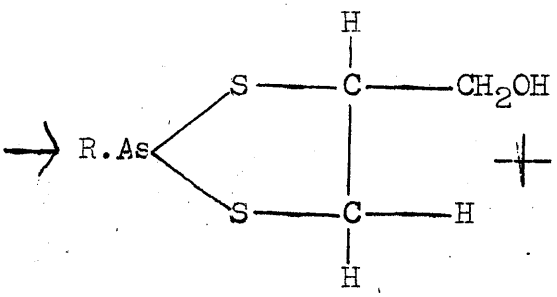
Cyclic B.A.L.—thioarsenite is much more stable than the tissue protein - thioarsenite and, therefore, B.A.L. when supplied to tissues poisoned with arsenic, removes the arsenic from the tissue protein - thioarsenite combination, thus detoxifying the tissue and allowing the enzymes to carry out once more their essential physiological actions.



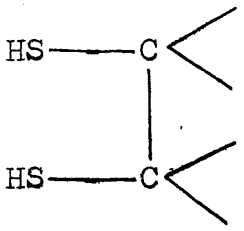
Tissue protein-thioarsenite



B.A.L.



B.A.L. - thioarsenite



tissue protein.

Animals poisoned with arsenic are protected and restored by B.A.L., which greatly promotes urinary excretion of arsenic.

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4. Toxicological and Pharmacological Researches on B.A.L.

(a) In animals.

B.A.L. is itself a toxic compound and this toxicity has been investigated by various workers.

Durlacher, Bunting, Harrison, Ordway and Albrink (1946) investigated the toxicological action of B.A.L. on experimental animals. Sublethal doses of B.A.L. given to rats, mice, guinea pigs, rabbits and dogs produced initial apathy, which was accompanied by lachrymation, blepharospasm and conjunctival oedema. In the case of dogs there was increased salivation, which was frequently accompanied by retching and vomiting. These symptoms, however, were found frequently to regress, leaving no residual upset. With larger doses of B.A.L. the above effects were produced with the addition of the following symptoms:- gradually increasing muscle tremors with inco-ordination, accompanied by clonic and tonic convulsions proceeding to coma and death.

The effect on the cardiovascular system was shown by an early transient rise of blood pressure and with lethal amounts of B.A.L., all animals exhibited preterminally signs of peripheral vascular collapse, as

evidenced by rapid pulse, hypotension and increased packed cell volume of the blood, indicating haemoconcentration.

The convulsions following B.A.L. were not due to hypoglycaemia and were alleviated by anaesthesia with sodium pentobarbital. Toxic doses of B.A.L. resulted in a diminished H⁺ ion concentration of the blood and a diminished blood carbon dioxide content, accompanied by an increased serum lactic acid and amino nitrogen content, thus producing a metabolic acidosis. An initial rise of blood sugar, a reduction in liver potassium and liver glycogen with a corresponding increase of liver sodium and chloride were noted.

Modell, Chenoweth and Krop (1946) carried out a detailed investigation of the toxicity of B.A.L. on cats. The earliest signs of B.A.L. poisoning in these animals were blinking, blepharospasm, lachrymation, conjunctival oedema and salivation. With larger doses ataxia respiraroty stimulation and increased urination were produced. With fatal doses there were produced, in addition to the above signs, respiratory depression, pulmonary oedema and convulsions. These workers considered that the eye signs and salivation were produced by the circulating B.A.L., and not by the B.A.L. present in the expired air. Prolonged daily administra-

tion of about one third of the L.D.50 dose of B.A.L. for cats had no effect on the red cell count, prothrombin time, carbon dioxide combining power of the blood, blood glucose or blood creatinine levels. On the other hand, the haemoglobin percentage of the red blood cells was reduced, the white cell count increased and the blood non-protein nitrogen raised. B.A.L. was shown to be absorbed through the intact skin of cats but the amount absorbed was small and variable. Apart from lethal doses, the toxic reactions of B.A.L. were, in the main, found to be reversible and the substance was rapidly eliminated.

Chenoweth (1946) investigated the cardiovascular action of B.A.L. on cats. B.A.L. given by the intravenous route produced a fall in the systemic and pulmonary arterial pressure and a rise in the portal venous pressure. On the other hand, when B.A.L. was administered by percutaneous application or by very small repeated intravenous injections, it caused a rise in blood pressure instead of a fall in blood pressure. Associated with the fall in blood pressure, there was a marked rise in the peripheral resistance of the blood vessels of the limbs with no rise of pressure in the vessels of the liver and vessels of the splanchnic area.

B.A.L. produced a marked increase in the rate of lymph flow from the thoracic and cervical lymph ducts. As a result of these experiments it was concluded that B.A.L. produced primarily a reversible constriction of certain peripheral arterioles producing a rise of blood pressure from small doses, but with larger doses damage to the capillaries occurred with the development of signs of peripheral vascular failure.

Hitchcock (1946) studied the effect of B.A.L. and other enzyme inhibitors on the blood vessels of cats. It was found that blood vessels of the different organs behaved differently to enzyme inhibitors, B.A.L. caused intense constriction of the femoral arterioles but was without effect on renal, hepatic or coronary arterioles.

The action of B.A.L. on skeletal muscle was investigated by Krop (1946) on the isolated skeletal muscle of the frog. It had been shown that B.A.L. inhibited the take up of oxygen when added to liver and bone marrow slices and Krop sought corroborative evidence on the functional changes occurring in the isolated sartorius muscle of the frog. Reduction and abolition of oxidative heat occurred, indicating that B.A.L. inhibited oxidative processes and thereby imposed anaerobic conditions upon the muscle. Lactate, normally

removed by oxidative processes, accumulated in the bath and gave chemical proof of the physical evidence of the arrested oxidation of the muscle due to B.A.L. This work was also indirect confirmation of the finding by Durlacher et al (1946) that in dogs there was an increase in blood lactate after administration of B.A.L. It indicated that increased blood lactate in the intact animal was not a consequence of pulmonary oedema but rather a direct interference with cellular oxidation.

(b) In man.

Eagle, Magnuson and Fleischman (1946) pointed out that although B.A.L. was unstable in aqueous solution it was stable in a vehicle of peanut oil and benzyl benzoate and in this medium it could be sterilised in glass ampoules with very little loss of activity. This finding led to the systemic use of B.A.L. for the treatment of manifestations of arsenic poisoning. Experiments with rabbits, poisoned with lewisite, showed that the longest interval between intramuscular injections of B.A.L. in peanut oil - benzyl benzoate mixture consistent with a continuing effect on arsenic excretion in the urine was about four hours and this interval was chosen for preliminary trials in man.

Eagle (1946) had found that the dosage of B.A.L. given by four hourly intramuscular injections in the treatment of experimental arsenic poisoning in animals, was in the region of 2.5mg. to 3.0mg./kg. Eagle (1946), and Eagle and Magnuson (1946) demonstrated in human volunteers that doses of 2.5mg. to 3.0mg./kg. of B.A.L. could be injected intramuscularly with only temporary local discomfort at the site of the injection and with only transient subjective symptoms in a small number of cases. At doses of 4mg. to 5mg./kg. there was an increased incidence of toxic reactions. The most common symptoms complained of were nausea, vomiting, headache; a burning sensation of the lips, mouth and throat, tongue and eyes, lachrymation, salivation, and pain in the teeth; generalised muscular aches in the trunk and extremeties, with burning and tingling in the extremities; and a sense of constriction in the chest, with a feeling of anxiety. The systolic and diastolic blood pressure were frequently elevated. The above reactions usually subsided in thirty to ninety minutes but with the larger doses they distressed the patient and sometimes alarmed the physician. In view of these findings the therapeutic dose of B.A.L. was limited to 2.5mg. to 3.0mg./kg.

Carleton, Peters, Stocken, Thompson and Williams (1946) and Longcope and Luetscher (1946), treating arsenical toxic reactions and mercuric chloride poisoning in man respectively, reported some or all of the above symptoms but considered that B.A.L. therapy at a dosage of 2.5mg./kg. was in the majority of patients free from toxic reactions and when they occurred they were mild and transient in most cases.

Summary.

B.A.L. is itself a toxic compound.

In animals B.A.L., in sublethal doses, produces an initial apathy accompanied by lachrymation, blepharospasm, oedema of the conjunctivae and increased salivation. Larger doses produce muscle tremors, inco-ordination with clonic and tonic convulsions proceeding to coma and death. The effect on the cardiovascular system is peripheral vascular collapse. Toxic doses of B.A.L. result in diminished H'ion concentration, diminished blood CO_2 content, accompanied by an increased serum lactic acid and animo nitrogen content. B.A.L. produces an initial rise of blood sugar, a reduction in liver potassium and liver glycogen with an increase in liver sodium and chloride. Oxidative processes in muscle tissue are inhibited. Apart from lethal doses, the toxic reactions of B.A.L. are in the main reversible and the substance is rapidly eliminated.

In man the therapeutic dose of B.A.L. in oil by intramuscular injection is 2.5-3.0mg./kg. With this dosage only local discomfort at the site of injection and with only transient subjective symptoms in a small number of patients is to be expected. At doses of 4mg. to 5mg./kg there is an increased incidence of toxic reactions, such as nausea, vomiting, headache; a burning sensation of the lips, mouth and throat, tongue and eyes; generalised muscular aches in the trunk and extremities with burning and tingling in the extremities; and a sense of constriction in the chest, with a feeling of anxiety. The systolic and diastolic blood pressure may be elevated. In the majority of cases the above reactions subsided in 30-90 minutes.

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5. The action of B.A.L. on Metals.

(a) In animals.

In addition to the importance of the discovery of B.A.L. as an effective antidote to lewisite, there arose the possibility of using the compound as a therapeutic agent for the treatment of manifestations of arsenical poisoning, of which the most serious are encephalopathy, dermatitis and hepatitis.

Following upon the investigations of the protective action of B.A.L. against arsenical poisons such as lewisite and phenyldichlorarsine by Harrison, Durlacher, Albrink, Ordway and Bunting (1946), the substance was tried against such therapeutic agents as Mapharside by Riker (1946). Riker, in the treatment of experimental arsenic poisoning in cats, showed that B.A.L. in peanut oil-benzyl benzoate mixture in suitable dosage, given intramuscularly, provided complete protection to cats acutely poisoned with mapharside.

Ginzler, Gilman, Philips, Allen and Koelle (1946) experimenting with rabbits showed that amelioration of the signs of acute cadmium intoxication, produced by giving cadmium chloride intravenously, was obtained by the administration of B.A.L. In this series of

experiments on cadmium poisoning, it is interesting to note that therapy with B.A.L. glucoside, (O-glucoside of B.A.L. introduced by Danielli, Mitchell, Owen and Shaw (1946). was more successful than therapy with B.A.L. and that the degree of renal damage in surviving rabbits was significantly less than had been previously observed with B.A.L. therapy.

Tobias, Lushbaugh, Patt, Postel, Swift and Gerard (1946) in experimental cadmium poisoning, produced in mice by inhalation of cadmium salts, showed that B.A.L. was therapeutically effective in cadmium poisoning if injected promptly after exposure. In an optional course of repeated injections the mortality was reduced from 93 to 7 per cent. In contrast with this result it was found that B.A.L. was deleterious if given prophylactically for cadmium poisoning produced by inhalation. Using the radioactive isotope Cd^{115} it was found that over half the inhaled cadmium chloride ($CdCl_2$) had passed beyond the lung after thirty minutes exposure. When B.A.L. was present at the time of the inhalation of cadmium, the cadmium was fixed in the lung, presumably as an insoluble cadmium-B.A.L. complex. This increased lung content of cadmium, due to its fixation in the lung by B.A.L., was associated with

increased lung damage. The authors considered that B.A.L., administered prophylactically, caused damage, by holding a large amount of cadmium for slow release, to lung tissue; while B.A.L. administered therapeutically, reaching the lung after most of the cadmium had left, diverted the cadmium already combined with lung tissue constituents and released it slowly enough so that most of the cadmium was removed. In the untreated animal, most of the cadmium passing from the lung eventually left the body via the gastro-intestinal tract. B.A.L. therapy was found to shift the route of excretion of the cadmium in the direction of elimination via the kidneys.

The efficacy of B.A.L. therapy, in the treatment of poisoning by compounds of antimony, bismuth, chromium, mercury and nickel on rabbits, was investigated by Braun, Lusky, and Calvery (1946). B.A.L. was found to be an effective antidote in acute poisoning, caused by the administration of toxic doses of salts of antimony, bismuth, chromium, nickel and mercury. B.A.L. was found to be ineffective in the treatment of rabbits acutely poisoned by salts of lead, thallium and selenium. In the case of lead and selenium, the action of B.A.L. was additive and in thallium poisoning B.A.L. had no effect whatever.

Gilman, Allen, Philips and St. John (1946), in the treatment of acute systemic mercury poisoning in rabbits, with B.A.L. and B.A.L. glucoside, showed that efficient therapy with these two compounds was effective in preventing mercury poisoning. B.A.L. was found to be a highly effective antidote to acute mercury poisoning in rabbits and dogs. In order to obtain complete or even partial protection against the poisonous effects of mercuric chloride it was found necessary to give B.A.L. shortly after the injection of the mercuric chloride (corrosive sublimate - $Hg.Cl_2$) solution. When the first intramuscular dose of B.A.L. was given, five minutes after the intravenous injection of mercuric chloride fatal to control animals, all the treated experimental animals survived. If a thirty minutes interval was allowed between the intravenous injection of mercuric chloride and the intramuscular dose of B.A.L., it was found that only approximately seventy-five per cent of the rabbits could be saved. In dogs B.A.L. was found to be an effective antidote to mercuric chloride, when given several hours after the oral administration of lethal doses of the metallic salt.

(b) In Man.

The therapeutic applications of B.A.L. are now being actively pursued. It was early realised that B.A.L.

offered a means of combating the toxic effects of arsenical drugs used for therapeutic purposes, such as encephalopathy, dermatitis and possibly hepatitis. These aspects developed much more rapidly in the United States, where the success of B.A.L. by inunction in cases of arsenical dermatitis was early proved.

Chiesman (1944) used B.A.L. ointment successfully, for the treatment of accidental arsenical contamination in factories. The method of inunction therapy with B.A.L. was soon superseded by the manufacture in the United States of ampoules of B.A.L. in ten per cent benzyl benzoate in peanut oil (arachis oil). This oil preparation of B.A.L. was found to be suitable for intramuscular injection and this method of administering B.A.L. has now been used extensively in the United States and Britain.

In an interim report to the medical Research Council Carleton, Peters, Stocken, Thompson and Williams, (1946), in a series of thirty cases of arsenical dermatitis, showed that intramuscular B.A.L. therapy was much more successful than any other means of treatment. The urinary excretion of arsenic was found to be increased during treatment.

Wexler, Eagle, Tatum, Magnuson and Watson (1946), showed that a single intramuscular injection of B.A.L.

(3.5Mg./kg. given as a ten per cent solution in peanut oil and benzyl benzoate), given to human volunteers exposed to controlled concentrations of arsenical smoke was followed by a significant and regular increase in the rate of urinary arsenic excretion.

Eagle (1946) and Eagle and Magnuson (1946) carried out the systemic treatment of arsenic poisoning with B.A.L. in 1943. A dosage of 2.5mg. to 3mg./kg. made up as a ten per cent solution in peanut oil-benzyl benzoate vehicle for intramuscular injection was used as a routine. Injections were given every four hours for the first two days, four injections on the third day and twice daily thereafter for ten days or until complete recovery. In fifty-five cases of "haemorrhagic encephalitis" caused by intensive arsenotherapy, forty cases were in coma or convulsing at the initiation of B.A.L. therapy. Forty-four cases recovered within a week and the remaining eleven cases died. These results suggested that early and adequate B.A.L. therapy could reduce the mortality in toxic arsenical encephalopathy. In eight-eight cases of arsenical dermatitis, fifty-one being typical exfoliative cases, B.A.L. intramuscular therapy stopped the progression of the inflammatory reaction and accelerated the healing process. The only serious

complications of intramuscular B.A.L. therapy occurred in this series of cases and consisted of five cases which developed cellulitis or gluteal abscesses at the site of injection. This complication did not develop with the other types of arsenical poisoning and was probably due to infection on the surface carried into the deeper tissues with the needle. In ten out of eleven cases of arsenical agranulocytosis, B.A.L. therapy was followed by an increase in the total number of white blood cells, especially the polymorphonuclear leucocytes. The eleventh patient had a reduced platelet count and died on the fourth day. In three cases of aplastic anaemia B.A.L. therapy had no effect. In fourteen cases of so-called post-arsenical jaundice it appeared that B.A.L. therapy could favourably affect the course of the disease in a definite but small proportion of the cases. In four cases of massive overdosage of mapharside, given in error, early and adequate B.A.L. therapy saved the lives of three of the patients. In forty-four patients who developed "arsenical fever" prompt recovery occurred after the administration of B.A.L. but the therapeutic evaluation of this group was difficult as most of these cases would have recovered without B.A.L. therapy.

Csonka and Graham (1947) had a similar experience

with small numbers of cases of arsenical poisoning in 1943-44. The patients in question were undergoing an intensive 30 day course of mapharside medication for early syphilis. Administration of B.A.L. in oil intramuscularly and cessation of arsenic therapy brought about speedy recovery in all cases.

Longcope and Luetscher (1946) tested the efficacy of B.A.L. therapy in the treatment of acute mercury poisoning. Twenty-three patients, admitted to John Hopkins Hospital with a history of having swallowed from 0.5g. to 20g. of mercuric chloride, were treated with intramuscular B.A.L. therapy. Eight of the patients, who had swallowed less than 0.5g., promptly recovered. Of six patients, who swallowed 1.0g., one died and he had been initially treated with small doses of B.A.L. The remaining patients, who had swallowed doses between 1.5g. to 20g., all recovered with adequate treatment.

Cohen, Goldman, and Dubbs (1947) successfully treated five cases of acute poisoning due to gold with intramuscular injections of B.A.L. As the authors state the number of cases is too small to justify definite conclusions, but the prompt clinical effects were impressive and were considered sufficient to warrant the more extended use of B.A.L. in the relief of gold

intoxications. In their series of cases transient symptoms of B.A.L. toxicity were observed, including a sense of warmth in the mouth, salivation, flushing of the face, injection of the conjunctivae with lachrymation and limb pains.

Ragan and Boots (1947) treated five cases of dermatitis produced by gold therapy in the treatment of rheumatoid arthritis. In all five cases there was a significant excretion of gold in the urine following the administration of intramuscular B.A.L. In four patients the dermatitis had been present less than two months and the rash and pruritus markedly cleared up with B.A.L. therapy. In the remaining patient the rash had been present for three months before therapy with B.A.L. was instituted; the rash and pruritus continued despite treatment. During B.A.L. therapy twenty-four hour urine specimens were collected and the concentration of gold present determined. All five patients showed a definite increase in the excretion of gold in the urine during the course of B.A.L. therapy. As the authors suggest, since it is known that B.A.L. reactivates sulphydryl containing enzyme systems poisoned by heavy metals, the relief of pruritus and dermatitis by B.A.L. would seem to indicate that the rash and pruritus following gold therapy are due to the toxic

effect of the metal on some enzyme system containing a sulphydryl group. It was noted that the rapid elimination of gold, brought about by B.A.L. was followed by the recurrence of arthritic symptoms and the authors considered that the therapeutic effect of gold is due to a temporary suppression of the processes which cause activity in rheumatoid arthritis.

Lockie, Norcross, and George (1947) treated two patients with serious toxic reactions to gold therapy, one with thrombopenic purpura and the other with granulocytopenia. The patient with purpura did not respond to liver extract, ascorbic acid, blood transfusion vitamin K therapy, and folic acid. The platelet count remained low. Within twenty-four hours of the first dose of B.A.L. the patient was conscious and his spectacular improvement was continued. Ten per cent B.A.L. in oil intramuscularly was given four hourly for forty-eight hours and then twice daily for ten days. The platelet count rose from about 15,000 to 120,000. During the course of treatment, inflammation of the right buttock developed, but subsided in forty-eight hours with conservative treatment. The patient with granulocytopenia was treated with B.A.L. therapy when the blood smear showed only three granular cells present.

B.A.L.-in oil was given intramuscularly every six hours for forty-eight hours and then twice daily for eight days. After three days the patient felt much improved and the granulocytes increased in number daily. The differential white cell count became normal. During the course of therapy an abscess formed in the left buttock at the site of injection, which necessitated incision and drainage.

Davison (1947) treated three cases of gold dermatitis, arising in patients under treatment with gold for rheumatoid arthritis, with B.A.L. and claimed that the speedy disappearance of the marked dermatitis in each patient was due to intramuscular B.A.L. therapy. The author does not state whether any toxic reactions developed due to the B.A.L. at the site of injections.

Summary.

In animals B.A.L. was an effective antidote to poisoning by arsenic, cadmium, antimony, bismuth, chromium, mercury and nickel. B.A.L. was found to be ineffective in the treatment of poisoning produced by lead, thallium and selenium.

In man B.A.L. has been successfully used in the treatment of toxic reactions due to arsenic viz. dermatitis and encephalopathy. The urinary excretion of arsenic was found to be increased during treatment. Overdosage of arsenic was successfully treated with B.A.L. The drug was successfully used in the treatment of acute mercury poisoning. The drug has been reported to be successful in the treatment of toxic reactions due to therapy with gold salts in cases of rheumatoid arthritis. The schedule of treatment was found to be intramuscular injections of B.A.L. in peanut oil-benzyl benzoate in doses of 2.5mg. to 3.0mg./kg. every four hours for the first two days, four injections on the third day and twice daily thereafter for 10 days or until complete recovery. A small proportion of cases developed cellulitis or gluteal abscesses at the site of injection.

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6. B.A.L. Glucoside.

Cameron, Mitchell and Danielli (1942), showed that in systemic lewisite poisoning a shock-like condition was produced during the first few days and in some particulars this bore resemblance to traumatic shock, being marked by haemoconcentration. The administration of plasma or other fluids was of no value in reducing the mortality produced by a given dose of lewisite.

Danielli, Danielli, Mitchell, Owen and Shaw (1946) carried out research, during the war years, for the developement of a chemotherapy for systemic arsenical poisoning and decided that the first step in the treatment of lewisite poisoning would be the inactivation of at least that part of the arsenic causing shock. They found that the capillary damage due to the lewisite was widely distributed throughout the body, with the greatest loss of protein from the circulation occurring in the viscera and skeletal muscles. Based on this widespread capillary damage, it was postulated that the required antidote would need to possess the following properties: be capable of reaching the whole vascular system; be relatively non-toxic; prevent arsenicals from penetrating from the blood stream into the tissue

cells; be capable of removing arsenic from cells into which it had penetrated, and finally the product of reaction between the drug and the arsenical should be readily excreted.

Voegtlin (1923), had shown that glutathione protected tissue cells against the action of arsenicals and Peters, Stocken and Thompson (1945) had shown in 1940 that 1:2 dithiols had a protective action against lewisite due to their sulphydryl (-SH) groups. B.A.L. was considered by Danielli et al (1946) to be too toxic when given in large doses and did not fulfil the above desiderata. Hence these workers sought a non-toxic thiol which would detoxicate any arsenic in the blood stream and prevent its passage into the tissue cells and at the same time trap the intracellular arsenic passing into the blood. A number of thiols with the required properties were synthesised and of these O-glucoside of B.A.L. was discovered. It was given the name of B.A.L. Intrav. (B.A.L. glucoside) and of all the compounds prepared it answered best to the required properties. The L.D.50 of B.A.L. glucoside for rats was found to be 7.5g./kg. while the L.D.50 of B.A.L. for rats was found to be 50mg./kg. i.e. B.A.L. glucoside was 150 times less toxic to rats than B.A.L. At doses of 1-2g./kg. B.A.L. glucoside caused no pathological symptoms in rats, rabbits,

guinea pigs and goats and was found to be protective against lewisite poisoning in these animals. When treatment of the arsenic poisoning was delayed for six and a half hours the mortality from lewisite poisoning was fifty per cent and it was considered that this was due to the slow removal of the arsenic from the tissues by the B.A.L. glucoside. By giving B.A.L. in conjunction with the B.A.L. glucoside it was found that B.A.L. acted as a carrier of arsenic between the cell and the B.A.L. glucoside in the blood and the mortality was reduced.

McCance and Widdowson (1946) showed that 100mg./kg. of B.A.L. glucoside given intravenously to male volunteers produced no ill-effects. This result is in striking contrast to intramuscular B.A.L., which when given in single doses of 3.0mg. to 5.0mg/kg. produces toxic reactions previously discussed.

Further interesting observations by McCance and Widdowson (1946) showed that the urinary excretion of copper and zinc was increased in six male volunteers after the administration of B.A.L. glucoside. The drug increased the excretion of copper about twenty times, zinc about five times, and had no consistent effect on the urinary excretion of iron. With regard to urinary

excretion McDonald (1946) showed that B.A.L. glucoside increased the urinary output of copper in sheep.

Summary.

B.A.L. glucoside (B.A.L. Intrav.) is 150 times less, toxic to rats than B.A.L. It is an antidote to arsenic poisoning. B.A.L. glucoside in a dose of 100mg./kg. given intravenously to male volunteers produced no ill-effects. This is in striking contrast to intramascular B.A.L., which when given in single doses of 5mg./kg. produces toxic reactions.

Cameron. G.R., Mitchell. P.D., and Danielli J.F.,
quoted by Danielli et al (1946) Nature 157, 217

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Nature 156, 616

SECTION TWO.

I. The object of the present investigations.

It was decided to investigate the effect of B.A.L. on the toxicity of heavy metals deemed to be of importance from a clinical and industrial viewpoint, and to this end the effect of B.A.L. on the toxicity of mapharside, mercuric chloride, mersalyl, tartar emetic, lead acetate, sodium bismuth tartrate, sodium aurothiomalate and chromium trioxide was investigated in mice.

Mapharside (meta-amino-para-hydroxy-phenylarsine-oxide hydrochloride) was chosen to represent the toxic effects of arsenic and on account of its wide use as the arsenical for intensive arsenotherapy, during which arsenical toxic reactions such as encephalopathy and dermatitis, are more liable to occur. Mercuric chloride ($HgCl_2$) or corrosive sublimate was taken to represent mercury due to its solubility, stability and known lethal effects. Antimony was represented by tartar emetic because of its solubility and greater stability than most other antimony compounds, and as representative of antimonial preparations used in the treatment of tropical diseases, such as leishmaniasis, filariasis and schistosomiasis, which treatment can give rise to toxic

effects from antimony. Lead is a well known industrial hazard, particularly in ship-breaking using the oxy-acteylene burner, and it was most important to test the effects of B.A.L. on such a metal. Lead acetate was chosen for its solubility and stability in preference to other basic and insoluble lead salts. Bismuth enjoys a recognised place in the treatment of disease and in particular as adjunct metallo-therapy in syphilis. Bismuth can give rise to toxic reactions and since it is widely used, a knowledge of the effect of B.A.L. on its toxicity would be clinically useful To evaluate B.A.L. in the treatment of acute bismuth poisoning, a water-soluble salt, sodium bismuthyl tartrate was used. The absorption of soluble bismuth salts occurs more rapidly and consequently they are the most toxic group of bismuth salts used in therapeutics. By many authorities gold therapy is considered to have a useful place in the treatment of rheumatoid arthritis. The risk of toxic reactions are well known and a serious drawback to therapy with gold salts. In view of the possible developement of toxic gold reactions such as exfoliative dermatitis, toxic nephritis, toxic hepatitis, purpura, and agranulocytosis it was decided to test the action of B.A.L.

on the toxicity of sodium aurothiomalate - a gold compound much used in the chrysotherapy of rheumatoid arthritis under the trade name of "myocrisin". Chromium compounds have been long known as an industrial hazard in the mining of ores containing chromium as well as in those branches of industry where chromates and dichromates are used in dyeing, electrotyping and plating. The effect of B.A.L. on the toxicity of chromium was investigated with the object of determining the possible use of B.A.L. as an effective therapeutic agent for chrome ulceration of the skin and nasal mucous membrane and chrome dermatitis due to chrome compounds. The preparation of chromium used was chromium trioxide because of its solubility and well known nephrotoxic action.

"Mersalyl" B.P. was taken as representative of the many mercurial diuretics now used in clinical medicine and B.A.L. was used as a possible antidote to the acute toxic action which these organic mercurial diuretics can produce. The effect of B.A.L. and "mersalyl" on the diuresis of normal rats was investigated.

The induction of chronic mercury poisoning was attempted in rabbits and guinea pigs and the effect of B.A.L. on such poisoning observed. Chronic arsenical poisoning was attempted in guinea pigs and the effect of B.A.L. noted. The effect was observed in the tissues

of the animals by fixing the tissues in picro-formol and routinely staining with haematoxylin and eosin.

As a preliminary to the above investigation the toxicity of B.A.L. itself was investigated in rats, mice, guinea pigs, rabbits and cats. The effect of B.A.L. on the blood pressure, respiration, heart, spleen volume, leg volume was observed in rabbits and cats. Isolated rabbit auricles, isolated perfused cat hearts, and isolated strips of gut and uterus from the rabbit, cat and guinea pig were examined in the usual way.

Finally the effect of B.A.L. on the physiological action of insulin in rabbits and the effect of B.A.L. upon necrosis of the liver caused by carbon tetrachloride in rats were examined.

II. METHODS.

1. The Toxicity of B.A.L.

The toxic effects of B.A.L. were investigated by injecting freshly prepared watery solutions of the drug by intraperitoneal, intravenous, intramuscular and subcutaneous injections into rabbits, rats, guinea pigs and mice.

Rabbits, anaesthetised with 25% urethane given intravenously and cats, anaesthetised with ether and chloralose 80mg./kg. intravenously were used to determine the actions of B.A.L. on the carotid blood pressure, heart (myographic records), spleen volume (plethysmograph and piston recorder), leg volume (plethysmograph and tambour), and respiration (stethographic lever). Blood pressure readings were recorded from the carotid artery with mercury manometer on a smoked drum. The respirations were recorded by the lever connected to the chest of the animals.

Isolated rabbit auricles, isolated perfused cat hearts (Langendorff preparation) and isolated strips of gut and uterus from rabbit (Magnus preparation), cat and guinea pig were perfused with B.A.L. in the usual way.

The isolated rabbit auricles were prepared in the following manner. If a rabbit is stunned, bled and the heart excised and put in cold Tyrode solution, the heart continues to beat. If the auricles are carefully separated with clean scissor cuts from the ventricle, great vessels, and all adventitious tissue they may be mounted in oxygenated Locke solution at 28°C., and will beat regularly. A record may be obtained on the kymograph by attaching a lever to one end of the preparation. Drugs in solution may be put in the vessel containing the preparation and the effects of the drugs on the rate, force and rhythm of the auricles recorded.

In the Langendorff preparation the whole heart is perfused with filtered oxygenated Locke solution at 37°C., and constant pressure, via a cannula tied in the aorta and directed towards the heart. Fluid circulates via the coronary arteries, the output of which can be measured as a minute volume. Drugs may be injected in solution above the cannula or put in the reservoir of perfusing fluid.

The vessels of the rabbit's ear were perfused according to the method of Gaddum and Kwiatkowski (1938), but as no recording apparatus was available the outflow was measured in drops per 15 seconds and hence larger doses of the drugs were used than otherwise necessary to

show clear effect. A rabbit with large ears was anaesthetised with ether and the common carotid artery was dissected out. The internal carotid artery was tied just above the carotid sinus and all the arteries cephalad to this point, except the artery to the ear were tied and divided. The artery to the ear runs laterally from the carotid at about the level of the superior cervical ganglion and was left undisturbed. The arterial cannula was tied in the carotid artery and perfusion with Locke's solution was started. The arterial cannula has two arms for perfusing different fluids and the cannula is largely filled with air, which separates the fluid coming from the reservoir, from the fluid in the mouth of the cannula. Injections of the drug were made through the rubber cap in the cannula.

As soon as the perfusion started, a piece of thin glass tubing was tied in the great auricular vein to act as a venous cannula. The head of the rabbit was then removed from the body after a strong ligature had been tied round the neck. Transfusion fluid and blood were allowed to drain from the vertebral region and when no more blood appeared the vertebral canal was blocked with plasticine and the outflowing fluid soon became clear. The outflow from the great auricular vein of the rabbit's ear was measured in drops per 15 seconds.

The method of investigating the effects of B.A.L. on the diuretic activity of mersalyl was that described by Burn (1937), for the assay of the antidiuretic potency of extracts of the posterior pituitary body. A group of sixteen male rats of about the same weight and not less than 140g. and not more than 240g. were chosen. They were kept with no food from the evening before the experiment began. Each rat was given 5ml. water per 100g. wt. by stomach tube, and saline or solution of drug subcutaneously. After the injection of saline the rats were placed in circular metal cages with a wire floor of large mesh, the cages resting on glass funnels which conducted the urine to a measuring cylinder below. Four rats were placed in one cage so that the rats injected with the saline occupied two cages and those injected with mersalyl occupied two more cages: 10ml. graduated measures collected the urine from each cage and were emptied as required into larger measures.

The time taken for the administration of water by stomach tube and saline by injection to a group of four rats was noted and the mid-point of this period was taken as the starting time of the group. This time point was determined for each group. The time of the first excretion of urine from each group was noted and recorded

every 15 minutes, until the excretion for the last four periods was constantly small. The time from the point of watering the group to the time of maximum excretion was calculated from the readings and gave a measure of the rate of diuresis. Three days later the process was repeated with the groups reversed so that those previously given saline subcutaneously were now given mersalyl subcutaneously and vice versa. The results were combined so that the diuresis caused by 5.0ml. water given by mouth in 16 rats had been measured, and the effect on the diuresis of mersalyl subcutaneously.

The experiment was repeated a week later with the same rats and the two sets of eight received respectively, subcutaneous and intraperitoneal saline and subcutaneous mersalyl and intraperitoneal B.A.L. The intraperitoneal saline given to the first group was equal in volume to the volume of B.A.L. given intraperitoneally. The time to the maximum rate of excretion of urine was again calculated from the readings of urine excreted at 15 minute intervals as above. The results of the two complete experiments thus gave an indication of the effect of mersalyl on water-induced diuresis (32 rats) in rats and the action of B.A.L. on this effect (16 rats)

2. The action of B.A.L. on the Toxicity of Heavy Metals.

The L.D.50 of the following salts of heavy metals - mapharside, mercuric chloride, tartar emetic, lead acetate, sodium bismuth tartrate, sodium aurothiomalate and chromium trioxide - was roughly determined by intraperitoneal injection in small groups of mice. After preliminary intraperitoneal injection of graded doses of each salt, in watery solution, to small groups of white mice had given an indication of the range of toxicity of the salt, a precise estimate was made as follows. 160 white mice weighing between 20 and 30g. in weight were taken in groups of 40 and given graded doses of the solution of the metal salt intraperitoneally, so as to cover an adequate range of toxicity. To half of each group of 40 mice, B.A.L. was given in a freshly prepared aqueous solution in a standard dose of 40mg/kg. intraperitoneally and the mortalities were recorded after twenty-four hours. The B.A.L. and metal solutions were so prepared that 0.1ml. of the solution was equivalent to 10g. by weight of mouse and hence the intraperitoneal injections were of small volume and constant volume/kg. wt

Where the detoxifying action of B.A.L. was such as to cause all the mice so protected to survive, the injections were repeated using higher doses of the metallic salt in those animals receiving protection with B.A.L. All the injections for one metal were completed at a single session. The animals were kept under standard conditions of warmth with food and water ad lib.

A graph was prepared plotting log. dose against probit of lethality after the method of Bliss (1936) from the "Statistical Tables for Biological, Argricultural and Medical Research Workers" by Fisher and Yates (1943). The L.D.50 was read from this graph by interpolation at the mid point of lethality.

3. Chronic poisoning with Mapharside and Mercuric Chloride.

Twenty-four guinea pigs, of 350-400g. weight were given mapharside 15mg./kg. subcutaneously every forty-eight hours for ten days. Half of the guinea pigs were given 40mg./kg. of B.A.L. intraperitoneally at the same time. Eight of the guinea pigs, receiving mapharside alone, developed symptoms of restlessness, twitching, anorexia, diarrhoea, wasting and weakness on the fifth day and were dead by the tenth day. The remaining four guinea pigs, receiving mapharside alone, suffered from the

above symptoms to a lesser degree and were killed on the tenth day. The twelve guinea pigs receiving B.A.L. remained well and active and were sacrificed on the tenth day. From both sets of animals fresh specimens of liver, kidney, spleen and gut were sectioned, stained with H and E and examined microscopically.

Chronic mercurial poisoning was induced in twelve rabbits by giving them a solution of mercuric chloride (corrosive sublimate) intraperitoneally daily for ten days. To half the above rabbits B.A.L. was administered in a dose of 2.5mg/kg. intraperitoneally. The rabbits receiving mercuric chloride alone lost weight, developed diarrhoea, tremors and died between the twelfth and sixteenth day. The rabbits receiving both mercuric chloride and B.A.L. survived in good health until sacrificed. From both sets of animals fresh sections were made of liver, kidneys, and gut, stained with H and E and examined for histological changes.

Chronic mercuric chloride poisoning was produced in eight guinea pigs by giving them 5.0mg./kg. of mercuric chloride in solution intraperitoneally daily. Eight guinea pigs were given the same dose of mercuric chloride daily, with the addition of 40mg./kg. of B.A.L. intraperitoneally. The guinea pigs, receiving mercuric

chloride alone, were dead by the fourth or fifth day. The animals receiving B.A.L. survived in good health until sacrificed. Fresh sections of liver, kidney, and gut were taken and stained sections prepared for histological examination.

4. The production of chrome ulceration in guinea pigs.

Twelve guinea pigs were shaved over their hind quarters and 0.25ml. of 5% chromium trioxide solution was injected intracutaneously into the right and left shaved areas to raise a wheal in each part. After an interval of five days, well established chrome ulcers were produced around the site of injection, with a black eschar of approximately half an inch to three-quarters of an inch in diameter, surrounded by a raised red areola for another quarter of an inch. By this time, the animals had recovered from the constitutional upset resulting from the absorption of some of the chrome solution. The animals were then divided into two groups. Half were treated with daily applications of acriflavine ointment B.P.; the other half were treated similarly with acriflavine ointment B.P. containing 10% by weight of B.A.L. The activity of the B.A.L. was maintained during the experiment by storing it between applications

in the refrigerator and its pungent odour was maintained to the end of the experiment. The chrome ulcers were measured at intervals and the clinical signs of healing were noted during the course of the experiment.

5. The effect of B.A.L. on the action of Insulin.

A group of five rabbits, each of about 2.0kg. in weight were starved overnight and next day at the start of the experiment a blood sample of 0.5ml. was drawn from the marginal ear vein of each rabbit and pooled in a heparinised tube. The blood sugar level was estimated by the method of Hagedorn and Jensen. The animals were now given 0.5 unit/kg. of soluble insulin subcutaneously and pooled blood samples were collected every hour for five hours and the blood sugar level estimated in each pooled sample of blood.

After an interval of three days, the experiment was repeated with the same five rabbits, with the difference that they were each given 25mg./kg. B.A.L. intraperitoneally in saline, immediately after the injection of 0.5unit/kg. of soluble insulin. The fasting blood sugar levels were determined by the method of Hagedorn and Jensen from the pooled blood samples as previously.

The two curves were plotted for comparison.

6. Carbon Tetrachloride poisoning in Rats.

Twelve male white rats weighing between 140g. and 180g. were divided into two groups of six. To one group, on alternate days, 0.5ml./kg. of carbon tetrachloride was given by intraperitoneal injection. To the other group, on the same alternate days, 0.5ml./kg. of carbon tetrachloride and 10mg./kg. of B.A.L. in saline were given consecutively by intraperitoneal injection. After five injections both groups of rats were in poor condition and were sacrificed. Sections of the liver, spleen and intestine were immediately prepared from each group, stained with H. and E. and examined microscopically.

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III. Results.

1. Properties of B.A.L.

(a) Toxic symptoms.

The L.D.50 for B.A.L. dissolved in water was found to be 100mg./kg. for white mice when injected intraperitoneally. This result agrees well with the results of Durlacher, Bunting, Harrison, Ordway and Albrink (1946), who found that the L.D.50 for mice injected intraperitoneally with B.A.L. was 0.8mM/Kg. (99.2mg./kg.).

Three guinea pigs, given 150mg./kg. of B.A.L. in water by the intraperitoneal route died, while three given 50mg./kg. of B.A.L. in water survived.

Twelve white rats survived, after receiving 40mg./kg. of B.A.L. in water by the intraperitoneal route and of six rabbits given 100mg./kg. of B.A.L. in water by the intravenous route two died.

In mice a lethal dose of B.A.L. given by the intraperitoneal route caused immediate weakness of the legs. The leg muscles were flaccid and ataxia was marked. At this early stage analgesia was marked, severe nipping of the tail producing no response. Lachrymation, blepharospasm, and oedema of the conjunctivae were noted. The animals showed increasing loss of co-ordination. Clonic and tonic convulsions followed, interrupted by periods of coma.

Respiration increased in depth and rate, increasing coma alternating with convulsions preceded death, which was accompanied by the signs of asphyxia. Sublethal doses of B.A.L. given to mice were followed by an initial period of apathy, which was accompanied by lachrymation, blepharospasm and conjunctival oedema. With small doses, these symptoms disappeared leaving no residual signs of toxicity. With larger sublethal doses, apathy, lachrymation, blepharospasm and conjunctival oedema preceded muscle tremors and weakness of the legs, producing well marked ataxia but no convulsions.

In rabbits and guinea pigs small doses of B.A.L. (20mg./kg. intraperitoneally) were followed by a brief period of depression and apathy, accompanied by blepharospasm and sneezing, which developed in fifteen minutes. This was followed by weakness and spasticity of the legs, and muscular tremor and ataxia which was well developed in thirty to sixty minutes. Both rabbits and guinea pigs showed increased salivation and passed urine and faeces frequently. The respirations were observed to be deep and hurried. With lethal doses of B.A.L. given intraperitoneally, there was a phase of apathy and depression, accompanied by blepharospasm and lachrymation with weakness of the leg muscles. This was followed by the development of muscular tremors which

gradually increased to generalised convulsions. Respiration was markedly impaired and death occurred in tonic convulsion. The animals offered no resistance to painful stimuli.

In the twelve rats, the intraperitoneal injection of B.A.L. in a dose of 40mg./kg. was followed by apathy and depression, accompanied by lachrymation and blepharospasm. The gait became ataxic, due to the weakness of the leg muscles and the animals were able to take up abnormal postures, such as lying flat with the head extended and the hind limbs also extended. There was a marked response to painful stimuli and all the rats were awkward, apathetic and showed no initiative. Convulsions did not develop and there were no deaths. The effects of the B.A.L. injection lasted some twenty-four hours, when it was observed that there was still some apathy, muscle weakness and loss of appetite.

Post mortem examination of the internal organs of the animals, dying as a result of lethal doses of B.A.L., was carried out immediately after death. The liver, spleen, kidneys and gut showed no conspicuous morphological changes, apart from congestion. The lungs were oedematous and the surface showed many small haemorrhages. On section, pulmonary oedema was evident with haemorrhagic exudate, and scattered throughout the lungs were small haemorrhages.

Fig. 1. Rabbit, ♂, 2.4kg. wt. Urethane 25% intravenous ether.

Upper record: carotid blood pressure

Lower record: respiration (stethograph, inspiration down).

Time in 30 secs.

B.A.L. 4mg./kg. intravenously causes a rise in blood pressure and stimulation of respiration.

(b) Cardiovascular and Respiratory Effects.

Anaesthetised rabbits were given various doses of B.A.L., dissolved in physiological saline solution, intravenously by the usual method. In a dosage of 0.5-1.0mg./kg. B.A.L. produced no appreciable effect on the blood pressure or respiration, whereas a dosage of 4.0mg./kg. caused a sharp rise in blood pressure of some 20-30mm.Hg., accompanied by a stimulation of respiration which was maintained for several minutes. This stimulating effect of small doses of B.A.L. on the cardiovascular and respiratory systems in the rabbit is illustrated in figure I.

Larger doses of B.A.L., 20-40mg./kg., caused a transient rise in blood pressure and stimulation of the respiration, which was followed by a steady decline in the blood pressure and failure of breathing. When the lethal dose was approached, the course of the steady decline of blood pressure and failure of respiration might be interrupted by the animal convulsing on the table, but more commonly the blood pressure declined to zero and the animal died quietly, after a few final respiratory gasps.

The effect of lethal doses, as detailed above, was in striking contrast to the mode of death of the

unanaesthetised animal, which convulsed violently. The lethal dose was no smaller in the unanaesthetised animal nor was the toxic effect of B.A.L. on the cardiovascular and respiratory systems modified by atropine sulphate, vagotomy or by the application of artificial respiration.

Even in the presence of high concentrations of B.A.L. (1 in 10,000) no change was noted in the action of isolated rabbit auricles and the auricles continued to beat regularly and strongly, thus indicating that B.A.L. had apparently little or no direct effect on heart muscle.

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 every fifteen seconds, using larger doses of drugs than would have otherwise been necessary. Single injections of 3mg. of B.A.L. dissolved in physiological saline solution caused a sharp but transient vasoconstriction. Constant perfusion with B.A.L. in the strength of 1 in 10,000 produced a reduction in the rate of flow of the perfusing fluid through the vessels of the ear of the rabbit. Hence the addition of B.A.L. to the perfusing fluid caused vasoconstriction of

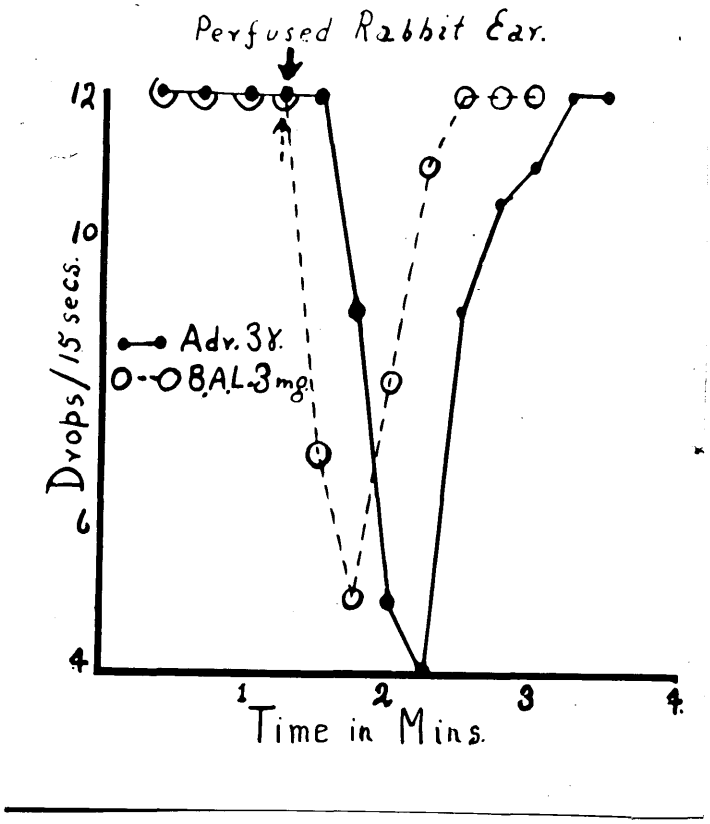


Fig. 2. Rabbit's ear perfusion. Perfusion with 1 in 10,000 solution of B.A.L. produces a reduction in the rate of flow of the perfusing fluid through the vessels of the ear of the rabbit and hence vasoconstriction.

the vessels of the rabbit's ear. This vasoconstriction effect of B.A.L. on small blood vessels is illustrated in figure 2, and explains the rise in blood pressure seen in man after intramuscular B.A.L. in oil 5mg./kg.

In cats anaesthetised with ether, chloralose or nembutal, intravenous B.A.L., dissolved in physiological saline solution, invariably caused an abrupt fall in blood pressure, accompanied by transient shrinkage of the spleen. When doses of 20mg./kg. of B.A.L. were given there was an abrupt fall of blood pressure, which was quickly restored, but in the majority of observations this restoration was only partial, though sometimes the blood pressure rose above the initial level for a few minutes. The abrupt fall in blood pressure was accompanied usually by passive shrinkage of the spleen. Sometimes active dilatation of the spleen was recorded. The phase of recovery, whether partial or complete, was always accompanied by active dilatation of the spleen; thus splanchnic dilatation may account for the initial fall in blood pressure after intravenous injection of B.A.L. When B.A.L. was given, the leg volume (i.e. vessels) constricted, while the heart was unchanged in vigour and amplitude of its beat. From these results it would appear that splanchnic dilatation could account for the fall of

blood pressure in the early stages, while the relative degree of alteration in the blood flow in the splanchnic and limb circulations would account for the varying responses of the blood pressure. Respiration was stimulated. Within a few minutes, however, a progressive fall in blood pressure, accompanied by an inhibition of the respiration ensued and this blood pressure fall and respiratory inhibition steadily progressed. Neither the abrupt nor the progressive fall in blood pressure was prevented by administering atropine sulphate in a dose of 1.5mg./kg., by bilateral section of the vagal nerves or by artificial respiration. From these observations there can be no question of vagal inhibition playing a part in the initial fall of blood pressure nor the failure of oxygenation of the blood playing a part in the later fall in the blood pressure. After a few minutes, when the blood pressure was steadily falling, the spleen contracted maximally and the blood pressure at this stage might be temporarily restored by the injection of adrenaline or by the infusion of saline. This contraction of the spleen is considered to be a physiological response to diminished blood volume rather than a direct pharmacological action of the B.A.L. on spleen tissue. The restorative effect of saline transfusion soon passed and the blood pressure

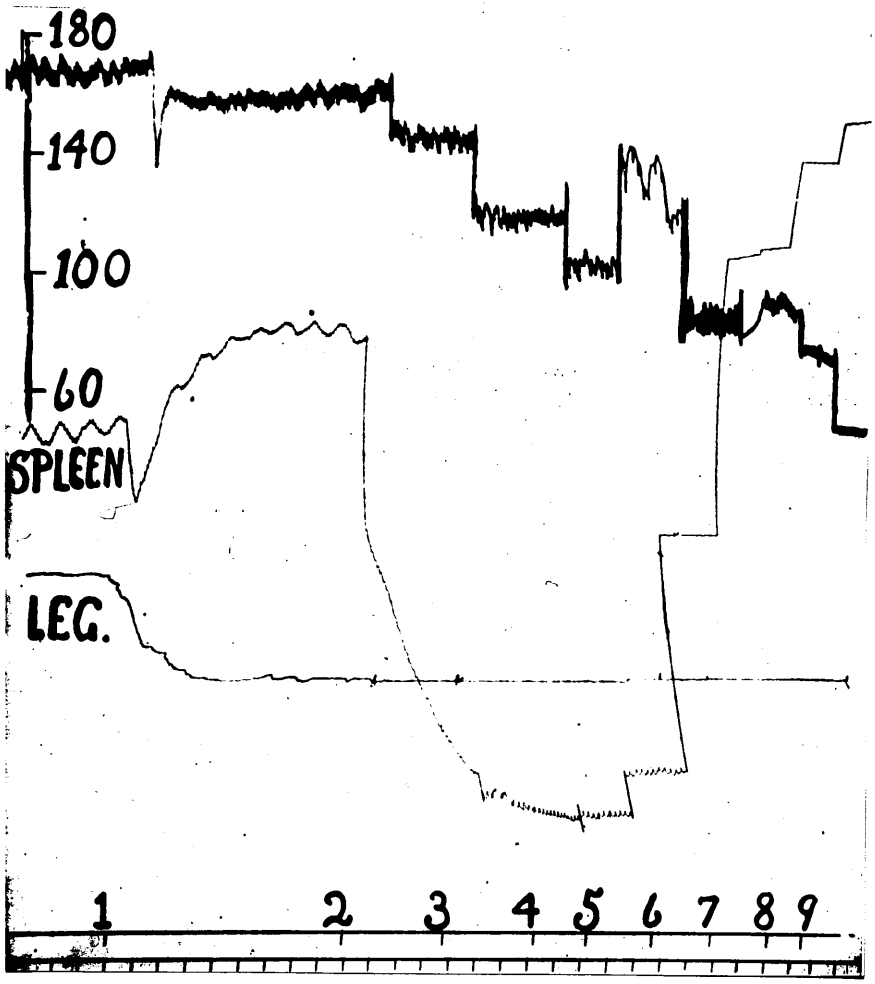


Fig. 3. Cat, ♂, 3kg. wt. Ether and Chloralose 80mg./kg.
Upper record: carotid blood pressure
Middle record: splenic volume (plethysmograph-tambour). Injection points and time in 30 secs. are marked.
At 1: B.A.L. 40mg./kg. was given intravenously at 11.43a.m. and 20mg./kg. repeated at 12.0a.m., 1.56p.m. and 2.10p.m. (total 100mg./kg.).
1 = 11.43a.m.; 2 = 11.50a.m.; 3 = 11.54a.m.;
4 = 12.06p.m.; 5 = 12.24p.m.; 6 = 1.45p.m.;
7 = 1.53p.m.; 8 = 2.10p.m.; 9 = 2.20p.m.

Fig. 4. Cat, ♂, 3kg. Chloralose 80mg./kg.
Upper record: carotid blood pressure.
Lower record: respiration.
Time 30 secs.
B.A.L. 80mg./kg. intravenously injected at arrow.

1. Fall and rise of blood pressure, then decline to extinction.
2. Stimulation and then slow failure of respiration. Circulation fails before respiration.

progressively fell, the spleen dilated once more (a terminal effect), while the respiration became slow and gasping. The leg volume, meanwhile, remained reduced and with sufficiently large doses death occurred, despite artificial respiration. These changes are illustrated in figures 3 and 4.

The heart continued to act well, when oxygenation was maintained by artificial respiration, and only finally failed 5-10 minutes after the blood pressure had reached zero. The isolated perfused heart of the cat (Langendorff preparation) showed no ill-effects from the injection of up to 5.0mg. of B.A.L. and the rate of coronary flow was unchanged. This is illustrated in figures 5 and 6.

The progressive fall in the blood pressure with an active heart, in the presence of peripheral vaso-constriction would suggest a steady leakage of fluid from the circulation. The petechial haemorrhages, seen in the lungs and liver on post-mortem examination, indicated damage to capillaries and small blood vessels. The mean packed cell volume was determined in five anaesthetised cats and found to be 36.6 per cent (this is less than the normal clinical finding in man but the blood was spun in Wintrobe tubes at 5,000 revolutions for twenty-

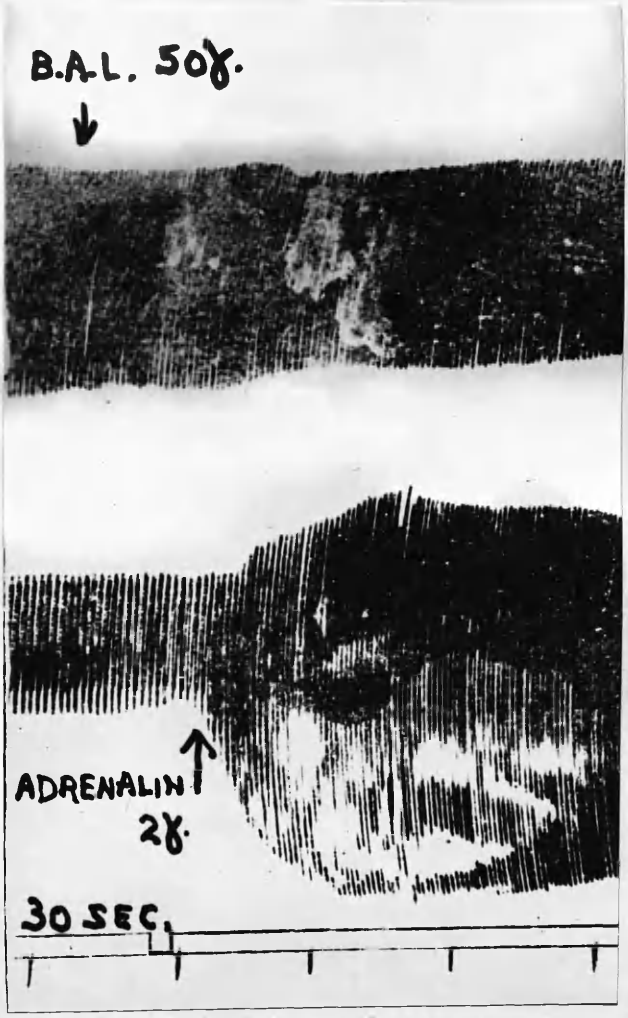


Fig. 5. Perfused cat heart (Langendorff preparation).
Upper record: shows minimal effect of B.A.L. 50γ.
Lower record: shows action of adrenaline 2γ.
Time in 30 secs.

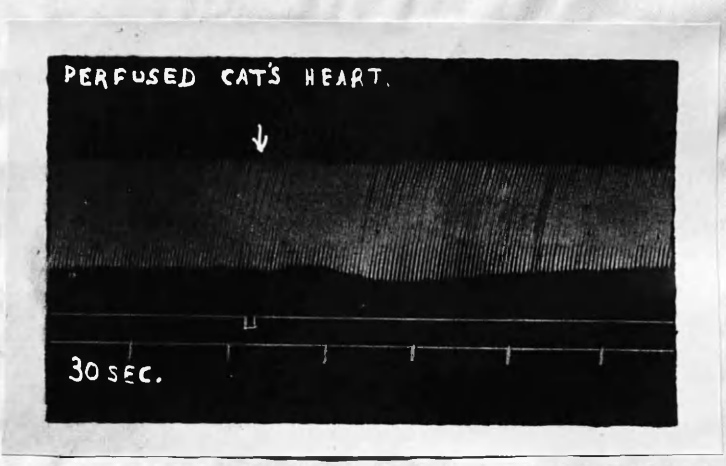


Fig. 6. Perfused cat heart (Langendorff preparation).
Upper record: shows effect of B.A.L. 0.5mg./kg.
Time in 30 secs.

five minutes). Three hours after giving B.A.L. in a dosage of 100mg./kg. and shortly before the onset of death, the packed cell volume was again determined by the same technique and found to be 48.3 per cent. This showed an increase of 31 per cent on the previous reading and indicated a severe degree of haemoconcentration despite the fact that an average of 25.0ml. of physiological saline and other fluids had been given intravenously in the course of the experiments. Haemoconcentration from capillary damage would appear to be the primary cause of death in anaesthetised animals, as Chenoweth (1946) indicated.

The odour of B.A.L. could be detected in blood, urine, tears, and the expired air, while post mortem examination showed that the odour was obtained from the freshly cut organs after death. B.A.L. would appear to be distributed to all the tissues and rapidly excreted by the kidneys and lung..

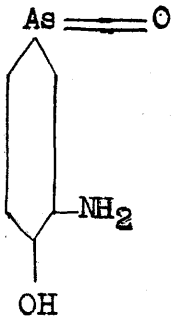
(c) The action of B.A.L. on Smooth Muscle.

Segments of duodenum of guinea pig and rabbit and of the uterus of rabbit, cat and guinea pig were mounted in Tyrode solution and the spontaneous movements recorded. B.A.L. added to the bath in a concentration of 1 in 10,000 had no effect, but higher concentrations stimulated movement and might cause spasm.

2. Action of B.A.L. on the Toxicity of Metals.

(a) Arsenic.

The arsenical preparation used was "mapharside" (m-amino-phenyl arsenoxide)



and fresh solutions were prepared as required by dissolving the appropriate amount from ampoules in physiological saline solution. Preliminary trial doses on small groups of white mice demonstrated that the L.D.50 for mapharside, by intraperitoneal injection, lay between 30mg./kg. and 42.5mg./kg.

176 white male mice, each weighing between 20-30g. were taken in groups of 44 and given graded doses of the metal by intraperitoneal injection so as to cover an adequate range of toxicity as determined by the trial doses. To half of each group of 44 mice, a standard solution of B.A.L. 40mg./kg. was injected intraperitoneally, immediately after the injection of the required dose of the metal. The results of the experiment were read after twenty-four hours. Table I. shows the dosage of metal and B.A.L. given and the effect of B.A.L. on the toxicity of the metal.

Table I.

The effect of B.A.L. 40mg./kg. on the lethality of mapharside in white mice.

Marpharside			Marpharside & B.A.L.	
Dose mg./kg.	No. Mice	No. Deaths	No. Mice	No. Deaths.
30	22	2	22	0
34	22	9	22	0
38	22	16	22	0
42.5	22	21	22	0

Table 2 records the necessary data for plotting the log. dose of the metal as abscissa against the probit of lethality as ordinate and from this graph the L.D.50 was read.

Table 2.

Data for plotting mortality curve of mapharside in white mice.

Dose Mapharside mg/kg.	Log. Dose	Percentage dead	Empirical Probit of lethality
30	1.48	9.1	3.67
34	1.53	40.9	4.77
38	1.58	72.7	5.60
42.5	1.63	98.1	7.09

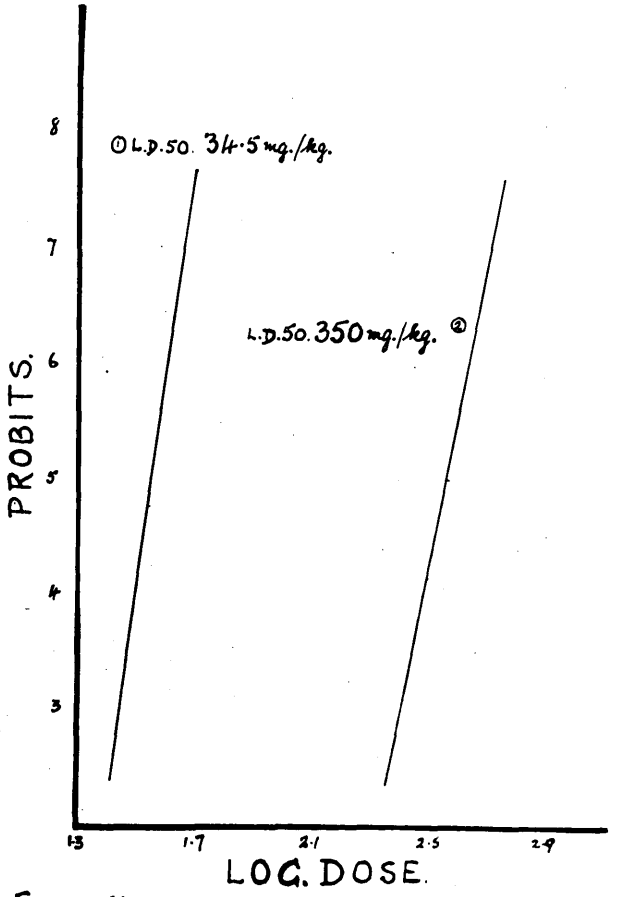


FIGURE 7. ① L.D.50 MAPHARSIDE IN WHITE MICE (i.p.)
② L.D.50 MAPHARSIDE + B.A.L. IN WHITE MICE (i.p.).

Fig. 7. Illustrates the protective action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of arsenic ("mapharside") given intraperitoneally to groups of white mice. The ordinates are probits of lethality, the abscissae logs. of the dosage. B.A.L. causes a shift to the right indicating decrease in toxicity.

The L.D.50 for white mice for mapharside given intraperitoneally was found to be 34.5mg./kg.

It will be seen from the tables that B.A.L. had such a strong protective action against arsenic poisoning that all the mice receiving it were protected from the lethal effects of the arsenic.

The experiment was repeated using a higher series of doses of mapharside as shown in table 3., and the graph plotted from the data recorded in the table.

Table 3.

Data for plotting mortality curve of mapharside in white mice treated with B.A.L.

Dose mg./kg.	Mapharside & B.A.L.				
	Log. dose.	No. Mice.	No. Deaths.	Percentage dead.	Empirical probit of lethality.
300	2.48	20	4	20	4.16
350	2.54	20	10	50	5.00
400	2.60	20	14	70	5.52

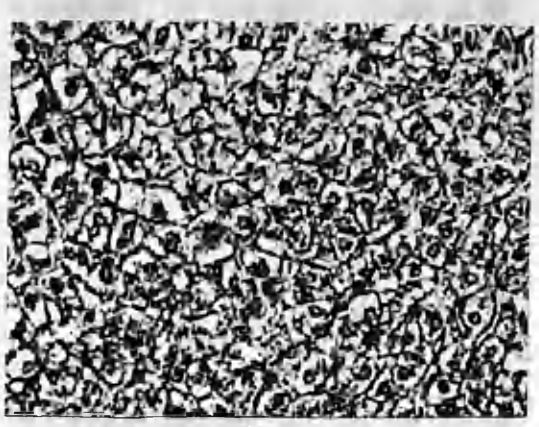
Figure 7 illustrates in graphical form the protective action of B.A.L. on mice given increasing doses of arsenic. It will be seen from figure 7 that B.A.L. raised the L.D.50 of mapharside from 34.5mg./kg. to 350mg./kg., that is, approximately a ten fold rise. Hence with mapharside B.A.L. has a strongly protective action against

lethal doses of the arsenical compound.

Of the twenty-four guinea pigs used in the arsenic poisoning experiment, the twelve guinea pigs receiving mapharside alone were early distinguishable from those twelve receiving mapharside plus B.A.L. Eight of the twelve guinea pigs, receiving arsenic alone, developed more marked symptoms of restlessness, twitching, diarrhoea, wasting and weakness and after the third injection died, while the remaining four guinea pigs, receiving arsenic alone, showed the above symptoms in a less marked form and were obviously very ill by the fifth injection and were killed. As the guinea pigs receiving mapharside died a guinea pig receiving the arsenic plus B.A.L. and corresponding in weight was sacrificed. By the fifth injection all the guinea pigs had either died or had been sacrificed.

The guinea pigs receiving B.A.L. remained well and their condition was very much better than the group receiving mapharside alone. They were active, took their food and there was little or no loss of weight.

When dead, fresh specimens of liver, kidney and gut were fixed, sectioned, stained and examined. The chief lesions caused by arsenic poisoning were necrosis of the liver cells, disintegration of the tubules and glomeruli of the kidney, catarrhal and haemorrhagic changes in the



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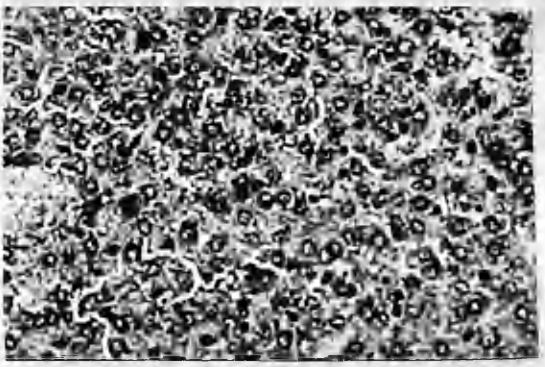

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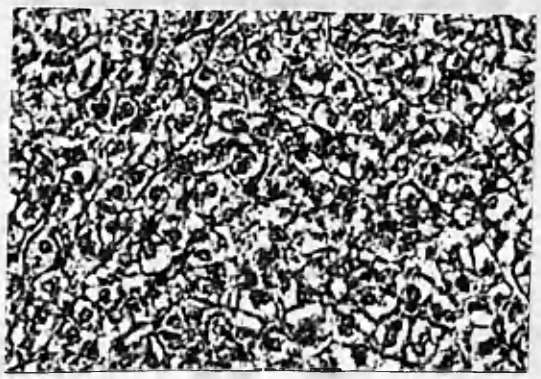
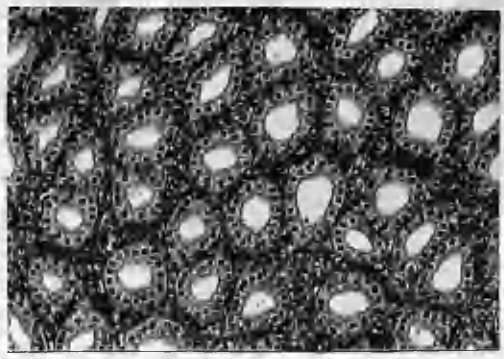


C.

Fig. 8. Illustrates the protective action of B.A.L. (40mg./kg. intraperitoneally) on the liver of guinea pig receiving arsenic ("mapharside") 15mg./kg. subcutaneously. (×150).
A : Normal guinea pig liver.
B : Liver showing necrotic effect of arsenic.
C : Liver showing protective effect of B.A.L.



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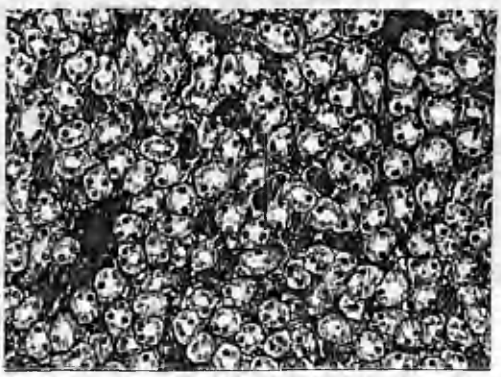

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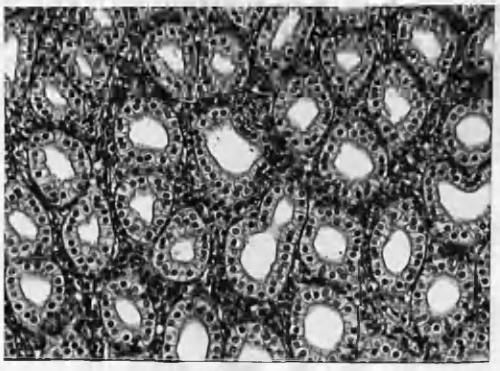


C.

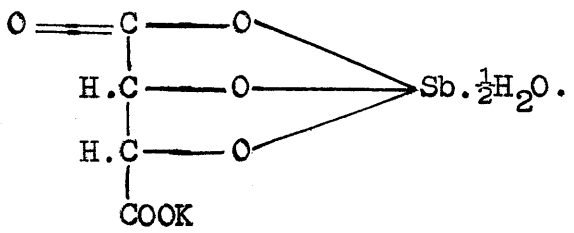
Fig. 9. Illustrates the protective action of B.A.L. (40mg./kg. intraperitoneally) on the kidneys of guinea pig receiving arsenic ("mapharside") 15mg./kg. subcutaneously. (×150).
A : Normal tubules guinea pig kidney.
B : Kidney showing necrotic effect of arsenic.
C : Kidney showing protective effect of B.A.L.

mucous membrane of the gut, with epithelial débris and exudate. The changes in the liver and kidney are shown in figures 8 and 9.

On examination of sections from the guinea pigs receiving mapharside and B.A.L. it was evident that B.A.L. had prevented wholly or to a great extent the above changes in the organs. The most striking difference was in the kidney sections and it is evident that the combination of arsenic and B.A.L. causes little damage during its excretion. These results are shown in figures 8 and 9.

(b) Antimony.

The preparation of antimony used for determining the L.D.50 in white mice by intraperitoneal injection was tartar emetic (potassium antimony tartrate).



As in the determination of the L.D.50 for mapharside, trial doses were first used to determine a suitable range of toxic doses of tartar emetic. The action of B.A.L. on the acute toxicity of antimony was then examined as described using 200 white mice.

Table 4 records the necessary data obtained from the experiment.

Table 4.

The effect of B.A.L. 4Cmg./kg. on the lethality of tartar emetic in white mice.

Tartar Emetic			Tartar Emetic & B.A.L.	
Dose mg./kg.	No. Mice	No. Deaths.	No. Mice	No. Deaths.
50	20	5	20	3
60	20	14	20	4
70	20	17	20	7
80	20	19	20	14
90	20	20	20	17

Table 5 records the data necessary for plotting the graph and from this the determination of the L.D.50 of tartar emetic given by intraperitoneal injection to white mice.

Table 5.

Data for plotting mortality curve of tartar emetic in white mice with and without B.A.L.

Tartar Emetic				Tartar Emetic & B.A.L.	
Dose mg./kg.	% dead.	Log. dose.	Empirical probit of lethality	% dead.	Empirical probit of lethality
50	25	1.70	4.33	15	3.96
60	70	1.78	5.52	20	4.16
70	85	1.85	6.04	35	4.61
80	95	1.90	6.64	70	5.52
90	100	1.95	-	85	6.04

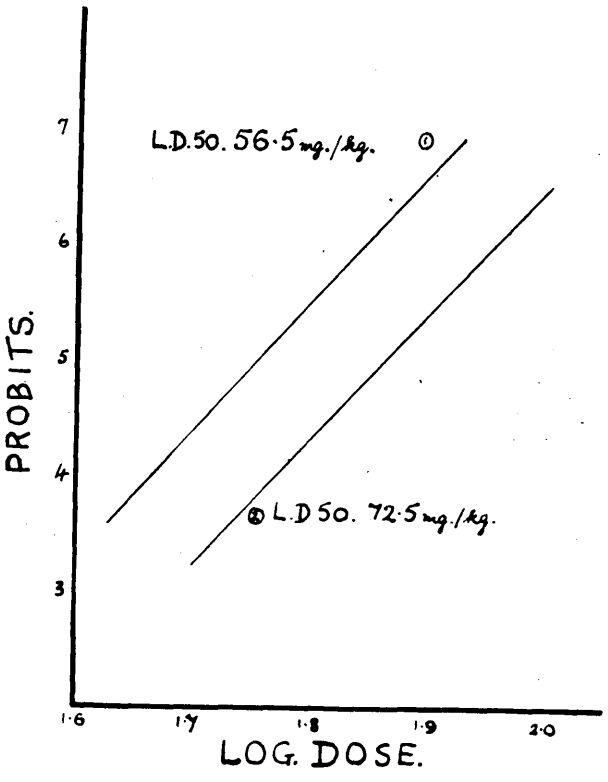


FIGURE 10. ①L.D.50. TARTAR EMETIC IN WHITE MICE (i.p.).
②L.D.50. TARTAR EMETIC plus B.A.L. (i.p.).

Fig. 10. Illustrates the protective action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of antimony (tartar emetic) given intraperitoneally to groups of white mice. The ordinates are probits of lethality, the abscissae logs. of the dosage.
B.A.L. causes a shift to the right indicating decrease in toxicity.

Figure 10 shows that the L.D.50 for tartar emetic was 56.5mg./kg. and that B.A.L. given in a standard dose of 40mg./kg. by intraperitoneal injection had a protective action on mice poisoned with this compound of antimony, raising the L.D.50 from 56.5mg./kg. to 72.5mg./kg. These results are illustrated in figure 10.

(c) Mercury.

The preparation of mercury used was mercuric chloride ($Hg.Cl_2$), (corrosive sublimate) and the experimental technique for determining the L.D.50 for mercuric chloride given intraperitoneally in white mice was as described under arsenic.

Table 6 records the doses of mercuric chloride used and the results obtained with the exhibition of B.A.L.

Table 6.

The effect of B.A.L. 40mg./kg. on the lethality of mercuric chloride in white mice.

Mercuric chloride			Mercuric chloride & B.A.L.	
Dose mg./kg.	No. Mice.	No. Dead.	No. Mice.	No. Dead.
90	20	2	20	0
120	20	12	20	0
150	20	15	20	1
185	20	19	20	1

Due to the powerful protective action of B.A.L. on mercuric chloride the experiment was repeated using higher doses of the mercury salt as shown in table 7.

Table 7.

The effect of B.A.L. 40mg./kg. on the lethality of mercuric chloride in white mice.

Mercuric chloride & B.A.L.					
Dose mg./kg.	Log. dose.	No. Mice.	No. dead.	% dead.	Empirical Probit of lethality.
220	2.34	20	3	15	3.96
250	2.39	20	12	60	5.25
380	2.58	20	18	90	6.28

Table 8 in conjunction with table 7 gives the necessary data for plotting the graph.

Table 8.

Data for plotting mortality curve of mercuric chloride in white mice.

Mercuric chloride				Mercuric chloride & B.A.L.	
Dose mg./kg.	% dead	Log. dose	Empirical Probit of lethality.	% dead.	Empirical Probit of lethality.
90	10	1.95	3.61	0	-
120	60	2.08	5.23	0	-
150	75	2.18	5.64	5	3.36
185	95	2.27	6.33	5	3.36

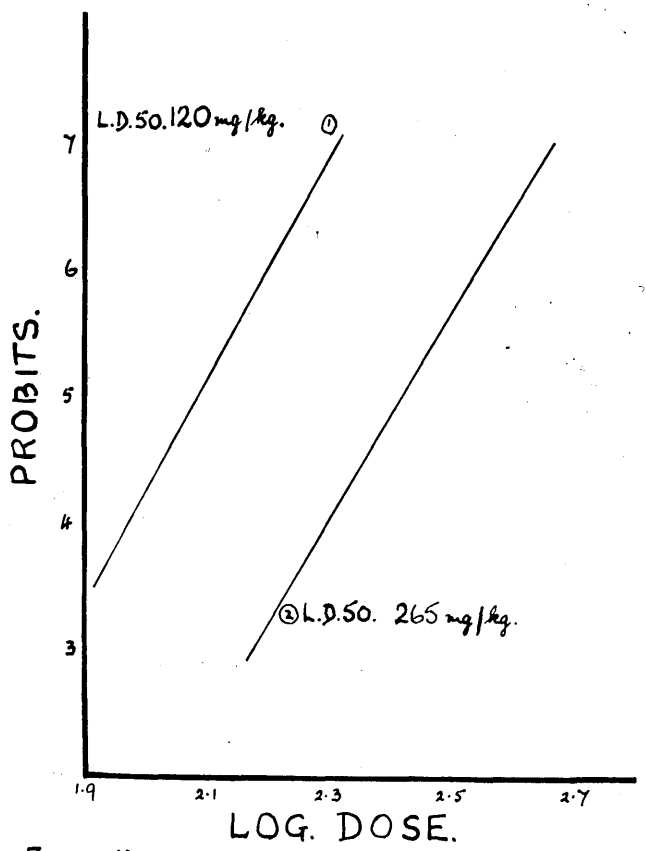


FIGURE 11. ① L.D. 50 MERCURIC CHLORIDE IN WHITE MICE (i.p.).
② L.D. 50 MERCURIC CHLORIDE + B.A.L. (i.p.).

Fig. 11. Illustrates the protective action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of mercuric chloride given intraperitoneally to groups of white mice. The ordinates are probits of lethality, the abscissae logs. of the dosage. B.A.L. causes a shift to the right indicating decrease in toxicity.

The L.D. 50 of mercuric chloride, given by intraperitoneal injection, was found to be 120mg./kg. in white mice. B.A.L. had a protective effect on animals poisoned with this salt and raised the L.D.50 to 265mg./kg. This protective action of B.A.L. is shown in figure 11.

Chronic mercurial poisoning was induced in twelve rabbits by giving them mercuric chloride solution in a dosage of 1.0mg./kg. by intraperitoneal injection. The injections were repeated daily for ten days. Half the rabbits were also given B.A.L. in a dosage of 2.5mg./kg. by intraperitoneal injection just after they had received their dose of mercuric chloride. The rabbits, receiving mercuric chloride alone, showed loss of weight and strength, anorexia, diarrhoea, and general cachexia. Tremor developed in the muscles of the limbs and death occurred between the twelfth and sixteenth day. The rabbits given B.A.L. in addition to mercuric chloride did not seem to be upset and retained their weight and appetite until sacrificed. From clinical observation it was obvious that B.A.L. had a protective effect on the toxicity of mercuric chloride. Post mortem examination of the rabbits poisoned with mercuric chloride revealed swelling and necrosis of the liver and kidney. The liver and kidneys appeared somewhat swollen with minute haemorrhages on the surface of the organs. On section

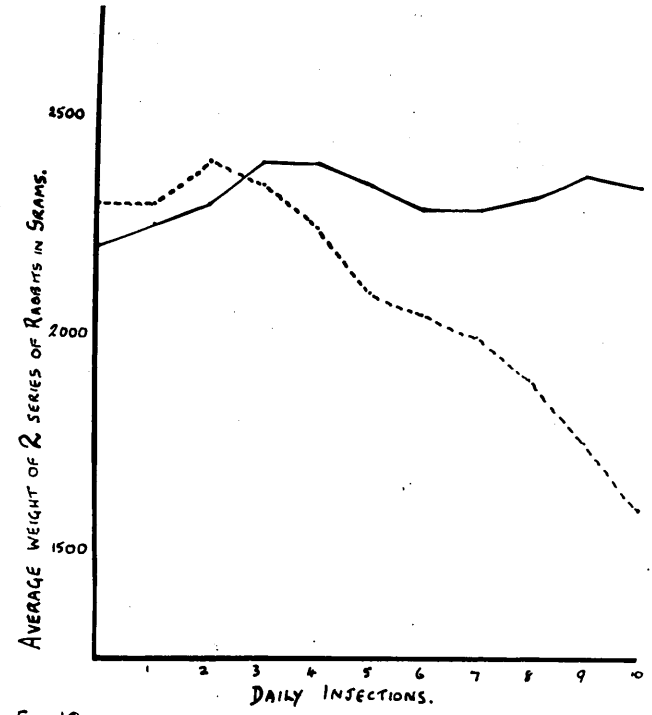


FIG. 12. ---REPRESENTS EFFECT $Hg.Cl_2$ ON COMBINED AVERAGE WEIGHT
——REPRESENTS EFFECT $Hg.Cl_2$ + B.A.L.

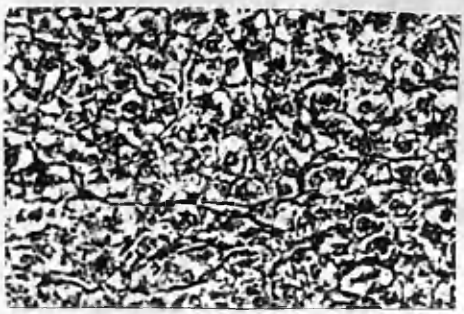
Fig. 12. Illustrates the protective effect of British Anti Lewisite on the loss of weight in rabbits due to poisoning with mercuric chloride.

the cortex of the kidney was pale in colour with a blurred appearance. The gut showed necrotic changes with haemorrhage into the lumen while the walls were discoloured and showed pregangrenous changes. At death fresh specimens of the liver, kidney and intestine were taken from both groups of rabbits. The tissue specimens were fixed, stained and prepared for histological examination. Histological examination of the sections, prepared from the rabbits poisoned with mercuric chloride, showed necrosis and degeneration of the liver cells. The kidney sections showed that the main onslaught of the toxic action of the mercuric chloride had fallen on the tubules which showed damage, consisting of desquamation and necrosis of the tubular epithelium. The lumen of the intestine and wall showed degeneration and haemorrhages. Histological examination of the above organs in the rabbits, receiving both mercuric chloride and B.A.L., showed that the above changes were absent or were very much reduced. Figure 12 illustrates the weight difference between the group of rabbits receiving mercury and B.A.L. and the other group without B.A.L.,

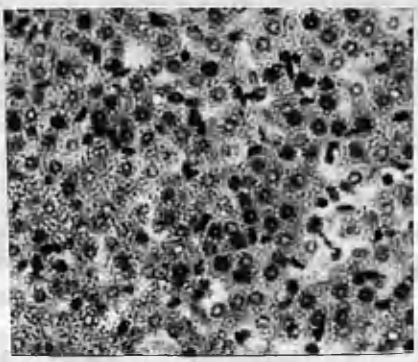
An attempt was made to induce chronic mercury poisoning in sixteen guinea pigs by dosing them with 5mg./kg. of mercuric chloride by intraperitoneal injection.

Half the guinea pigs were also given 40mg./kg. B.A.L. by intraperitoneal injection daily, immediately after the injection of the mercuric chloride. The guinea pigs given mercuric chloride alone developed anorexia and diarrhoea, showed loss of weight and apathy with muscular weakness, particularly in the limbs. Tremors developed and the animals were dead in four days from the beginning of the experiment. The animals given B.A.L. in addition to the mercuric chloride remained fit and active. There was no loss of weight or appetite and the development of diarrhoea or tremors was not observed. The guinea pigs were sacrificed on the fourth day. Fresh specimens of liver, kidney and gut were fixed and stained for histological examination.

Post mortem examination showed that the effect of the mercuric chloride had fallen mainly on the kidney, liver and gut. The kidney appeared somewhat enlarged with minute haemorrhages on its surface and on section showed a pale cortex. The liver was slightly enlarged and had a congested appearance. The spleen was congested, while the gut wall was discoloured in places and showed haemorrhagic and necrotic changes in the lumen. These post mortem findings were absent or very much reduced in the guinea pigs which had received B.A.L. in addition to the mercuric chloride.



A.



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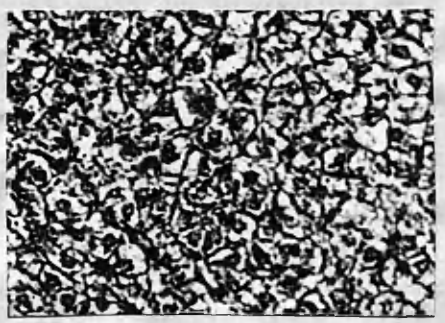


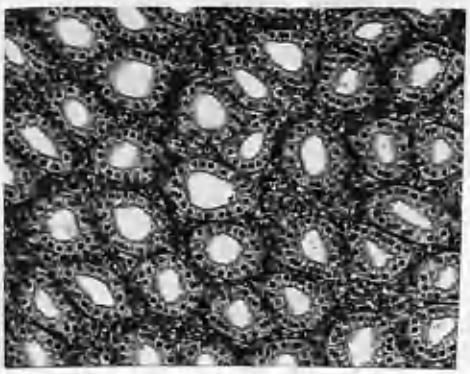
C.

Fig. 13. Illustrates the protective action of B.A.L. (40mg./kg. intraperitoneally) on the liver of guinea pig receiving mercuric chloride 5mg./kg. by intraperitoneal injection. (×150).

A : Normal liver.

B : Liver showing necrotic effect of mercury.

C : Liver showing protective effect of B.A.L.



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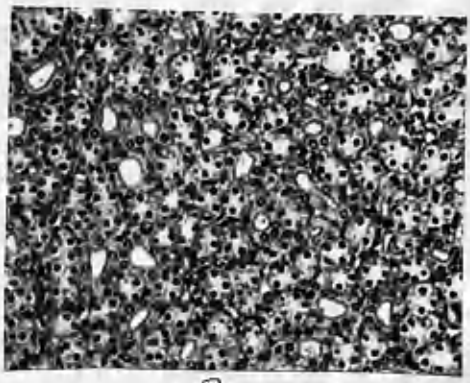

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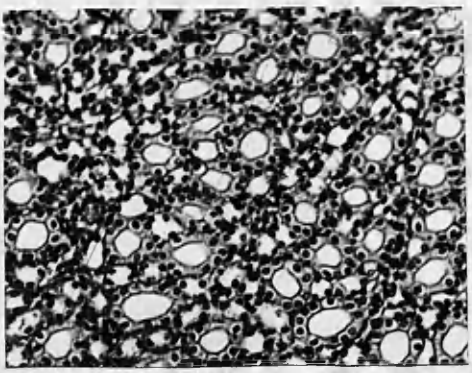


C.

Fig. 14. Illustrates the protective action of B.A.L. (40mg./kg.) intraperitoneally on the kidney of guinea pig receiving mercuric chloride 5mg./kg. by intraperitoneal injection. (×150).
A: Normal kidney.
B: Kidney showing necrotic effect of mercury.
C: Kidney showing protective effect of B.A.L.

Histological examination of the kidneys of the guinea pigs who had received mercuric chloride alone showed desquamation and necrosis of the tubular epithelium. The liver showed degenerative and necrotic changes while the guts showed necrotic and haemorrhagic changes in the wall with necrosis and desquamation of the epithelial cells of the lumen. Examination of the sections of the above organs from the animals, receiving both B.A.L. and mercuric chloride, showed that the above changes were either absent or much reduced. Figures 13 and 14 illustrate the histological changes produced in guinea pigs, with and without the protection given by B.A.L., against the poisoning of mercuric chloride.

(d) Mersalyl.

According to Long and Farah (1946) the intravenous injection of B.A.L. reduced the lethality of intravenous "Salyrgan" in mice and protected the cardiovascular system of anaesthetised dogs from the toxic effects of this mercurial compound. In this present work the L.D.50 of "mersalyl B.P." was determined by the same technique as previously described. The effect of B.A.L. on the L.D.50 of "mersalyl" was also determined.

Table 9 records the experimental results.

Table 9.

The effect of B.A.L. 40mg./kg. on the lethality of mersalyl in white mice.

Mersalyl			Mersalyl & B.A.L.	
Dose mg./kg.	No. Mice.	No. Dead.	No. Mice.	No. Dead.
80	20	0	20	3
110	20	0	20	5
115	20	3	20	9
130	20	7	20	7
150	20	9	20	16

Table 10 shows the data from which the graph was drawn to determine the necessary L.D.50 for "mersalyl" and "mersalyl" plus B.A.L.

Table 10.

Data for plotting mortality curve of mersalyl in white mice.

Mersalyl				Mersalyl & B.A.L.	
Dose mg./kg.	Log. Dose.	% dead.	Empirical Probit of lethality.	% dead.	Empirical Probit of lethality.
80	1.90	0	-	15	3.96
110	2.04	0	-	25	4.33
115	2.06	15	3.96	45	4.87
130	2.11	35	4.61	35	4.61
150	2.18	45	4.87	80	5.84

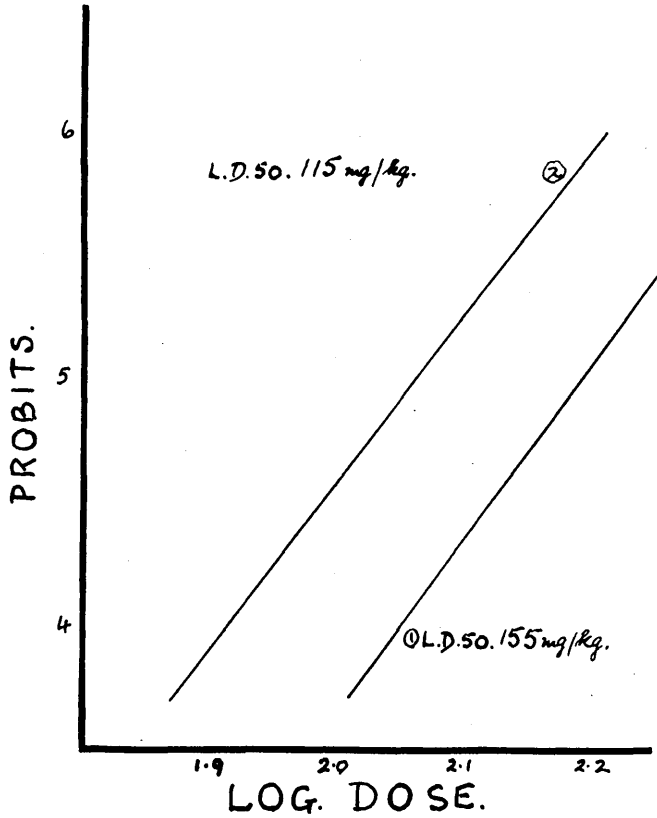


FIGURE 15. ①L.D.50 MERSALYL IN WHITE MICE (i.p.).
②L.D.50 MERSALYL + B.A.L. (i.p.).

Fig. 15. Illustrates the potentiating action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of "mersalyl" given intraperitoneally to groups of white mice. The ordinates are probits of lethality, the abscisseae logs. of the dosage. B.A.L. causes a shift to the left, indicating an increase in toxicity.

Figure 15 illustrates the effect of B.A.L. on the L.D.50 of mersalyl given intraperitoneally to white mice. The L.D.50 of mersalyl B.P. was found to be 155mg./kg. and the L.D.50 of mersalyl B.P. was lowered to 115mg./kg. when B.A.L. in a dosage of 40mg./kg. was given intraperitoneally immediately after the diuretic. The results clearly indicate that B.A.L. increases the toxicity of mersalyl when both are given by the intraperitoneal route.

The L.D.50 for "mersalyl" given intravenously to mice was found to be 120mg./kg., and when B.A.L. in dosage of 20mg./kg was also given intravenously to mice receiving intravenous "mersalyl", many of the mice were protected from the violent convulsions caused by intravenous "mersalyl". B.A.L., given intravenously raised the L.D.50 of "mersalyl" given intravenously to 165mg./kg. This apparent anomaly between the effects of B.A.L. and "mersalyl" when given intraperitoneally and intravenously is discussed later but some clarification was provided by the results of tests of the effect of "mersalyl" on water diuresis in groups of rats.

B.A.L. at a dosage of 40mg./kg., given intraperitoneally to rats acted as an antidiuretic, suppressing urine for three to four hours. "Mersalyl" at a dosage of 100mg./kg., given intraperitoneally caused immediate anuria, and death followed after forty-eight hours.

If B.A.L. and "mersalyl" were given intraperitoneally within a few minutes of one another in the above doses, suppression of urine was much less than when either alone was given, and the rats survived.

The method of investigating the effect of B.A.L. on the diuretic activity of "mersalyl" was that described by Burn (1937) for the assay of the antidiuretic potency of extracts of the posterior pituitary body and has been described already under methods.

Table 11 shows the method of recording the necessary data required in the antidiuretic experiments with rats.

Table 11.

Group A. 4 rats.

Time watered 9.51 hours.

Saline intraperitoneal to balance B.A.L.

Saline subcutaneous to balance mersalyl.

Time	Total Volume of Urine in ml.	Volume of Urine in ml. in last 15 mins.
10.13	0.1	-
10.28	1.2	1.1
10.43	5.4	4.2
10.58	10.9	5.5
11.13	15.0	4.1
11.28	17.5	2.5
11.43	18.9	1.4
11.58	19.4	0.7
12.13	19.8	0.4
12.28	20.3	0.5
12.43	20.4	0.1

The method of calculating the time to maximum excretion is detailed below:-

Total urine excreted 20.5ml.

Half this plus the initial output is (10.25 + 0.1)ml.
= 10.35ml.

This was excreted between 10.43hrs. and 10.58hours.

Point of maximum excretion = 10.56 hours.

Time to this point = 65 minutes.

The rats, when watered and given subcutaneous saline to balance mersalyl and intraperitoneal saline to balance B.A.L. in further experiments, had a diuresis, the peak of which occurred after 65 minutes. When given mersalyl in a dose of 1.0mg./kg. and saline (to balance the B.A.L. used in the next experiment) the peak of the diuresis occurred after 72 minutes. Repetition of the experiment one week later using mersalyl in a dosage of 1.0mg./kg. and saline showed that the peak of diuresis occurred at 73 minutes, whereas the mersalyl given subcutaneously in the above dosage with B.A.L., in a dosage of 4mg./kg. given intraperitoneally, gave a figure of 90 minutes for the peak of the diuresis. It follows from these results that B.A.L., in small or large doses, and mersalyl, in large doses, each has a delaying effect on the excretion of water by normal white male rats. With large doses these effects are opposite to one

another but with small doses this is not so, the antidiuretic effect of the B.A.L. being greatly increased.

(e) Chromium.

Chromium has been known for a long time to be an industrial hazard in the mining of the metallic ores, as well as in those industries which use salts of chromium for dyeing, electrotyping and plating. The effect of B.A.L. on the toxicity of chromium was investigated, with the object of determining the possible use of B.A.L., as an effective therapeutic agent for chrome ulceration of the skin and nasal mucus membrane and chrome dermatitis due to chrome compounds.

The preparation of chromium used was chromium trioxide and the L.D.50 of this compound with and without the addition of B.A.L. was determined as previously described in white mice. The data necessary for obtaining the required L.D.50 for chromium trioxide and chromium trioxide plus B.A.L. is shown in table 12.

Table 12.

The effect of B.A.L. 40mg./kg. on the lethality of chromium trioxide in white mice.

Chromium Trioxide			Chromium Trioxide & B.A.L.	
Dose mg./kg.	No. Mice.	No. Dead.	No. Mice.	No. Dead.
55	20	2	20	0
65	20	9	20	0
75	20	16	20	4
82.5	20	19	20	7
90	20	20	20	12
95	20	20	20	14

Table 13 gives the required readings to plot the graph of log. dose against empirical probit of lethality.

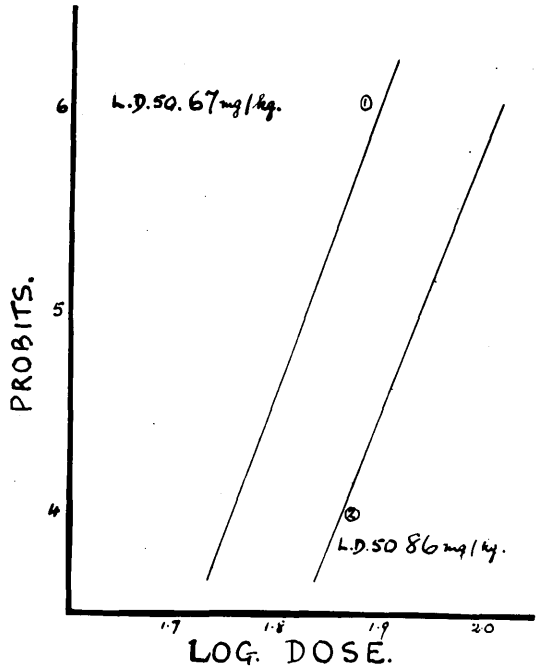


FIGURE 16. ⊙ L.D.50 CHROMIUM TRIOXIDE IN WHITE MICE (i.p).
⊗ L.D.50 CHROMIUM TRIOXIDE + B. A. L. (i.p).

Fig. 16. Illustrates the protective action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of chromium given intraperitoneally to groups of white mice. The ordinates are probits of lethality, the abscissae logs. of the dosage.
B.A.L. causes a shift to the right indicating decrease in toxicity.

Table 13.

Data for plotting mortality curve of chromium trioxide in white mice.

Chromium Trioxide				Chromium Trioxide & B.AL.	
Dose mg./kg.	Log. dose.	% dead.	Empirical Probit of lethality.	% dead.	Empirical Probit of lethality.
55	1.74	10	3.72	0	-
65	1.81	45	4.87	0	-
75	1.88	80	5.84	20	4.16
82.5	1.92	95	6.64	35	4.61
90	1.95	100	-	60	5.25
95	1.98	100	-	70	5.52

Figure 16 illustrates that B.A.L. has a protective action against chromium. The L.D.50 for chromium trioxide given intraperitoneally was raised from 67mg./kg. to 86mg./kg. when B.A.L. was also given intraperitoneally in dosage of 40mg./kg.

Twenty-four guinea pigs were shaved and chrome ulcers were produced by the intracutaneous injection of 5% chromic acid solution. The intracutaneous wheal produced by the 5% chromic acid solution had broken down by the fifth day producing well established circular ulcers at each site of

injection. There was a black sloughing eschar, with a diameter of half to three quarters of an inch, while the edge of the ulcer was surrounded by a raised red areola, approximately quarter of an inch in width. During the formation of the ulcers the animals suffered from some systemic absorption of the chromic acid from the site of the intracutaneous injection. This was evidenced by varying degrees of apathy, loss of appetite, malaise and weakness but, by the time the ulcers were established, all the animals had recovered from these symptoms. At this stage of the experiment the guinea pigs were divided into two groups and the ulcers of half the animals were treated with daily application of the acriflavine ointment while the ulcers of the other half of the animals were treated with acriflavine ointment containing 10% by weight of B.A.L. The B.A.L. ointment was stored in a refrigerator to ensure the minimal loss of potency. Observations were made on the rates of healing of the ulcers of both groups. The ulcers treated with B.A.L. softened, induration was progressively lost and they were completely healed in thirty days. The ulcers treated with acriflavine ointment only were still indurated and approximately one quarter of their original size after forty days treatment. Complete healing took another twelve to eighteen days. There was no doubt that

the treatment of the chrome ulceration with B.A.L. was a distinct advance on treatment without B.A.L. As a result of these observations it is suggested that treatment with B.A.L. ointment and, if necessary, the administration of B.A.L. systemically, by intramuscular injection in peanut oil-benzyl benzoate would be of value in the treatment of cases of chrome ulceration of the skin, the nasal mucous membranes and chrome dermatitis in man.

(f) Lead.

During the present century, the incidence of lead poisoning has been greatly reduced, due to the greater industrial preventitive measuresnow adopted. One of the main sources of lead poisoning still present is caused by the inhalation of lead vapour, formed as a result of the action of the oxy-acetylene burner on the metal plates of ships containing lead, as occurs during the course of ship breaking. In shipbreaking, preventitive measures are difficult to apply and this industry is one of the main sources of clinical lead poisoning. For these reasons, it was considered that B.A.L. was worthy of trial to determine its effect on the toxicity of lead.

The lead salt used experimentally was lead acetate B.P. $(Pb(CH_3.CCO)_2.3H_2O)$, and an acute toxicity experiment with

white mice was carried out using a suitable range of toxic doses. Suitable dosage was determined by means of a series of trial doses using small groups of mice.

Table 14 records the series of doses of lead acetate, given to groups of white mice by intraperitoneal injection with and without the addition of B.A.L., given intraperitoneally in doses of 40mg./kg.

Table 14.

The effect of B.A.L. 40mg./kg. on the lethality of lead acetate in white mice.

Lead acetate			Lead acetate & B.A.L.	
Dose mg./kg.	No. Mice.	No. Dead.	No. Mice.	No. Dead.
400	20	0	20	5
425	20	0	20	9
450	20	8	20	15
475	20	12	20	19
500	20	17	20	20
525	20	19	20	20

Table 15 gives the data necessary for constructing the graph based on plotting the log. dose of the lead acetate against the empirical probit of lethality with and without the addition of B.A.L.

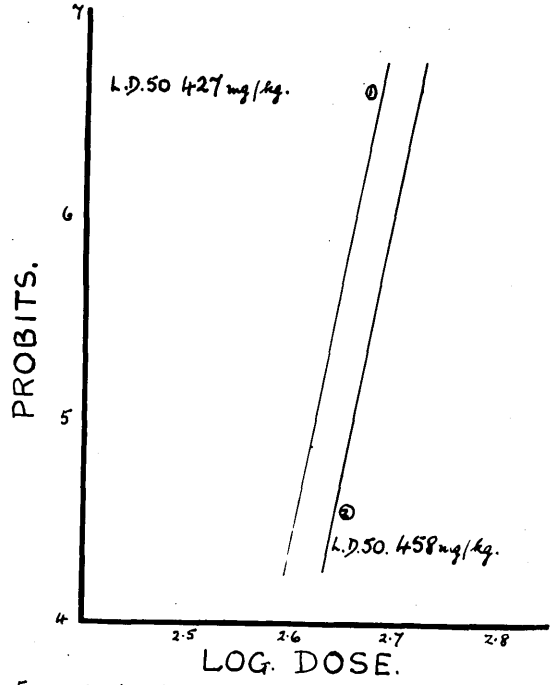


FIGURE 17. ① L.D.50 LEAD ACETATE + B.A.L. IN WHITE MICE (i.p.).
② L.D.50 LEAD ACETATE IN WHITE MICE (i.p.).

Fig. 17. Illustrates the potentiating action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of lead given intraperitoneally to groups of white mice.
The ordinates are probits of lethality, the abscissae logs. of the dosage.
B.A.L. causes a shift to the left indicating increase in toxicity.

Table 15.

Data for plotting mortality curve of lead acetate in white mice.

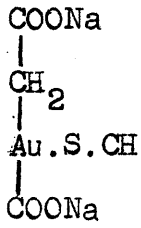
Lead Acetate.				Lead acetate & B.A.L.	
Dose mg./kg.	Log. dose.	% dead.	Empirical Probit of lethality	% dead.	Empirical Probit of lethality.
400	2.60	-	-	25	4.33
425	2.63	-	-	45	4.87
450	2.65	40	4.75	75	5.67
475	2.68	60	5.25	95	6.64
500	2.70	85	6.04	100	-
525	2.72	95	6.64	100	-

The L.D.50 of lead acetate given in solution intraperitoneally to white mice was found to be 458mg./kg. while the L.D.50 of lead acetate plus B.A.L. given intraperitoneally was found to be 427mg./kg. These results prove that B.A.L. had an additive effect on the toxicity of lead. This additive effect of B.A.L. on the toxicity of lead is illustrated in figure 17.

(g) Gold.

One of the most important advances in the treatment of rheumatoid arthritis was the introduction of gold salts on the continent by Forestier (1932). Many British and European physicians are agreed that careful and judicious exhibition of these salts can be expected to produce

beneficial results in approximately two-thirds of suitable cases, and that this undoubted percentage of improvement justifies the risk of severe toxic reactions which are liable to occur, in many cases quite unexpectedly. In view of the possible development of such toxic reactions to gold as exfoliative dermatitis, toxic nephritis, toxic hepatitis, purpura, and agranulocytosis it was decided to test the action of B.A.L. on the toxicity of sodium aurothiomalate



a gold compound much used in the chrysotherapy of rheumatoid arthritis.

Trial doses of the gold salt were carried out in small groups of white mice in order to obtain a suitable range of toxic doses. With a suitable range of dosage determined two series of groups of white mice were injected intraperitoneally with the gold salt in varying dosage, while one series of white mice also received intraperitoneal B.A.L. in a dose of 40mg./kg. Table 16 records the results obtained.

Table 16.

The effect of B.A.L. 40mg./kg. on the lethality of sodium aurothiomalate.

Sodium aurothiomalate			Sodium aurothiomalate & B.A.L.	
Dose mg./kg.	No. Mice.	No. Dead.	No. Mice.	No. Dead.
650	20	1	20	4
750	20	3	20	9
1250	20	11	20	18
1500	20	18	20	19

Table 17 supplies the necessary data for graphing the log. dose against the empirical probit of lethality.

Table 17.

Data for plotting mortality curve of sodium aurothiomalate in white mice.

Sodium aurothiomalate				Sodium aurothiomalate & B.A.L.	
Dose mg./kg.	Log. Dose.	% dead.	Empirical Probit of lethality.	% dead.	Empirical Probit of lethality.
650	2.81	5	3.35	20	4.16
750	2.88	15	3.96	45	4.87
1250	3.10	55	5.13	90	6.28
1500	3.18	90	6.28	95	6.64

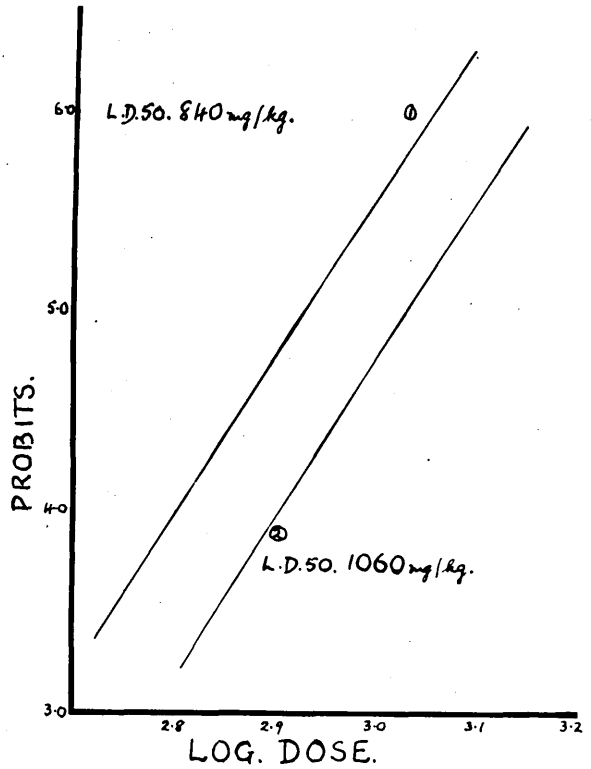


FIGURE 18. ①L.D.50 SODIUM AUROTHIOMALATE + B.A.L IN WHITE MICE (i.p).
②L.D.50 SODIUM AUROTHIOMALATE IN WHITE MICE (i.p).

Fig. 18. Illustrates the potentiating action of British Anti Lewisite (40mg./kg. given intraperitoneally) on the toxicity of gold given intraperitoneally to groups of white mice.
The ordinates are probite of lethality, the abscissae logs. of the dosage.
B.A.L. causes a shift to the left indicating increase in toxicity.

Figure 18 illustrates the effect of B.A.L. on the toxicity of sodium aurothiomalate on white mice when both are given by intraperitoneal injection. The L.D.50 of sodium aurothiomalate in white mice by intraperitoneal injection was found to be 1060mg./kg. B.A.L. had an additive effect on the toxicity of sodium aurothiomalate reducing the L.D.50 of sodium aurothiomalate to 840mg./kg.

It should be noted, however, that Cohen, Goldman and Dubbs (1947), Ragan and Boots (1947), Lockie, Norcross and George (1947), and Davison (1947), have reported the successful use of B.A.L., given by intramuscular injection, in cases of toxic reactions resulting from gold therapy, used in the treatment of rheumatoid arthritis. Cohen et al (1947) treated five cases of acute poisoning due to gold and noted prompt clinical improvement in their cases once therapy with B.A.L. was started. As they themselves admit, the number of cases was too small to justify definite conclusions. Ragan and Boots (1947) treated five cases of dermatitis due to gold therapy and in four of the cases the dermatitis cleared up with B.A.L. therapy. In all five cases there was noted a significant excretion of gold in the urine following the administration of B.A.L. Lockie et al (1947) successfully treated two patients with B.A.L. - one with thrombocytopenic purpura and the other with granulocytopenia due to gold therapy. Davison (1947)

treated three cases of gold dermatitis with B.A.L. and claimed that the rapid disappearance of the dermatitis in each patient was due to the B.A.L.

(h) Bismuth.

The bismuth salt tested was sodium bismuthyl tartrate given by intraperitoneal injection to white mice and the effect of B.A.L., given by intraperitoneal injection is shown by the results in table 18.

Table 18

The effect of B.A.L. 40mg./kg. on the lethality of sodium bismuth tartrate.

Sodium bismuth tartrate.			Sodium bismuth tartrate & B.A.L.	
Dose mg./kg.	No. Mice.	No. Dead.	No. Mice.	No. Dead.
200	20	0	20	3
350	20	0	20	11
450	20	2	20	15
600	20	7	20	19
800	20	14	20	20
875	20	17	20	20

Table 19 gives the necessary data for the construction of a graph showing the relation of the log. dose to the empirical probit of lethality.

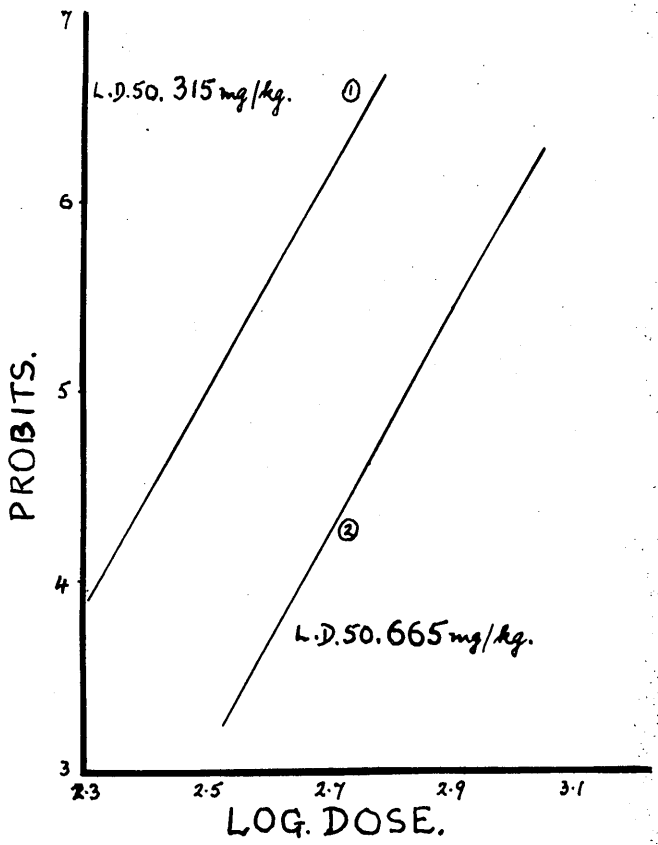


FIGURE 19. ①L.D.50 SODIUM BISMUTH TARTRATE + B.A.L. (i.p.).
②L.D.50 SODIUM BISMUTH TARTRATE IN WHITE MICE (i.p.)

Fig. 19. Illustrates the potentiating action of British Anti Lewisite (40mg./kg. on the toxicity of bismuth given intraperitoneally to groups of white mice. The ordinates are probits of lethality, the abscissae logs. of the dosage. B.A.L. causes a shift to the left indicating increase in toxicity.

Table 19.

Data for plotting mortality curve of sodium bismuthyl tartrate in white mice.

Sodium bismuth tartrate				Sodium bismuth tartrate & B.A.L.	
Dose mg./kg.	Log. dose.	% dead.	Empirical Probit of lethality.	% dead.	Empirical Probit of lethality.
200	2.30	0	-	15	3.96
350	2.54	0	-	55	5.13
450	2.65	10	3.72	75	5.67
600	2.78	35	4.61	95	6.64
800	2.90	70	5.52	100	-
875	2.94	85	6.04	100	-

The graph illustrated in figure 19 shows that B.A.L. had an additive effect on the toxicity of the bismuth salt, the L.D.50 of which was found to be 665mg./kg., intraperitoneally in white mice, B.A.L. reducing the L.D.50 to 315mg./kg.

3. Effect of B.A.L. on Enzyme Systems.

Peters, Stocken and Thompson (1945) showed that B.A.L. protected the arsenic-sensitive pyruvic oxidase in vitro and was also capable of reversing the poisoning, which had occurred due to its high affinity for arsenic. B.A.L. itself is toxic and in order to study the cause of this toxicity, Webb and Heyningen (1947) investigated the action of B.A.L. on a large number of enzymes in vitro. The seven enzymes polyphenol oxidase, carbonic anhydrase, catalase, peroxidase, aldehyde mutase, phosphorylase and glyoxylase were strongly inhibited by B.A.L. Four of these enzymes are metallo-proteins. Polyphenol oxidase contains copper, carbonic anhydrase zinc, and catalase and peroxidase contain iron. This result is strong evidence that B.A.L. specifically inhibits enzymes containing metals and that the inhibition is due to B.A.L. combining with the metals, as the substance has a high affinity for metals. Papain and the glycolytic system of muscle acetone powder were both found to be activated by B.A.L. Cysteine is also capable of activating papain and the glycolytic system of muscle acetone powder, and Webb and Heyningen considered that this effect was probably due to the reducing properties of the thiol (-SH) groups.

Herbert, Gordon, Subrahmanyan, and Green (1940) showed that the action of the enzyme zymohexase was inactivated by

traces of heavy metals. Webb and Heyningen found that B.A.L. slightly activated this enzyme and considered that the apparent activation was probably due to B.A.L. removing traces of these heavy metals in the undialyzed solution of muscle acetone powder used.

In the case of polyphenol oxidase, Webb and Heyningen considered that the effect of B.A.L., in inhibiting the enzyme, was certainly due to the formation of an insoluble copper-B.A.L. compound. They found that the enzyme could be reactivated by the addition of excess of copper salt. As a result of these experiments the authors considered that B.A.L. probably acts as a general inhibitor of enzymes containing metals and as an antagonist of metal activation of enzymes. The authors concluded that they could not decide whether or not the toxicity of B.A.L. was due to the inhibition of metal-containing enzymes by B.A.L.

Barron, Miller and Meyer (1947) studied the effect of B.A.L. on the activity of enzyme systems and on tissue metabolism, in an attempt to elucidate the toxic effects observed when large amounts of dithiols were injected into animals. Barron et al confirmed the findings of Webb and Heyningen (1947) that B.A.L. produced inhibition of some enzyme systems by combining with the heavy metals forming the prosthetic group of the protein moiety of the enzyme. They found that, during the oxidation of B.A.L.,

there was inhibition of enzyme systems which contained essential thiol groups. The authors considered that the reducing power of B.A.L. was also responsible for some of the toxic effects. The physiological activity of insulin was found to be destroyed by B.A.L. and it was considered that the destruction was probably due to the reducing action of B.A.L. in reducing the -S-S- groups of insulin.

(a) The effect of B.A.L. on the action of Insulin.

In the present experiments a group of five rabbits, weighing each approximately 2kg. were starved overnight and a blood sample of 0.5ml. was drawn from the marginal ear vein of each rabbit the following morning and pooled by mixing in a heparinised tube. The fasting blood sugar level was then estimated. The animals were then given 0.5 unit of soluble insulin/kg. subcutaneously and blood was again collected from the marginal ear vein of the rabbits, at hourly intervals and pooled in a heparinized tube by thorough mixing. The blood sugar level was estimated by the method of Hagedorn and Jensen in each pooled sample of blood.

After three days, the above experiment was repeated with the same rabbits, with the addition of 25mg./kg. of B.A.L. given intraperitoneally after the insulin had been injected subcutaneously. At hourly intervals,

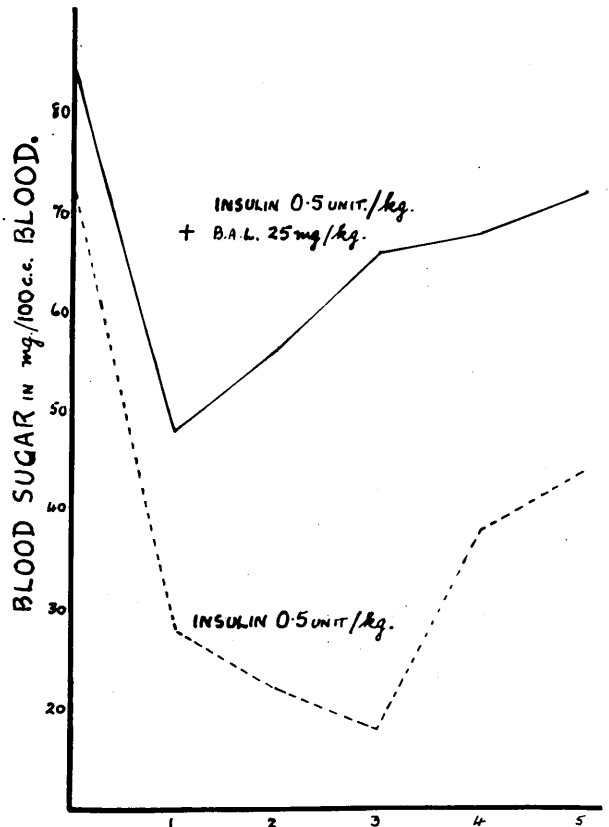


FIGURE 20. GLUCOSE TOLERANCE TEST.
BLOOD SUGAR CURVE OF FIVE RABBITS.

Fig.20. Illustrates the mean blood sugar curve in five rabbits after 0.5 unit/kg. of soluble insulin given subcutaneously, and the effect of B.A.L. 25mg./kg. given intraperitoneally in inhibiting the action of the same dose of insulin in the same animals.

blood was collected from the marginal ear vein of the rabbits and pooled in a heparinized tube by thorough mixing. The blood sugar level was estimated in each pooled sample.

Tables 20 and 21 record the experimental data obtained in these experiments and supplies the data for figure 20, which illustrates the effect of B.A.L. on the action of insulin.

Table 20.

The effect of 0.5 unit/kg. of soluble insulin on the blood sugar level of five rabbits.

Soluble insulin 0.5 unit/kg. subcutaneously.		
Readings.	Time in hrs.	Blood Sugar mg./100ml.
Mean fasting blood sugar level	10.45	0.72
Insulin given at	11.00	-
Mean blood sugar level at	12.00	0.28
Mean blood sugar level at	1.00	0.22
Mean blood sugar level at	2.00	0.18
Mean blood sugar level at	3.00	0.38
Mean blood sugar level at	4.00	0.44

Table 21.

The effect of B.A.L. 25mg./kg. on the action of insulin.

Soluble insulin 0.5 unit/kg. subcutaneously plus B.A.L. 25mg./kg. intraperitoneally.		
Readings	Time in hrs.	Blood sugar mg./100ml.
Mean fasting blood sugar level	10.45	0.84
Insulin plus B.A.L. given at	11.00	-
Mean blood sugar level at	12.00	0.48
Mean blood sugar level at	1.00	0.56
Mean blood sugar level at	2.00	0.66
Mean blood sugar level at	3.00	0.68
Mean blood sugar level at	4.00	0.72

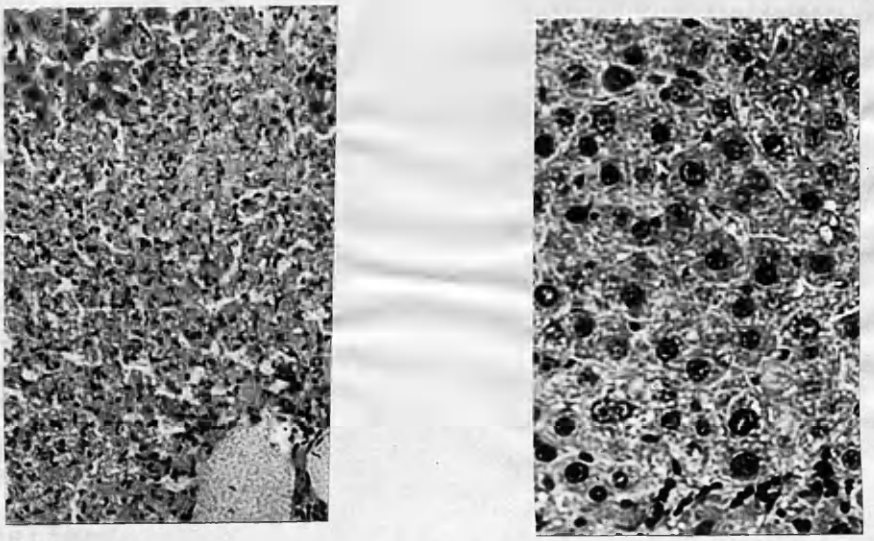
The mean blood sugar level after three hours fell by seventy-five per cent, when the rabbits were given soluble insulin alone, whereas with insulin and B.A.L. the mean blood sugar level fell by twenty-one per cent. In addition, it will be observed from figure 20 that insulin by itself produced a more precipitate and a more prolonged fall in the blood sugar level than did the insulin in the same animals treated with B.A.L.

4. The effect of B.A.L. on carbon tetrachloride poisoning in rats.

Glynn, Himsworth and Neuberger (1945) showed that necrosis of the liver in rats on synthetic amino-acid mixture diets did not develop if an adequate amount of cystine was added to these diets. Five amino-acid mixture diets were used; sulphur free, high methionine, high cystine, low methionine and low methionine plus cystine. The sulphur-free amino-acid mixture diet was common to the remaining diets but each of these had different supplements, namely, high methionine, high cystine, low methionine and low methionine plus cystine. Cystine could be replaced by methionine, which is converted into cystine in the body.

Both cystine and methionine contain a sulphydryl (-SH) group and Witts (1947) in his review of dietetic factors in liver disease stated that the above results in rats suggested that the essential factor in the prevention of dietary liver necrosis was the sulphydryl group, just as the methyl ($-CH_3$) group was the essential factor in preventing dietary cirrhosis.

In view of the above experimental findings it was considered worth while to observe if B.A.L., possessing two



A. B.

Fig. 21. Action of B.A.L. on the liver necrosis in rats produced by carbon tetrachloride. (× 150).

A. Carbon tetrachloride: necrosis of liver cells.

B. Carbon tetrachloride plus B.A.L.: necrosis of liver cells.

series showed necrosis of the liver cells. This is illustrated in figure 21.

Summary.

British Anti Lewisite (B.A.L.) has an L.D.50 of 100mg./kg. intraperitoneally in mice. It causes conjunctivitis, ataxia, rapid then impaired respiration and convulsions in small animals.

In anaesthetised rabbits, B.A.L. in small doses (4mg./kg.) causes a temporary rise in blood pressure, but in cats only a fall in blood pressure is seen. This is considered initially to be due to splanchnic dilatation, but constriction of the leg and spleen with an active beating heart and progressive fall in blood pressure is the main feature of the action of lethal doses of B.A.L. in anaesthetised cats. Loss of fluid from the capillaries, leading to haemoconcentration and a state of shock, is held to be the cause of death in anaesthetised animals, as convulsion is the cause of death in intact animals.

B.A.L. 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. Tissue damage caused by chronic poisoning with arsenic, mercury and chromium is prevented or relieved by B.A.L.

B.A.L. intravenously has a protective action on mice poisoned with mersalyl given intravenously. B.A.L. intraperitoneally has a deleterious effect on mice poisoned with mersalyl given intraperitoneally. Mersalyl and B.A.L. in large doses have each a delaying effect on the excretion of water from normal rats and in small doses effects are additive, but in large doses opposite.

B.A.L. inhibits the physiological action of insulin.

B.A.L. does not prevent necrosis of the liver in rats poisoned with carbon tetrachloride.

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SECTION III.

DISCUSSION.

B.A.L. and the metals.

The present work and that of other workers quoted has clearly shown that B.A.L. is an effective antidote to poisoning by arsenic, mercury, antimony, chromium, nickel and cadmium. In addition, McCance and Widdowson (1946) have shown that the closely related B.A.L. glucoside (O-glucoside of B.A.L.) promotes the urinary excretion of copper and zinc salts, while iron is unaffected. Braun, Lusky and Calvery (1946) showed that B.A.L. was ineffective in the treatment of rabbits acutely poisoned by salts of lead, selenium and thallium. In the case of lead and selenium the action of B.A.L. was additive. These findings with lead in rabbits confirm the present findings with lead in mice where B.A.L. was found to have an additive effect on the toxicity of the metal. Braun et al found that B.A.L. was an effective antidote in the treatment of acute bismuth poisoning in rabbits produced by sodium and potassium bismuth tartrate. The L.D.50 for bismuth in rabbits was found to be 55mg./kg. while with B.A.L. the L.D.50 was found to be 85mg./kg; thus showing that B.A.L. reduced the toxicity of the bismuth salt by 56%. This protective action of B.A.L. on acute bismuth poisoning in

rabbits is in contradistinction to the present findings with white mice. The L.D.50 for bismuth in white mice injected intraperitoneally was found to be 665mg./kg. while with B.A.L. the L.D.50 was found to be 315mg./kg; thus showing that B.A.L. increased the toxicity of the bismuth salt. Braun et al used sodium potassium bismuth tartrate while in the present work sodium bismuthyl tartrate was used. Both salts are water soluble and were chosen because of their ready solubility, rendering them more toxic and more easily absorbed than insoluble bismuth salts and therefore better able to produce acute bismuth poisoning readily. Braun et al gave the bismuth salt in a single large toxic dose intramuscularly into the gluteal muscles of the right leg, while the treated animals received, in addition, an intramuscular injection of B.A.L. into the gluteal muscles of the left leg according to the following schedule: a dose of 30mg./kg. of B.A.L. was administered one hour after the administration of the toxic dose of bismuth salt; doses of 15mg./kg. of B.A.L. were given at intervals of 6, 24, and 48 hours later. The rabbits were kept under observation, and death, when it occurred, was in two to nineteen days, with the greatest mortality from the second to the sixth day. At autopsy, the kidneys of the treated animals were essentially normal, while the kidneys of the untreated animals were severely

damaged. In the present experiments with white mice, the bismuth salt was given by intraperitoneal injection, likewise the B.A.L.; one injection following upon the other so that the probability of mutual reaction in the peritoneal cavity between the bismuth salt and B.A.L. is strong. The same conditions prevailed in the mice when B.A.L. was found to be an **effective antidote** for arsenic, antimony, mercury and chromium poisoning.

B.A.L. with gold and "mersalyl".

Cohen, Goldman and Dubbs (1947), Davison (1947), Lockie, Norcross and George (1947), and Ragan and Boots (1947), claimed that B.A.L. was effective in the treatment of toxic effects due to gold therapy arising in the treatment of rheumatoid arthritis with gold salts. It is interesting to note, however, that Cohen, Goldman and Dubbs pointed out that, although they achieved successful results clinically with B.A.L. in humans suffering from toxic effects of gold therapy, they were unable to substantiate these claims in the laboratory and were seeking an explanation of this anomaly. The present work on the effect of B.A.L. on the toxicity of gold in mice bears out their inability to substantiate the successful clinical use of B.A.L. on gold toxicity. In mice it was

found that, by intraperitoneal injection, B.A.L. increased the toxicity of gold salts. In the clinical trials quoted above, the B.A.L. was given by the intramuscular route some considerable time after gold therapy had ceased.

According to Long and Farah (1946) the intravenous injection of B.A.L. reduces the lethality of intravenous "salyrgan" in mice and protects the cardiovascular system of anaesthetised dogs from the toxic effects of this mercurial compound. In the present work the L.D.50 of "mersalyl", B.P. was found to be 155mg./kg. intraperitoneally in mice. B.A.L. 40mg./kg. given intraperitoneally immediately after the diuretic lowered the L.D.50 to 115mg./kg. In other words B.A.L. increased the toxicity of the "mersalyl". In contradistinction to this, the L.D.50 for "mersalyl" given intravenously to mice, was found to be 120mg./kg., and when B.A.L. was also given intravenously it raised the L.D.50 to 165mg./kg. In this case, when B.A.L. was given intravenously, it reduced the toxicity of the "mersalyl". Some clarification of this anomalous behaviour is provided by the results of tests of the effect of "mersalyl" in water diuresis in rats in the present work. B.A.L. 40mg./kg. intraperitoneally to rats acted as an antidiuretic, suppressing urine for three to four hours. "Mersalyl" 100mg./kg. given intraperitoneally to the rats

caused immediate anuria, and death followed after forty-eight hours, but if B.A.L. and "mersalyl" were given intraperitoneally, in the above doses, within a few minutes of one another, suppression was much less than when either had been given alone, and the rats survived. Further the animals, when watered and given subcutaneous saline, had a diuresis of which the peak occurred after 65 minutes; when given "mersalyl" 1mg./kg. and subcutaneous saline the peak occurred at 85 minutes. Repetition of "mersalyl" 1mg./kg. and subcutaneous saline after one week gave a figure of 83 minutes for the peak to occur, whereas "mersalyl" subcutaneously and B.A.L. 4mg./kg. intraperitoneally gave a figure of 90 minutes. It follows, therefore, that "mersalyl" and B.A.L. each have a delaying effect on the excretion of water from normal rats and that in small doses these effects may be additive, but in large doses these effects are opposite.

The anomalous behaviour of B.A.L. with certain metals.

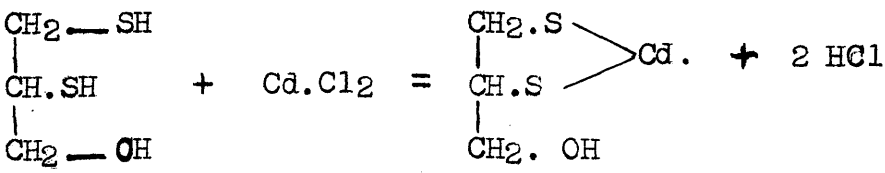
These differing results, obtained in the present work and the apparently contradictory results obtained by other workers, are capable of explanation by the outstanding feature of the chemical constitution of B.A.L., namely, the readily available supply of thiol (-SH) groups which it provides. Barron, Miller and Meyer (1947) and Webb and

and Heyningen (1947) point out, that B.A.L. inhibits the activity of any enzyme system by combining with the heavy metals which form the prosthetic group of the protein moiety of the enzyme. In other words, B.A.L. may be considered as a potent inhibitor of metal - containing enzymes, with the exception of the cytochrome system. Such an activity would account for the widely differing phenomena of B.A.L. poisoning, namely the convulsive action on the central nervous system, drop in blood pressure, interference with respiration and presumably also damage to the small blood vessels, which produces such marked alterations in the circulation. 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 in the lungs and liver, indicate damage to the capillaries and small vessels. The smooth muscle of the peripheral vessels appears to be much more sensitive to B.A.L. than the smooth muscle of the gut, uterus, the heart or coronary arteries and the differing initial response of the spleen and limb volumes to intravenous injection of B.A.L. needs further examination.

The formation of B.A.L. -metal complexes.

Peters, Stocken and Thompson (1945) postulated that heavy metals are toxic to biological enzyme systems, because they unite with the sulphydryl group (-SH) of the protein moiety of cellular enzymes, forming reversible mercaptides. It was a reasonable extension of this hypothesis that the dithiol B.A.L. reactivated poisoned enzyme systems or exerted therapeutic benefit in the treatment of heavy metal poisoning by the formation of mercaptides of sufficiently low dissociability to reverse effectively the combination of heavy metals with sensitive cellular enzyme systems. This concept received support in the treatment of arsenical poisoning when Peters et al demonstrated that B.A.L. reacted with lewisite to form a stable mercaptide, and that B.A.L. reversed effectively the in vitro inhibition of pyruvic acid oxidase, caused by arsenicals. The effectiveness of B.A.L. therapy in systemic poisoning with arsenic, both in experimental animals and in man has already been commented on and is well illustrated by the work of Peters et al, Eagle, Magnuson and Fleischman (1946) and Eagle and Magnuson (1946). These results prove the ease with which B.A.L. forms stable thioarsenites in vivo. The observation by Barron, Miller and Kalnitsky (1947), that the inhibition of -SH containing

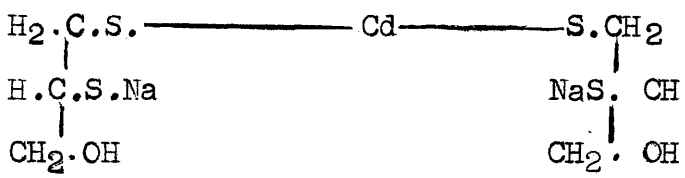
enzymes by mercury can be reversed by B.A.L., has its clinical application in the efficacy of B.A.L. in the treatment by experimental mercury poisoning, as shown by Gilman, Allen, Phillips and St. John (1946), and in the treatment of human mercury poisoning, as shown by Longcope and Luetscher (1946). The generalization, that heavy metals have a common basic mechanism of toxic action and can be antagonized by B.A.L., receives further support by the studies of Gilman, Phillips, Allen and Koelle (1946) on the action of the drug on cadmium *in vitro* and *in vivo*. Although systemic cadmium poisoning is uncommon clinically, the actions of the metal afford, better than those of arsenic and mercury, an experimental method for elucidating the mechanisms by means of which mercaptans reverse the toxic effects of heavy metals *in vivo*. Gilman *et al* studied the reactions between mercaptans and Cd + + *in vitro*. When solutions of cadmium chloride ($Cd.Cl_2$) were added to aqueous solutions of B.A.L., a copious white precipitate of Cd.-B.A.L. complex was formed and the reaction may be formulated as:-



B. A. L. Cd. - B.A.L. complex

The Cd.-B.A.L. complex was insoluble in water and organic solvents.

Under alkaline conditions it was found that 2 molecules of B.A.L. reacted with 1 molecule of Cd^{++} to form a soluble complex which was highly dissociated with respect to its components. The soluble complex was formulated as $Cd(B.A.L.)_2$ with the following structure:-



This soluble $Cd(B.A.L.)_2$ complex was presumed to be formed <u>in vivo</u>, when B.A.L. reacted with cadmium, and was used to explain the changes affected by B.A.L. therapy in the course of cadmium intoxication. The effect of B.A.L. (mercaptan) therapy on systemic cadmium poisoning was studied in the rabbit. The prophylactic administration of B.A.L. to rabbits, receiving lethal doses of cadmium chloride intravenously, resulted in a noted amelioration of signs of acute cadmium intoxication. The B.A.L. therapy was, however, without lasting benefit due to the rabbits developing fatal renal insufficiency. When B.A.L. therapy was started half to one hour following the intravenous injection of cadmium chloride, a significant reduction in mortality ensued but once again the symptoms and signs of acute cadmium intoxication were allayed

and the animals which died, exhibited extensive renal insufficiency. Therapy with B.A.L. glucoside gave the results obtained with B.A.L. but in addition, it was noted that the incidence and degree of renal damage in surviving animals was significantly less than that obtained with B.A.L. Tentative formulae for the mercaptides, formed when B.A.L. reacts with cadmium in vitro, were postulated and it was assumed that similar compounds were formed in vivo. The toxicities of these complexes were determined by intravenous injection to rabbits. The $Cd(B.A.L.)_2$ complex was found to be as toxic as $CdCl_2$ on a molar basis and had a marked nephrotoxic action. It is interesting to note that the cadmium mercaptides of B.A.L. glucoside, Cd-B.A.L. glucoside and $Cd(B.A.L.\ glucoside)_2$ were found to be less toxic than $CdCl_2$, and that relatively high doses of these cadmium B.A.L.-glucoside mercaptides did not produce evidence of nephrotoxic action. From these results it was concluded, that B.A.L. reacted with cadmium chloride in vivo to form a mercaptide of low dissociation, which, in the presence of excess B.A.L., directs the cadmium towards the kidney for excretion and thereby prevents poisoning of extrarenal loci. In the kidney, however, the mercaptide is concentrated in the epithelium of the renal tubule by glomerular filtration and absorption, and in the tubule intracellular oxidation of the mercaptide results in the

release of toxic amounts of cadmium. In the case of B.A.L. glucoside, mercaptides are found *in vivo* of low dissociation but remain extracellular, and are directed towards the kidney for excretion and are not reabsorbed in the tubule to the degree evidenced by the $Cd(B.A.L.)_2$ complex.

Tobias, Lushbaugh, Patt, Postel, Swift and Gerard (1946) investigated the experimental cadmium poisoning resulting from inhalation of cadmium fumes. Animals, fatally poisoned with cadmium by inhalation, die in the course of hours to a few days with massive pulmonary oedema and symptoms chiefly due to anoxic anoxia. Later deaths, occurring in a few days to a week or so, are the result of diffuse pneumonic consolidation of the lung with abscess formation. Long delayed deaths, occurring weeks to months after the inhalation of the cadmium, occur with incompletely healed pulmonary lesions and gastro-intestinal upset of anorexia, malaise, occasionally bloody diarrhoea and marked generalised wasting. The authors assumed that the action of cadmium ions on animal tissues was similar to that of other heavy metal ions, namely that the cadmium combined with the tissue protein sulphydryl groups to form insoluble metal proteinates or mercaptides and thus interfered with and impaired enzyme and other cellular functions as suggested by Peters, Stocken and Thompson (1945)

As B.A.L. had been shown by Peters *et al* to be effective

in preventing and reversing such combinations in the case of arsenic, it was the first choice of Tobias et al. B.A.L. proved effective but had a lower margin of safety than with arsenic. They assumed that cadmium was removed from vital tissue combinations by B.A.L. with the formation of the Cd(B.A.L.) complex and this was excreted mainly through the kidneys. Cd(B.A.L.) was prepared and, as expected on the basis of insolubility and stability, proved to be far less toxic than cadmium chloride. Like Gilman, Philips, Allen and Koelle (1946) they found that the soluble $Ca(B.A.L.)_2$ complex was highly toxic to the kidney. It was found that B.A.L. was effective in cadmium poisoning but was deleterious when given prophylactically for cadmium poisoning produced by inhalation. This result was held to be due to the fact that B.A.L. fixed more cadmium in the lungs than otherwise would have remained. B.A.L. must be used promptly, after exposure, to be effective and it was found that, in an optional course of repeated intramuscular injections, the mortality in mice was reduced from 93 to 7 per cent. In addition, B.A.L. greatly reduced the structural damage due to the cadmium in the body. By using the radioactive isotope Cd^{115}, it was demonstrated that over half of the inhaled cadmium chloride passed beyond the lung, by the end of a thirty minute exposure period. If

B.A.L. was present at the time of the inhalation of cadmium chloride, the cadmium was fixed in the lung by the B.A.L., presumably as the insoluble Cd-B.A.L. complex, in greater amount and was associated with greater lung damage. B.A.L. given after exposure to cadmium inhalation neither accelerated nor delayed the removal of cadmium from the lung, despite the fact that it decreased the lung damage. Hence, when B.A.L. was prophylactically administered, it apparently produced damage to lung tissue by holding a large amount of cadmium for slow release, whereas, when B.A.L. was given therapeutically, it reached the lung after much of the cadmium had left, diverted the cadmium already combined with lung tissue constituents and then released it so that most of the cadmium was removed. In the untreated animal, it was found that most of the cadmium passing from the lungs, after inhalation, was not fixed elsewhere and finally left the body in the gastro-intestinal tract, little being excreted through the kidney. B.A.L. therapy was found to shift the excretion of cadmium in the direction of the kidney.

Peters and Stocken (1947) prepared and investigated the toxicity of the compound of B.A.L. and "mapharside", which they called Maph-B.A.L. Experiments showed that the combination of B.A.L. and lewisite, producing lewisite-thioarsenite, had a toxicity of less than one fifth of

that of the parent compound, lewisite. Peters and Stocken were surprised to find that Maph-B.A.L. under certain conditions was more toxic than "mapharside". However, in agreement with the lewisite experiments, the toxicity of Maph-B.A.L. was reduced to one fifth that of "mapharside" in the presence of extra B.A.L. These workers considered that the difference in the independent toxicities of lewisite-B.A.L. and Maph-B.A.L. was remarkable and explanation of the toxicity of Maph-B.A.L. could not be given. The authors felt fairly certain, in view of the fact that reversal of the poisoning effect in rats was possible, that the relative toxicity of the compound was due to dissociation. It was also postulated, that the more rapid toxic effects of Maph-B.A.L. were due to a penetration to the sites which are dangerous to the rats, by virtue of the difference in physical properties of the Maph-B.A.L. from those of "mapharside". In this manner the arsenic would be liberated locally from the maph-B.A.L. and would exert its maximum toxic effect. The increased toxicity could then be explained as due to a decreased chance of elimination, as compared with "mapharside". In this connection, "mapharside" itself has a high water solubility which encourages elimination from the system. Repeated doses of B.A.L. 4mg./kg. to the rats were sufficient to save the animals from the toxic effects of Maph-B.A.L.

Hence, clinically, there is a strong argument for giving the maximum amounts of B.A.L. tolerable, because excess protects against any toxicity due to Maph-B.A.L. as well as "mapharside". Peters and Stocken remark that, though caution in interpretation is necessary, there is no present reason for supposing that any Maph-B.A.L. reaching the circulation due to the liberation of arsenic in the tissues will not have with it enough uncombined B.A.L. to stop the development of arsenical toxic reactions. The present work has shown that B.A.L. increases the toxicity of lead in mice when both are given by the intraperitoneal route. Comparing the results with "mapharside" and Maph-B.A.L. it is possible that the lead-B.A.L. complex is more toxic than lead.

B.A.L. with its two thiol groups can thus form various complexes with metals and as Gilman, Philips, Allen and Koelle (1946) point out in the case of cadmium, the ring compound formed <u>in vivo</u> by B.A.L. is a soluble substance which proves on isolation to have a greater toxicity than cadmium chloride itself. This increased toxicity is due to increased pathogenicity to the kidney. In the present work in mice it was found that B.A.L. was an effective antidote to metallic poisoning by arsenic, antimony, mercury and chromium whereas, under the same experimental conditions in mice, it was found that B.A.L. increased the toxicity of lead, bismuth and gold.

The arsenic, mercury, chromium and antimony preparations used to determine the L.D.50 in mice in the present work are highly irritant to the peritoneum, are all soluble compounds and quickly absorbed and cause gross renal damage on excretion. Treatment with B.A.L. intraperitoneally reduced the toxicity of these compounds and prevented visible damage to the kidney. The products of the chemical linkages between B.A.L. and these metals must be of such a nature that they are not toxic to the kidney on excretion. The reduction of the acute toxicity of the metallic salt may be due to production of a non-irritant compound by reaction with B.A.L. in the peritoneal cavity of the mice, which compound may be slower to absorb and easier to excrete in a non-toxic form, thus deviating the metal from sensitive extra-renal sites of action. It may well be that B.A.L. deviates these metals in the direction of the kidney, as in the case of cadmium, where the routes of excretion of cadmium and cadmium plus B.A.L. are different. Cadmium inhaled passes from the lung mainly to the gastro-intestinal tract for excretion, whereas cadmium inhaled plus intramuscular injection of B.A.L., as followed by the radioactive isotope Cd^{115}, causes the cadmium to be mainly excreted by the kidney. During this excretion by the kidney, the cadmium-B.A.L. complex is evidently deviated from sensitive extrarenal sites of action.

The salts of lead, gold and bismuth used had a much higher L.D.50 than the salts of arsenic, chromium, mercury and antimony and B.A.L. had no protective action against the former metals. The salts of lead, bismuth and gold, when given intraperitoneally, are not so irritant as the salts of arsenic, mercury, chromium and antimony; they tend to precipitate and absorb more slowly and thus their mode of action in producing acute lethality is probably different from the others. The B.A.L.-metal complex formed with these three metals, may absorb more quickly or be more toxic than the metallic salt itself.

A point of importance is suggested by the findings of Gilman, Philips, Allan and Koelle (1946) that the in vitro B.A.L.-cadmium complex differs markedly from the complex formed in vivo. In the case of the present work with mice the solutions of the metallic salts were given intraperitoneally followed very shortly by the B.A.L. in the same locus. Since both the solution of the metal and B.A.L. were injected consecutively into the peritoneal cavity, it is reasonable to assume that an in vitro type of precipitate or complex was formed in the cavity. This complex might well differ in stability, rate of absorption and toxicity from the in vivo type of compound formed in rabbits, injected intramuscularly at different sites with bismuth and B.A.L. by Braun, Lusky and Calvery

(1946). Analogous to the giving of bismuth and B.A.L. at different sites is the successful treatment of gold intoxications, arising in the treatment of rheumatoid arthritis and reported by Cohen, Goldman, and Dubbs (1947), Lockie, Norcross and George (1947), Ragan and Boots (1947) and Davison (1947).

In the present work, it was found that B.A.L. intravenously had a protective effect in mice against "mersalyl", given intravenously, whereas it had an additive effect when both were given consecutively intraperitoneally. Since B.A.L. has been shown in the present work to have a powerful antidotal action against mercuric chloride in mice, when both were given intraperitoneally, it is surprising that B.A.L. does not repeat this protective action against the mercurial diuretic "mersalyl". "Mersalyl" is the sodium salt of a complex organic acid derived from salicylic acid, and its solution in physiological saline is apt to become toxic, owing to decomposition with formation of ionised mercury. This decomposition can be inhibited by the presence of theophylline which is used for this purpose in the preparation of the official injection used in the present work. It might be suggested, that any comparison between the action of B.A.L. intraperitoneally and "mersalyl" and mercuric chloride is unfair, as the mercury in the "mersalyl"

is obviously prevented from being ionised whereas the mercuric chloride yields mercuric ions, readily available in solution to unite with the -SH groups of the B.A.L. This cannot be the reason as B.A.L. intravenously does protect mice, when toxic doses of "mersalyl" are given intravenously. It is much more likely that the same phenomenon of the formation of different mercaptides with different toxicities according to the in vivo conditions of the reaction of the metal and B.A.L. may account for the opposite action of B.A.L. on the toxicity of "mersalyl" given intraperitoneally and intravenously. In the water diuresis in rats, testing the effect of "mersalyl" and B.A.L., the present work showed that each compound had a delaying effect on the time of maximum water excretion from normal rats and that, in small doses of each of the compounds, these effects were additive, but with large doses of the compounds the effects were antagonistic.

Clinical Applications of B.A.L.

The possible clinical applications of B.A.L. are many. The arsenicals have a wide clinical sphere of usefulness and have been used in the treatment of syphilis, trypanosomiasis, relapsing fever, Vincent's angina, yaws, rat-bite fever and amoebic dysentery. During therapy with arsenic, complications may arise, the more serious being encephalitis haemorrhagica, haemorrhagic nephritis, dermati-

tis, agranulocytosis and jaundice. Eagle (1946) and Eagle and Magnuson (1946) have clearly demonstrated the great therapeutic usefulness of B.A.L. in treating arsenical complications. In a series of eighty-eight cases of arsenical dermatitis, fifty-one were of the exfoliative type and B.A.L., in the majority of cases, stopped the progression of the inflammatory reaction and hastened healing. The average time for almost complete recovery was claimed to be thirteen days. It was pointed out by Eagle that, after the administration of B.A.L., the inflammation and oedema of the skin subsided, the temperature fell and there was marked subjective improvement. The longer the dermatitis had lasted before B.A.L. was given, the longer was the time required for definite improvement. Cases of mild dermatitis with only pruritus and erythema responded in twenty-four to forty-eight hours. Fifty-five cases of toxic encephalitis were treated with B.A.L. therapy with the result that forty-four recovered completely within one to seven days, while the remaining eleven died. Delay in initiating treatment or too small dosage may have been the cause of failure in some of these cases. Ten out of eleven cases of agranulocytosis recovered but in three cases of aplastic anaemia due to arsenicals, there was no beneficial effect from B.A.L. therapy. Of four cases of massive overdosage with oxyphenarsine hydrochloride three patients responded promptly

while one patient died, due to too small a dosage of B.A.L. being given. Forty-four cases of fever due to arsenic recovered promptly with B.A.L. therapy.

Eagle suggests that, in severe arsenical complications, a dosage of 3mg./kg. should be given at each injection. For the first two days, six injections are given at four-hourly intervals, on the third day four injections, and then two injections daily for ten days or until complete recovery. In mild cases 2.5mg./kg. are given at each injection in the same schedule as above. Side effects are rarely seen at a dosage of 2.5 to 3mg./kg.

Carleton, Peters, Stocken, Thompson and Williams (1946) treated thirty cases suffering from arsenical dermatitis, mostly following injections of neoarsphenamine. Twenty-one of the patients were treated by intramuscular injection, the remainder by inunction with a five per cent B.A.L. ointment. Injection therapy was much more satisfactory than inunction therapy and proved to be the method of choice. The authors reported that clinical evidence indicated a beneficial effect in a substantial number of cases. Longcope, Luetscher, Wintrobe and Jager (1946) reported on twenty-two cases of arsenical dermatitis treated with B.A.L. Seven of the patients had an intractable localized dermatitis, caused by diphenylamine chlorarsine, which improved within a few days as the result of inunction with

B.A.L. ointment up to ten per cent strength. There were fifteen cases of generalised exfoliative dermatitis, following the use of arsenicals for the treatment of syphilis, and symptomatic and objective improvement following B.A.L. therapy with inunction or by intramuscular injections. The duration of the dermatitis in over half of the patients was shorter than a comparable control group, who were not treated with B.A.L. The authors point out that B.A.L. in ointment was quite painful, when applied to inflamed skin and were of the opinion that intramuscular B.A.L. therapy was much less disturbing. No serious side effects were observed during the course of the treatments.

These remarkable advances in the treatment of arsenical intoxications by B.A.L. remove a great source of anxiety to the physician using the modern potent organic arsenical preparations. In peace and in war, when intensive therapy with arsenic might have to be carried out, it is comforting to know that in B.A.L. the physician has a drug, which if used promptly and in sufficient dosage, can overcome such a fatal complication of arsenical therapy as encephalitis. In exfoliative dermatitis due to arsenic the duration of treatment is greatly reduced. It is not too much to claim that, what adrenaline is to the asthmatic, B.A.L. is to the patient undergoing intensive courses of arsenical therapy for syphilis. Henceforth toxic reactions arising from

arsenical therapy in the treatment of syphilis, trypanosomiasis, yaws and amoebic dysentery need no longer be so alarming. In industrial medicine toxic reactions to arsenicals have a useful antidote in B.A.L.

Longcope and Luetscher (1946) successfully treated twenty-three cases of acute poisoning by mercuric chloride with intramuscular injections of B.A.L. Although acute poisoning with mercuric chloride is rare clinically, it is a matter of supreme clinical importance that in B.A.L. physicians have such a powerful antidote to mercury poisoning. It is reasonable to suggest that B.A.L. therapy should be tried in industrial plants manufacturing mercury preparations and where dermatitis and mercurialism are produced, either industrially or during therapy with mercury. B.A.L. would seem to be indicated for foetor of the breath, the soreness of the gums and painful teeth, increased salivation, anorexia, weakness, anaemia due to chronic poisoning with mercury, and for the rarer cases when the brunt of the mercury poisoning falls on the central nervous system, as evidenced by tremors and weakness of the affected muscles. Hence, for acute and chronic mercury poisoning, B.A.L. would seem to offer a more than useful adjunct to present forms of treatment.

Antimony is poisonous to protozoa and is widely used in the treatment of leishmaniasis, trypanosomiasis,

bilharzias, filariasis, yaws, oriental sore and relapsing fever. The toxic reactions arising from therapy with antimony resemble those due to arsenical poisoning. During treatment of the above diseases, constant watch has to be kept for the development of toxic reactions. With B.A.L. effective as an antidote to antimony poisoning it is recommended that this drug should be extensively tried when toxic reactions to antimony arise in the course of antimonial therapy.

Chromium has been recognised for some time as an industrial hazard, both in the mining of the metallic ores and in those industries which use chromates and dichromates for dyeing, electrotyping and chrome-plating. The fumes arising in the process of chrome-plating constitute an important industrial hazard. Chromic poisoning produces local irritation such as severe dermatitis, ulcers and nephritis. In industrial fume poisoning, the damage occurs mainly in the skin and mucous membranes. The exposed hands and forearms develop a diffuse dermatitis which progresses to simple or multiple ulcerations - these are known as "chrome holes". Inflammation of the nasal mucosa may proceed to perforation of the septum and sometimes extends to the bronchioles. The ulcerations are slow in developing but penetrate deeply and only heal with difficulty Treatment is very unsatisfactory, and the most effective protection is proper ventilation.

The present work has demonstrated that B.A.L. has an antidotal action on chromium poisoning in mice raising the L.D.50 of chromium trioxide from 67mg./kg. to 86mg./kg. It has also been shown that the treatment of chrome ulcers, produced in guinea pigs, healed much more quickly on treatment with B.A.L. in ointment form than the chrome ulcers in the control series of guinea pigs. This evidence of the effectiveness of B.A.L. in acute chromium poisoning in white mice is substantiated by the work of Braun, Lusky and Calvery (1946), who determined the effect of B.A.L. on acute chromate poisoning in rabbits. The L.D.50 for potassium chromate by intramuscular injection was 55mg./kg. and B.A.L. by intramuscular injection raised the L.D.50 to 85mg./kg.

These results, proving the antidotal effect of B.A.L. on chromium poisoning in experimental animals, are strong presumptive evidence for recommending therapeutic trial of B.A.L. in the various manifestations of chrome poisoning met with clinically. It would appear that chrome dermatitis would yield to a course of intramuscular injections with B.A.L. Where the chrome damage is deeper, as in chrome ulcers and perforation of the nasal septum, treatment both locally with B.A.L. ointment and systemic intramuscular B.A.L. is recommended.

Braun, Lusky and Calvery (1946) concluded that B.A.L.

is not effective in the treatment of either acute or chronic lead poisoning. Acute lead poisoning was produced in rabbits by a single toxic dose of lead nitrate given intraperitoneally and B.A.L. in doses of 10mg./kg. daily were given by intramuscular injection for ten days. The animals treated with B.A.L. had the greatest mortality. Chronic lead poisoning was produced in rabbits by daily intraperitoneal injections of lead nitrate for fourteen days. Seven days were allowed to elapse before therapy with B.A.L. was commenced. B.A.L.was administered by daily intramuscular injection and it was found that all the rabbits receiving B.A.L. died, while the control animals survived. The present work on mice confirms these findings. The acute poisoning effect of lead acetate given intraperitoneally in white mice was increased by the intraperitoneal injection of B.A.L. Both series of experiments would appear to indicate that the route of administration of B.A.L. in animals has no effect on decreasing the toxicity of lead. However, in view of the successful reports of B.A.L. therapy in the treatment of toxic reactions to gold salts, it would seem that B.A.L. might be given a careful trial in cases of human lead poisoning in the view that the results of animal experiments (acute) and human therapeutic trials (chronic) have proved to be contradictory in the case of gold and bismuth.

With regard to therapy with B.A.L. in bismuth and gold intoxications, the present work has indicated that B.A.L. increased the toxicity of both these metals in mice when B.A.L. and the metal were given intraperitoneally. As discussed, Braun, Lusky and Calvery (1946) found that B.A.L. was effective in acute bismuth poisoning, when both substances were given by the intramuscular route. The work of Cohen, Goldman, and Dubbs (1947), Davison (1947), Lockie, Norcross and George (1947), and Ragan and Boots (1947), has been discussed, wherein the authors reported successful treatment of gold intoxications, arising in the chrysotherapy of rheumatoid arthritis, with intramuscular B.A.L. therapy, although as Cohen, Goldman and Dubbs state there were no animal experiments to suggest this use of B.A.L. The authors also state that the number of cases is too small to make dogmatic statements. From the laboratory aspect and the clinical aspect it is evident that further experimental and clinical work will have to be carried out before the final assessment can be made of the value of B.A.L. in the treatment of clinical cases of bismuth and gold intoxications.

In all the therapeutic applications in which B.A.L. is used and to which it may be put, it must be realised that the drug itself is toxic. The present work has shown particularly, the toxic effects on the central nervous

system, as evidenced by its convulsant actions; on the respiratory system by impairing respiration; on the cardiovascular system by causing fall in blood pressure, and on the peripheral circulation by causing vasoconstriction and loss of fluid from the capillaries, leading to haemoconcentration and a state of shock, held to be responsible for the cause of death in anaesthetised animals. Convulsions were considered to be the cause of death in intact animals.

Although the therapeutic benefits to date by far outweigh the dangers due to the toxicity of B.A.L., nevertheless, the use of a less toxic drug with the same action would be advisable. B.A.L. glucoside discovered during the war years by Danielli, Danielli, Mitchell, Owen and Shaw (1947) would appear to be the answer to the problem. These workers considered that B.A.L. was too toxic in large doses and experimented to find, if possible, a non-toxic thiol which had the following therapeutic desiderata: be capable of penetrating the whole vascular system; prevent arsenicals from passing from the blood stream into the tissue cells; be capable of removing arsenic from the cells into which it had penetrated; be relatively non-toxic and finally the drug should be capable of uniting with the arsenic to form a compound readily excreted and non-toxic. O-glucoside of B.A.L. (B.A.L. Intrav.) was discovered and answered best to the required desiderata. The toxicity of

B.A.L. glucoside was found to be much less than that of B.A.L. The L.D.50 of the compound for rats was found to be 7.5g/kg., while the L.D.50 of B.A.L. for rats was found to be 50mg./kg., i.e. B.A.L. glucoside was 150 times less toxic to rats than B.A.L. Furthermore, at doses of 1-2g/kg. B.A.L. glucoside caused no pathological symptoms in rats, rabbits, guinea pigs and goats and was found to be protective against lewisite poisoning in these animals. When the treatment of experimental arsenical poisoning was delayed for six and a half hours the mortality from lewisite poisoning was fifty per cent, and it was considered that this was due to the slow removal of the arsenic from the tissues by the B.A.L. glucoside. By giving B.A.L. in conjunction with B.A.L. glucoside it was found that B.A.L. acted as a carrier of arsenic between the cell and the B.A.L. glucoside in the blood and the mortality was reduced.

This reduced toxicity of B.A.L. glucoside was confirmed by McCance and Widdowson (1946) in male volunteers. These workers found that 100mg./kg. of B.A.L. glucoside given intravenously to male volunteers produced no ill-effects. This result is in striking contrast to intramuscular B.A.L., which when given in single doses of 3-5mg./kg. to humans is apt to produce toxic results previously discussed.

The above results would suggest that B.A.L. glucoside is to be preferred to B.A.L. in therapy. Whether the B.A.L.

glucoside should be given alone or combined with B.A.L. demands further clinical trials.

The present work and the work of others quoted, clearly demonstrates that B.A.L. and B.A.L. glucoside occupy an important place in therapeutics. Until their advent there existed no cure for arsenical poisoning. It was essential that industrial hazards from arsenic such as might be encountered in the manufacture of glass, horticultural products, medicinal chemicals and pigments should be controlled by preventitive measures. Workers who contracted arsenic poisoning, even with the best preventitive measures in use, were doomed to a spell of poor health. These drugs now would seem capable of aiding considerably the present preventitive measures and capable of curing arsenic poisoning when it arises. Criminal poisoning by arsenic, foodstuffs contaminated with arsenic, and arsenical warfare agents can now be controlled. The use of arsenic derivatives in medicine has become of great importance particularly in the treatment of syphilis and many tropical diseases. With the proper use of B.A.L. and B.A.L. glucoside, the physician has a potent weapon for combating toxic reactions and can also consider more intensive treatment with arsenicals knowing that he has a potent antidotal agent at hand. These drugs are also effective in mercury, antimony, chromium, cadmium and nickel

poisoning and possibly with other metals not yet tried. Successful reports have been described in the treatment of toxic reactions due to therapy with gold salts and their clinical trial in lead poisoning should perhaps be considered.

In conclusion, British scientists have produced a therapeutic agent, due to the exigencies of war, which combats arsenic and mercury poisoning and probably other heavy metal poisoning in the various ways in which such poisoning may arise whether therapeutically, industrially, or as a result of war. The full potentialities of these drugs have yet to be explored and the rational biochemical approach to their discovery will no doubt help to unravel the importance of enzyme systems in their various complex functions in the body. B.A.L. also provides a "research tool" of great interest and value to the biochemist and pharmacologist, which may ultimately prove to be the greatest benefit accompanying the introduction of this potent and versatile substance.

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