REVIEW ARTICLE

THE NITROGEN MUSTARDS

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Introduction

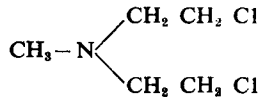
Mustard gas is well known for its local irritant action. It may also produce systemic disturbances if any is absorbed into the blood stream[1]. The nitrogen mustard derivatives were extensively investigated as potential chemical warfare agents in the recent war and particular attention was paid to their systemic effects. Animal studies showed that they inhibited the division of actively proliferating cells in the hæmatopoietic and lymphatic tissues. This action suggested that nitrogen mustard derivatives might be of value in the treatment of neoplastic conditions arising in the bone marrow or lymph glands. Clinical trials were started in the United States in 1943 and the first results collected from the various centres of investigation were published in 1946[2]. It was found that remission could be obtained in a number of neoplastic diseases. Unfortunately however serious toxic reactions were frequently encountered, because normal tissue is nearly as susceptible as tumour tissue to the action of the drug.

In the last five years widespread trials have made clear the therapeutic indications and the limitations of the original nitrogen mustard compounds. Research is now being directed to the synthesis and trial of new analogues. It is desirable to separate the toxic from the palliative action and if possible to find a curative agent.

The Chemistry of the Nitrogen Mustards

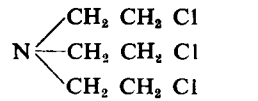
Original Derivatives. Gilman and Philips[3] in 1946 reviewed the chemistry of these compounds. The two simplest are

Methyl bis (b-chloroethyl) amine (the " bis " compound, or HN_2)



and

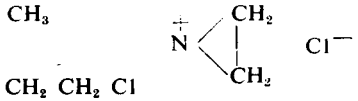
Tris (b-chloroethyl) amine (the " tris " compound, or HN_3)



Most of the clinical trials have been conducted with the " bis " compound; it has a powerful tumour-inhibiting action and is the only nitrogen mustard commercially available in this country to-day. In August, 1950, the British Pharmacopœia Commission selected " Mustine " as the Approved Name for methyl bis (β-chloroethyl) amine, it is however marketed and used clinically as the hydrochloride not as the free base.

The " tris " compound has an equally powerful action, but has been largely discarded on account of its toxic effects, particularly a tendency to cause thrombosis on intravenous injection.

When these compounds are dissolved in water the molecule undergoes rearrangement, the nitrogen becomes pentavalent and a cyclic imonium compound is formed.



This imonium compound is very active and may become attached to chemical groups of body substances or be hydrolysed to an inactive chlorhydrin. The second β-chloroethyl group then undergoes rearrangement to form a much less active imonium compound and that finally hydrolyses. The formation and hydrolytic inactivation of the first imonium derivative proceeds very rapidly, the half life period of the " bis " compound in neutral solution is about 90 seconds at body temperature[4]. In practice the preparation used is the hydrochloride, and this gives an acid solution in water which is more stable[5]. On injection into a vein the solution is brought to the *p*H of blood plasma, but the high concentration of the chloride ion probably reduces imonium formation. When the molecule enters the cell, the chloride concentration is minimal and imonium ions are formed[6]. They react with amine, carboxyl or sulphydryl groups of the nucleus. An attempt to determine the site of imonium formation has been made with diethyl-iodoethylamine synthesised with radioactive iodine and given to mice. Comparison of the distribution of radioactivity with that following the injection of radioactive iodide suggested that the iodine was not being liberated from the nitrogen mustard derivative in the blood plasma[7].

New Compounds. The search for possible new therapeutic agents led first to the substitution of other halogens for chlorine in the original derivatives. No useful progress has been made in this direction[8]. The chemical grouping necessary for nitrogen mustard activity consists apparently of two chloroethyl groups attached to a nitrogen atom. It has however been shown that di-epoxides produce similar changes[9].

The basic *bis* chloroethylamine grouping can be modified in many ways with different aliphatic radicals and by the formation of double compounds. Burchenal[10] in the United States has been working on this problem and clinical trials have been made with a number of derivatives. 1:3-*bis* [*bis* (β-chloroethyl)] aminopropane dihydrochloride (SK 136) has been used for a number of patients[11,12,13,14]. It has been claimed to cause rather less nausea and vomiting than the " bis " compound but it may give rise to bad dreams, dizziness and headache. It does not appear to have any appreciable advantage over the original derivatives. Haddow[15] in this country has been studying aromatic substitution compounds. He has synthesised β-naphthyldi-2-chloroethylamine (R 48) which has possible therapeutic value. It is active when given by mouth although the action is slower and less powerful than the " bis " compound.

It does not produce the troublesome nausea and vomiting that so frequently follow the injections, but has the same action on the blood forming organs and may in addition cause a hæmorrhagic cystitis[16].

Screening Techniques. Several accounts have been published of the methods used for preliminary testing of new compounds in this series[4,10,17,18]. The first step is usually to determine whether the compound inhibits the development of leukæmia or sarcomata in mice. Compounds that are shown to have considerable activity are then tested for toxicity in animals and finally clinical therapeutic trials are initiated.

Experimental Studies of the Actions of the Nitrogen Mustards

The most striking changes seen in animals after the administration of the nitrogen mustards are in the nervous and reticulo-endothelial systems. Less marked changes occur in the gastro-intestinal tract the adrenals, testicles and skin. It has also been demonstrated that the mechanisms of blood coagulation and antibody production are affected. Very detailed studies have been made of the actions of these drugs on tissue cultures and mammalian cornea, but these will be considered in a subsequent section.

Neurological Effects. Large doses of the " bis " compound given to small animals produce neurological disturbances that are rapidly fatal. Parasympathomimetic, convulsant or paralytic phenomena may be observed[19], and histologically demyelination has been found in about one third of the animals[6]. The parasympathomimetic action is apparently related to the cyclization of the first chloroethyl group[20], whereas the paralytic action is produced by the methyl β-hydroxyethyl ethylenimonium derivative[20,21,22,23]. With the " bis " compound doses sufficient to produce neurological signs are invariably fatal, other nitrogen mustards however may give rise to permanent cerebellar or brain stem disturbances of movement[24].

Bone-marrow Effects. Mitotic division of the primitive white cells or red cells is inhibited, and these cells may largely disappear from the marrow[25,26]. Marrow culture studies have confirmed that the cells that fail to divide are actually killed[27]. The reticulum cells are apparently more resistant to the action of nitrogen mustard, but it has been suggested that they may be affected by the abnormal phagocytic activity required for the removal of the damaged cells[26]. If the aorta is occluded for 15 minutes after the injection, the marrow of the caudal part of the body escapes damage[28]. There is evidence in animal studies that if the red cell series is stimulated by the administration of phenylhydrazine the cells may be more resistant to subsequent nitrogen mustard action[29].

Spleen. Reduction occurs in the size of the spleen[19,25,26].

Lymphatic Glands. Necrosis of the germ centres has been observed within 5 hours of an injection of the " bis " compound and the lymphatic glands and thymus become smaller[19,25,26]. These changes are not mediated through the suprarenal cortical steroids as in the alarm reaction for they may still be demonstrated after adrenalectomy[28,30].

The Blood. There is a rapid fall in the number of circulating lymphocytes and a more gradual decline in granulocyte and red cell counts[19,25,26]. It has been suggested that the rapid disappearance of the lymphocytes may be due partly to their escape through the damaged gut wall[26].

Gastro-intestinal Tract. Vomiting and hæmorrhagic diarrhœa are seen in some animals and may be fatal through losses of fluid and electrolytes[5,19,31]. The vomiting is probably due to a local action on the gastric mucosa, but it has been suggested that it is partly central in origin[3]. The diarrhœa may be reduced by occluding the blood supply to the intestine for 15 minutes after the injection, but although the fluid loss is largely prevented, the animals may still die[31]. Histological changes have been demonstrated in the gastro-intestinal tract[19].

Adrenal Glands. Hypertrophy of the suprarenals with marked reduction of cortical cholesterol follows injections of the " bis " compound[32].

Liver. No change has been observed in normal liver cells, but regeneration of a rat's liver after partial hepatectomy is delayed by nitrogen mustard[33].

Kidney. No histological evidence of kidney damage has been found, but functional disturbances may aggravate the loss of fluid and electrolyte associated with gastrointestinal tract lesions[3].

Testicle. Temporary testicular damage has been demonstrated after large doses[34].

Hair. Subcutaneous injections in animals may lead to permanent greying of the hair at the site of the injection[35].

Antibody Production. The disappearance of the lymphocytes, unlike that following suprarenal cortical hormone, is not followed by an increase of circulating antibodies[36,37]. If the " bis " compound is given shortly before or at the same time as an antigen, production of antibodies is partially inhibited[36,37,38], and the development of the Schwarzman phenomenon may be prevented[39]. " Hypersensitivity " responses to foreign proteins, including renal lesions, are reduced by the administration of nitrogen mustard[40,41].

Complement. There has been one report of inhibition of complement[42].

Blood Coagulation. Prolonged clotting time and reduced heparin tolerance may follow injections of the " bis " compound. These disturbances may be corrected with intravenous heparin or toluidine blue[43].

Tumorigenic Action. The injection of the " bis " compound may be followed after a considerable interval by the development of a tumour. The neoplasm may appear at the injection site[44], in the lungs[45] or elsewhere[46].

Distribution in the body. Studies with radioactive elements incorporated in the molecule have shown that iodoethylamines are accumulated principally in the lungs, lymph glands and blood[7]. Injected " mustard gas " synthesised with radioactive sulphur is found in highest concentration in the lungs and kidneys. The bone marrow takes up comparatively little and the changes produced there are probably related to the greater sensitivity of the cells[47].

Cause of Death in Animals. Early deaths from large doses are associated with neurological disturbances. In some animals it may result from the loss of fluid and electrolyte through gastrointestinal changes. Damage to the blood-forming organs rarely leads to death in animal studies. It has been shown in animals that a dose of nitrogen mustard produces greater changes if given as a single injection as opposed to several small injections at short intervals[33].

Viruses, Bacteria, Protozoa. If influenza virus[48] or trypanosomes[49] are exposed to nitrogen mustard, their capacity for infecting mice is reduced. *Bact. coli* may acquire resistance to dilute solutions of the " bis " compound[50]. Concentrations of 500 mg./l. are viricidal and bactericidal in blood, and it is claimed that if the blood is then kept for 5 days it becomes non-toxic[51].

Mutation. Solutions of the " bis " compound produce mutations in *Penicillin notatum*[52] in *Drosophila*[53] and in strains of *Bact. coli*[50].

The Mechanism of Nitrogen Mustard Action

Animal studies have shown that the larger doses of nitrogen mustard act chiefly on the nervous system whereas with smaller doses changes are seen in tissues where there are actively proliferating cells. Research into the mechanism of these actions has proceeded along two main lines—the effect of dilute solutions on enzyme preparations, and observation of details of cellular changes produced in tissue cultures and intact tissues.

Enzyme Studies. Very dilute solutions of the " bis " compound inhibit the choline enzymes, oxidase, esterase, and acetylase. The neurological symptoms of nitrogen mustard poisoning may be produced in this way[54]. The inhibition may result from combination of the ethylenimonium ring with the SH groups of the enzymes[55], or alternatively the imonium ion, a quaternary ammonium derivative, may compete with choline for attachment to the enzyme[54]. Barron[54] has also reported the action of dilute solutions on the enzymes of carbohydrate metabolism. Pyruvate oxidase and phosphorylation enzymes are inhibited.

Tissue Preparations. Mitotic activity is brought to a standstill in cultures of embryonic tissue coming in contact with nitrogen mustard solutions[56,57,58]. In cultures of tumour tissue similar cytological changes have been demonstrated[57]. Friedenwald and his collaborators have made detailed studies of the changes produced in the mammalian cornea by nitrogen mustards[59,60]. Mitotic arrest and nuclear fragmentation have been found and also disturbances of the metabolism of the corneal stroma. Disturbances of metabolism have been demonstrated in other tissue preparations. Glycolysis is inhibited in human and rat skin[61], and oxygen consumption by slices of normal viscera[54] or of mouse sarcoma[62] is reduced.

Abnormalities of mitosis. The effect of the nitrogen mustards on cell proliferation is intimately related to abnormalities produced in mitotic division. Dustin[63] has reviewed the action of the various mitotic poisons and pointed out that the nitrogen mustards, like irradiation, lead to an inhibition of the prophase. This is followed by nuclear fragmentation

and death of the cell. It has, however, been observed that this nuclear fragmentation is more conspicuous after nitrogen mustard than after exposure to X-rays and it is possible that the actions are not identical[60]. The mitotic disturbances are probably due to damage to the chromosomes. It has been suggested that " chromosome stickyness " is increased[35]. They may be held together by attachment to the two chloroethyl groups[64] or as a result of a physical change in their thymonucleate coating[65,66]. The mitotic inhibition cannot be related to interference with nucleic acid phosphorylation[67].

Relation of Nitrogen Mustard Action to Irradiation

Nitrogen mustard and irradiation produce similar changes in the cell nucleus. Further comparisons have shown that the effects of the " bis " compound are produced more rapidly and are more severe, whereas those following exposure to deep X-rays develop slowly and are generally less intense. Recovery on the other hand starts earlier and is completed more quickly after injections of the " bis " compound[4]. Attempts to equate the effects produced in small animals suggest that a dose of 0·4 mg./kg. of the " bis " compound corresponds to overall irradiation of 140 r.[68] or 1·0 mg./kg. to 300 to 400 r.[21].

Clinical Applications

The results of animal studies suggested that the nitrogen mustards might produce a selective inhibition of actively proliferating tumours, particularly those originating in the blood-forming organs. The best results in clinical trials have been obtained in neoplastic conditions of hæmatopoietic and lymphatic tissues—the leukæmias and the lymphomata. The results in other neoplastic diseases have with one exception proved disappointing. The inhibition of experimental hypersensitivity phenomena in animals has led to an extension of the therapeutic trials to human disorders that are thought to be caused by comparable mechanisms. Unfortunately the practical value of the nitrogen mustards has been greatly reduced by their action on normal hæmatopoietic and gastrointestinal tissues. The gap between therapeutic and toxic dose is virtually non-existent, anæmia, leucopenia and thrombocytopenia as well as troublesome nausea and vomiting follow the injections. The local irritant action which nitrogen mustard shares with the original sulphur mustard restricts the route of administration and may lead to additional complications.

Administration of Nitrogen Mustard. The hydrochloride of the "bis " compound is a white crystalline salt. It is available in 10 mg. bottles and immediately before injection should be dissolved in 10 ml. of sterile saline solution. If the solution is allowed to stand before being used some hydrolysis will occur. The " bis " compound must be administered intravenously. If injected straight into a vein with a syringe, thrombosis often follows. It is now usually injected into the tubing of a fast running intravenous saline drip infusion[69]. Veins partly occluded by tumour

should be avoided as they have a greater tendency to thrombose. If any of the solution escapes outside the vein severe inflammatory changes will be produced[70]. Solutions of the hydrochloride are not dangerous on the intact skin[21], but sensitisation dermatitis has been reported[71]. Intra-arterial administration has been tried in some cases[72] and intrapleural administration for patients with pleural effusion and multiple neoplastic deposits[73].

Dosage. The amount of nitrogen mustard that can be given as a single injection is limited by the nausea and vomiting produced. The amount that can be given in a course of injections over a short time is limited by the vulnerability of the bone marrow. It was originally suggested that a course should consist of four injections each of 0·1 mg./kg. of body weight on consecutive days[2]. The tendency now is to give fewer but larger doses [74], and up to 0·6 mg./kg. has been given as a single injection[75] and courses of as much as 3 mg./kg.[76]. In general, however, it is more satisfactory to adopt a conservative attitude and not to exceed 0·4 mg./kg. in each course and to limit the size of an individual dose to 0·2 mg./kg.[69].

Repetition of Courses. If two courses are given in rapid succession dangerous depression of the bone marrow may result. Provided the blood has returned to normal 4 weeks may be regarded as a safe interval[77].

"Prophylactic Therapy." If in any patient nitrogen mustard therapy is proving helpful, attempt may be made to keep the patient in prolonged remission by giving injections of 0·2 mg./kg. at regular intervals[69]. This is in contrast to the more usual procedure of awaiting a relapse before repeating the course of treatment. It has been suggested that the side reactions may be more severe after these "maintenance injections" [78].

Use in conjunction with X-ray therapy. Experimental studies in animals have shown that if irradiation is used before the "bis" compound the effects are not summated, whereas if the "bis" compound is given first more intense changes may be produced[4]. Attempts to combine the two forms of treatment have not led to spectacular results[73,78], and may be dangerous because of the difficulty of assessing the effects on the bone marrow.

Combination with Urethane treatment. Animal experiments have suggested that giving urethane with nitrogen mustard leads to increased action on lymphatic and neoplastic tissues[30,79]. Only one clinical trial of combined therapy has been reported and the details given are too meagre for critical evaluation[80].

Therapeutic Indications

Before nitrogen mustard therapy is recommended for any disease, a number of factors must be taken into account. It is not sufficient for the treatment to produce temporary reduction in the size of the tumour. Improvement in the patient's general condition and relief of symptoms for a reasonable period are criteria of satisfactory palliation[77,81]. The

discomforts and dangers of the toxic reactions must be considered and finally the results must be compared with those to be expected from alternative methods of treatment. Rhoads has summarised 1,100 reports of the use of the " bis " compound in the collective American trials and draws some, perhaps rather optimistic, conclusions concerning the indications for nitrogen mustard therapy[2,82].

Biopsy studies in a number of cases have confirmed the startling changes produced by this treatment in some neoplastic diseases[78,83,84,85,86]. There are several reports of autopsies carried out shortly after a course of the " bis " compound, in which little or no remaining neoplastic tissue could be found. The cause of death presumably being either the toxic action of the drug or previous disorganization of the patient's tissues produced by the neoplasm[87,88,89].

The alternative treatment for most of the diseases in which the " bis " compound has been tried is deep X-ray therapy. In assessing the value of the new agent, it is therefore necessary to attempt to compare the results obtained by the two methods. The suggestion is often made that nitrogen mustard therapy may be helpful when the neoplasm becomes " radio-resistant." This is an unsatisfactory term and it is seldom defined in case reports. It may mean that the tumour cells have ceased to respond to X-ray therapy, or alternatively that X-ray therapy has ceased to be practicable either because there are no tumours suitable for treatment or because the limit of skin tolerance has been reached[69]. Karnofsky has pointed out that the mechanism of action of the two agents is similar, and that if the tumour cells are resistant to irradiation, they will not respond to nitrogen mustard[68]. In the other types of so-called X-ray resistance the " bis " compound may produce a good remission.

The Lymphomata. Neoplastic conditions of the lymphatic system are grouped together under this heading. It has been divided into various subgroups in a number of systems of classifications. Pathologists unfortunately often differ in their interpretations of these classifications and it is simpler to consider only the well defined types of lymphomata.

Hodgkin's Disease. The best results of nitrogen mustard therapy have been obtained in this condition. In early cases with localised changes both X-rays and the " bis " compound will cause regression, that following irradiation lasts considerably longer, however, and therefore X-ray therapy is the treatment of choice. If the signs of the disease are widely disseminated and the patient is febrile, the " bis " compound is likely to lead to a more complete remission[2,74,78,90]. Certain manifestations usually respond well, for example skin lesions[70,91], paraplegia[92] or pulmonary infiltrations [93,94], whereas with generalised skin irritation or bone lesions the results are likely to be disappointing[70,90]. Large glandular masses causing obstructive symptoms may, with X-ray therapy, show initial enlargement before ultimately responding. Treatment with the " bis " compound does not produce any swelling and is therefore a safer method[91]. The remissions produced by nitrogen mustard vary in duration from a few days to a year or more. When the effects of the first course are beginning to disappear, provided the blood condition is satis-

factory, the treatment may be repeated. In some patients the symptoms have been kept in check for several years[81].

Lymphosarcoma. Results of nitrogen mustard therapy are less consistent, but if the disease is of the slowly progressing type satisfactory remission may be obtained[82,95].

Reticulosarcoma. This is the most rapidly fatal type of lymphoma. The tumours often respond dramatically to the "bis" compound, but the remissions are too transient to be of any value[95].

Giant Follicular Lymphoma. Although satisfactory remissions may be obtained the results are not so good as those following irradiation[90,95].

Mycosis Fungoides. In this condition the lymphomatous deposits are for the first few years confined to the skin. The results of therapy with the "bis" compound are very variable but quite good in some cases[89,95,96,97,98].

The Leukæmias. In acute leukæmias the "bis" compound is of no value. It may precipitate a fatal issue by damaging the remaining hæmatopoietic tissue. In chronic myeloid leukæmia useful remissions may be obtained[13,99], but if X-ray therapy is available, it is to be regarded as the treatment of choice. In chronic lymphatic leukæmia, the white blood cell count and lymph glands usually respond, but the general condition and any anæmia that may be present, rarely improve[13,77,99].

Multiple Myeloma. This condition shows little response to the "bis" compound although bone pains may be relieved for a time[100].

Polycythæmia Vera. Satisfactory remissions may be produced by the "bis" compound, but it is too early to compare the long term results with those following irradiation by deep X-ray or radioactive phosphorus[101]. Some clinicians regard nitrogen mustard therapy as dangerous in this condition[95].

Carcinoma. The results of therapeutic trials have with the exception of bronchial carcinoma proved disappointing[73,82]. Secondary deposits in the lungs may show slight, transient regression[102].

Primary Carcinoma of the Lung. Symptomatic remissions in cases of inoperable bronchial carcinoma follow nitrogen mustard therapy. Deep, but not pleural, pain and shortness of breath may be relieved, there is often improvement in the patient's general condition, and possibly on occasions some prolongation of life[35,73,76,102]. The results are comparable to those produced by palliative irradiation. It is not clear why bronchial growths should respond while others fail to do so. It has been suggested that these tumours occur in a crowded area where very slight reduction in size, that would pass unnoticed in less vital areas, may lead to considerable amelioration of symptoms[73]. Anaplastic tumours show most marked response but all histological types of bronchial neoplasm may be influenced by nitrogen mustard therapy.

Other Tumours. Patients with melanomata[82], primitive nerve cell tumours[75], testicular tumours[73], Kaposi sarcoid[89,98], eosinophil granuloma or Letterer Siwe disease[97] derive little benefit from nitrogen mustard.

Sarcoidosis. Several cases of this condition have been treated but no definite benefit can be claimed[103,104,105].

Skin Conditions. Nitrogen mustard therapy in the cutaneous manifestations of the lymphomata has already been mentioned. Relief of irritation in two cases of non-leukæmic dermatoses has been reported[95] and a satisfactory remission in a single case of disseminated lupus erythematosus[89]. Psoriasis is not influenced by it[97]. Aleksandrowicz considers that nitrogen mustard acts by stimulating reticuloendothelial activity, granulation tissue, and the occurrence of fibrosis. He claims to have treated neoplastic, decubitus and other chronic ulcers with considerable benefit[106,107,108].

Nephritis. In animals the " bis " compound inhibits hypersensitivity reactions. This has led to its trial in cases of human subacute nephritis but no definite benefit can yet be claimed[109].

Gastric Ulcer. The " bis " compound may inhibit the secretion of gastric acid. In a short series of cases of peptic ulcer treatment has been reported as being helpful[110]. The value of such therapy appears doubtful.

Tuberculosis. Aleksandrowicz, as a result of his unorthodox views on the action of the " bis " compound, has tried it for patients with chronic tuberculosis. He has claimed improvement in cases of spinal caries[111] and of cervical gland and pulmonary tuberculosis[108,112].

Toxic and Side Effects of Nitrogen Mustard Therapy

Venous Thrombosis. Thrombosis of the vein used for the injection of the " bis " compound has already been discussed. Transient local discomfort is usually produced and in one case a fatal pulmonary embolus has followed[113]. If many courses of the " bis " compound are given and widespread thrombosis occurs, blood transfusion, so often required as part of the treatment, may become very difficult.

Nausea and Vomiting. In the majority of patients troublesome nausea and vomiting begin about 2 hours after the injection and continue over the next 4 hours[35,78]. The severity of these reactions is to some extent proportional to the size of the dose, but may decrease after the later injections. Anorexia is usual during the course of treatment, but if the neoplasm has responded well, an excellent appetite soon returns. Various drugs have been tried to influence the nausea and vomiting. A barbiturate sedative is often employed but its value is doubtful. Pyridoxin, 100 mg. by intramuscular injection, has been given, again without any clear-cut benefit[70,78,95,102]. It should not be injected until 30 minutes after the " bis " compound as it may inactivate it. Benadryl and atropine are of no value[103]. Not only is the vomiting distressing to a seriously ill patient, but it may also be dangerous. Dehydration may be produced[90]. Serious hæmatemeses have been reported[78,95,114]. In two patients with thrombocytopenia, the vomiting has caused fatal cerebral hæmorrhage[90,93]. In a few patients nitrogen mustard treatment has been followed by the development of a peptic ulcer[95,115].

Diarrhœa. This has sometimes been noted, but is much less marked in patients than in animals[35,99].

Rigors. These have occasionally been described as complications of

nitrogen mustard therapy but are more likely to be related to the injection technique[35,78,88,100].

Bone Marrow. The changes observed in serial bone marrow punctures have been described by Spurr and his co-workers[83]. Depression of the red and white cell precursors is maximal in the second week and it takes several weeks for the marrow to return to normal. Recticulum cells and plasma cells are more resistant to the " bis " compound. Similar studies have been made in this country[116].

Lymphatic Glands. Serial biopsies have shown cytological changes with an apparent increase of fibrous tissue. The alterations in the lymphatic glands appear before those in the bone marrow[83,85].

Spleen. Splenic puncture shows reduction in the size of the sinusoids and often complete disappearance of the Malpighian corpuscles[83,85,86].

The Blood. Within a few hours of injection of the " bis " compound a lymphopenia develops accompanied by a transient increase in the granular cell count. This soon gives place to a neutropenia, maximal in the third week and recovering usually by the sixth. Reticulocytes become scanty soon after the injection and do not reappear until the third week. There is usually a slight fall in the red cell count but occasionally a sharp reduction occurs in the second week possibly as the result of hæmolysis. A few patients who are anæmic when the injections are given, and in whom a good response is obtained, may show a steady rise in the hæmoglobin level after treatment[69]. Thrombocytopenia is usually noticeable by the third week[70,116]. Various attempts have been made to protect the bone marrow from the action of nitrogen mustard. Choline and hexamine have been tried without any success[117,118]. Placing tourniquets round the limbs for 2 minutes after the injection of the " bis " compound has not altered the hæmatological sequelæ[73]. If the action on the bone marrow is unusually severe, agranulocytosis, severe anæmia or thrombocytopenic purpura may result.

Agranulocytosis. The development of this condition usually results from the administration of too much of the " bis " compound. In some patients and in some diseases, however, the bone marrow may be unusually sensitive. Patients in the terminal stages of lymphomatous diseases are more liable to develop hæmatological complications[88], whereas those suffering from bronchial carcinoma seem to be relatively resistant[35]. Pharyngitis[91], stomatitis[74,76] and lung infections may develop in the absence of the normal body defences. In some cases penicillin, with or without blood transfusion, has enabled the patient to recover from this complication, but it has not infrequenly proved fatal.

Extreme caution should be exercised in treating a patient with a leucopenia. Patients with lymphomata sometimes have very low white cell counts before any treatment has been given, and splenectomy has been advised to correct this prior to giving nitrogen mustard[119]. However, if the bone marrow can be shown to contain normal numbers of granulocyte precursors, the leucopenia may be disregarded, and a rise in the white cell count may be anticipated after the treatment[81].

Anæmia. It is difficult to dissociate the anæmia due to the disease from

that produced by the " bis " compound. The occasional sudden drop in red cell counts shortly after a course of injections has already been mentioned. This does not necessarily mean that treatment has failed, and if the patient is maintained by blood transfusion a remission may still follow[69].

Purpura. Although reduction of the platelet count is usual, purpuric eruptions are not very common[115,116]. Deaths from hæmorrhage have been reported[75] and these have been related to thrombocytopenia rather than to the defect of blood coagulation which has been observed[43]. The prolongation of the clotting time has been associated with the presence of a heparin-like substance in the plasma but others have been unable to confirm this finding[120].

Liver Damage. Histological studies in one series of patients dying shortly after nitrogen mustard therapy failed to reveal any specific changes in the liver[86]. There are, however, reports of liver cell necrosis[78] and of deaths from liver failure[75,97,103]. The latter may have been due to the underlying disease or homologous serum jaundice rather than the results of nitrogen mustard therapy.

Testicular Damage. A reduction of active spermatogenesis following the " bis " compound has been reported by several observers[85,86,88].

Neurological Disturbances. Although these were frequently observed in animal investigations, they are not common in man, probably on account of the relatively smaller dose used. Lightheadedness and drowsiness have been mentioned[35], toxic psychosis has been reported in one patient[121] and encephalopathy in another[75]. With some of the newer nitrogen mustards that have been used in more recent clinical trials, neurological symptoms have been quite common[12].

Skin Reactions. Maculo-papular rashes[115], a varicelliform eruption[88] and exfoliative dermatitis[103] have on rare occasions followed nitrogen mustard therapy. It has been claimed that the risk of general toxic reactions can be foretold by the result of a skin test with various dilutions of the " bis " compound[106,107]. It appears, however, that the results of this test depend entirely on the texture of the skin[118].

Other tissues. The suprarenals and kidneys do not show any specific changes in patients dying shortly after a course of the " bis " compound.

Balance Studies. Negative nitrogen and potassium balances have been demonstrated after nitrogen mustard administration[122]. Urinary coproporphyrin excretion is increased[123].

Plasma Proteins. Electrophoretic studies of plasma proteins after administration of the " bis " compound show no consistent change[118,124].

Conclusions

The nitrogen mustards, studied in the course of chemical warfare research, have proved a useful therapeutic agent in medicine. They are of value in the management of neoplastic conditions of the lymphatic tissues and blood-forming organs. Unfortunately, however, their action is only palliative, and unpleasant and dangerous side effects may be produced.

The original nitrogen mustards form the basis of numerous potential chemo-therapeutic agents and research is proceeding to find new compounds that are more active and less toxic. So far no new analogue has been found that is of greater value in practice than the original " bis " nitrogen mustard.

Since this article has been prepared, two extensive American reviews have arrived in this country. Philips (*Pharmacological Reviews* 1950, p. 281) discusses the pharmacological aspects, and Karnofsky (*Advances in Internal Medicine,* Vol. IV, Interscience Publishers London 1950, p. 1) covers a wider field.

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