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Nitrogen mustards and their use in Hodgkin's disease

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THE NITROGEN MUSTARDS AND THEIR USE IN HODGKIN'S DISEASE

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INTRODUCTION

The nitrogen mustards have been chosen by the writer as the theme for this thesis for three basic reasons. The first reason is that nitrogen mustard therapy represents the first time a chemical compound has efficiently been utilized to supplement and even to replace radiation therapy for a disease that borders on the malignant - for a disease that by its very nature presents the perplexing picture of the bridge that lies between benign tissue changes and the realm of malignancy. The early reports from those investigators utilizing this series of chemical compounds for clinical therapeutic purposes have consistently shown that in Hodgkin's disease the efficacy of the drugs is equal to, and perhaps greater than, the efficacy of radiation therapy.

The second reason for choosing the nitrogen mustards as the topic of this thesis is the promise they put forth as a therapeutic agent in the hands of the practitioner. It is true that at present the work being done in this field is being carried out only by special groups of investigators but already their reports show that the methods of administration of the drugs are uniformly simple. Rapidly the discoveries of these investigatory groups are being ac-

cumulated, combined, and correlated and from this data is emerging the knowledge of how best to administer the nitrogen mustards. The end result will be a thorough knowledge of dosage, toxicity and the other clinical factors that will enable us to use these compounds in such a way as to derive the maximum therapeutic value with a minimum of toxic effects. Thus an economical therapeutic agent will be placed into the hands of the practitioner to aid in the work that must now be done by the specialists in the field of roentgenology.

Thirdly, the nitrogen mustards hold forth promises of serving as important tools for the studies of fundamental cell metabolism. If we are ever to solve the problems of how and why body cells embark on the proliferating rampage we term malignancy, we must first obtain thorough knowledge of basic cell metabolism. We now possess a whole new field of chemical derivatives with which studies of cellular respiration, cellular enzymatic activities, and cellular metabolism in general may be studied.

HODGKIN'S DISEASE

It is not the purpose of this paper to treat widely of the disease which we term Hodgkin's disease. Yet if we are to interpret the results of nitrogen mustard therapy in this condition we must have some fundamental knowledge of the disease process. What is perhaps more important is that if we are to evaluate the use of these chemical compounds in malignancies, then we must have knowledge of the relationship of Hodgkin's disease to the malignancies.

It is regretful that the pathological process which to date has responded best to nitrogen mustard therapy should be one of the most complex, problematical disease processes suffered by man. The confusing picture presented by the disease is typified by the fact that of the seven original cases first described by Thomas Hodgkin in 1832 only three or four would actually be classified as Hodgkin's disease by present day pathological classification.

From the standpoint of etiological studies Hodgkin's disease has for years been the subject of bitter controversy. There are those who claim that the disease represents a variation of a tuberculous

infection: others maintain that it is a separate disease entity. Some investigators report that the etiological organism is a diphtheroid bacillus, some proclaim that it is an organism of the Brucella group, and others support a virus origin. Many maintain that it is but another aspect of lymphosarcoma. There are even those who maintain that the disease represents an allergic reaction to any of the usual pyogenic organisms.

An avid supporter of the relationship of Hodgkin's disease and tuberculosis was Sternberg(1). He, along with Dorothy Reed(2), first described the ever-present and diagnostic giant cells found in the affected lymph nodes. It is generally accepted (especially by the opponents of the tuberculosis theory) that Sternberg in his studies of the disease in 1898 had the misfortune to study a series of cases combined with active tuberculosis. It is said that in his later years he gave up the idea that in Hodgkin's disease he was dealing with a peculiar form of tuberculosis, and yet subsequent investigators continue to support this etiological viewpoint. Stewart and Doan(3) support this theory and cite the work of Sternberg(4), Von Baumgarten(5), Lichtenstein(6), Vasilu and Iriminoiu(7), Bernard,

Coste, and Lamy(8), Brandi(9) and others to verify their statements. These authors state that Hodgkin's disease differs from typical tuberculosis hardly more than the various manifestations of clearly recognized tuberculosis differ from one another.

Among the investigators contending that Hodgkin's disease is a variant of lymphosarcoma we may include Gibbon(10) and Coley(11). Oliver(12) in referring to Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma states "all constitute a series of neoplastic processes of the lymphatic glands, which differ not so much qualitatively as quantitatively", and that "it is the predominant cell type which allows one to class the tumor as lymphosarcoma, an endothelioma, or Hodgkin's disease". Mallory(13) included lymphocytoma, lymphoma, lymphosarcoma, pseudoleukemia, lymphatic leukemia, and Hodgkin's disease under the all inclusive term lymphoblastoma. Richter(14) reported on a case of lymphatic leukemia associated with reticulum cell sarcoma. McMahon and Parker(15) presented a case of combined lymphoblastoma, Hodgkin's disease and tuberculosis; Craver(16) reported a case of Hodgkin's disease with sarcomatous features associated with lymphatic leukemia. MacCarty(17) expressed belief that Hodgkin's disease and lymphosarcoma have a "common neoplastic cellular origin"; Levin(18) said

they are "phases of the same pathological entity and the two may exist in the same patient"; Ginsberg(19) "believed they were merely variations of the same disease"; Cohn and Richter(20) stated they were "genetically related"; Warthin(21) concluded that "transition forms exist between all of the group including lymphosarcoma, Hodgkin's disease and reticulum cell sarcoma and that one type might be transformed into the other"; and Callender(22) also described the close relationship between Hodgkin's disease and reticulum cell sarcoma.

Herbut, Miller and Erf(23) have presented six cases on whom biopsy and other studies were made during life and on whom follow-up autopsies were performed. In this study it was shown that at one time during the lives of these patients their illnesses were diagnosed as Hodgkin's disease and at another time as lymphosarcoma. At autopsy pathological studies showed various combinations of Hodgkin's disease, lymphosarcoma, and reticulum cell sarcoma. These investigators theorized that the three diseases arise from a common stem cell - the reticulum cell - and then differentiate in one direction or another according to the amount and type of stimulation.

Jackson and Parker(24) choose to break Hodgkin's disease down into three types proceeding from a fairly

benign paragranulomatous type to a more acute granulomatous type. The latter may in turn proceed to a sarcomatous type which is malignant in nature. They profess that the types may progress from the least malignant to the most malignant type but that the reverse transition is not possible.

To further accentuate the indefiniteness of "what constitutes Hodgkin's disease" we need only to review the synonyms found in the literature. It is also called "lymphogranuloma, pseudoleukemia, malignant granuloma, Sternberg's disease, Hodgkin's granuloma, Hodgkin's sarcoma, sarcomatous Hodgkin's disease" and many, many other descriptive terms(25).

Dorothy Reed(2) postulated, "We should limit the term Hodgkin's disease to designating a clinical and pathological entity, the main features of which are painless, progressive glandular enlargement, usually starting in the cervical regions, without the blood changes of leukemia. The growth presents a specific histological picture, not a simple hyperplasia, but changes suggesting a chronic inflammatory process. Eosinophils are usually present in great numbers in such growths but not invariably".

This discussion of Hodgkin's disease per se is not meant to be a complete interpretation of the disease. It is not the purpose of this paper to make

a study of this particular pathological process. Neither is it the writers purpose to convey to the reader the idea that our knowledge of the disease is a chaotic jumble of biased arguments. Much has been learned of the disease, but we must remain cognizant of the fact that still more remains to be learned before we may definitely place Hodgkin's disease in a category that meets with the approval of a decisive majority of its many investigators. It is important that we bear this in mind when we attempt to interpret the results of any form of therapy carried out in this disease. This is especially true if we are to evaluate such therapy in terms of application to malignancies and blood dyscrasias. Hence, it is probably best that we accept the beneficial results of nitrogen mustard therapy in Hodgkin's disease as just that and nothing more. Then if we desire to judge the effect on lymphosarcoma, reticulum cell sarcoma, lymphatic leukemia or other pathological conditions we should make separate studies on definitely diagnosed cases of these diseases until such time as the status of Hodgkin's disease is clarified.

THE NITROGEN MUSTARDS

Historical Background

It is generally accepted that Victor Meyer in 1886 first reported the preparation of the compound that ultimately led to the development of the nitrogen mustards. This compound is dichloroethylsulfide. It is described as a colorless, oily liquid, boiling freely at about 217 degrees Centigrade, only very slightly soluble in water, but very soluble in all organic solvents, and possessing a characteristic odor suggestive of mustard or garlic. It is evident how the popular term "mustard gas" developed from this last property.

Because of the noxious qualities of the compound studies of mustard gas were carried out during the first World War to evaluate its capacity as an offensive gas. As with all offensive weapons there existed the possibility that the enemy possessed this same weapon. This possibility, plus the knowledge that our own troops and investigators were liable to exposure to the gas even if it existed solely in our hands, necessitated the search for measures to be used as antidotes. As a result investigative research was begun. These investigators realized that from the standpoint of treatment of the vesicant war

gas casualties an understanding of the basic mechanism of cellular action must be obtained. From their studies came forth the conception that this gas exerted its vesicant action by the intracellular release of hydrogen chloride. In addition a few remote reports described the systemic effects of mustard gas on hematopoietic tissues, gastro-intestinal tract, and electrolyte and fluid balance. By and large, however, little extensive progress was made in the investigations before the end of the war brought an end to the stimulus for exhaustive research.

Some of the more important contributions during the period described in the preceding paragraph included the work of Lynch, Smith and Marshall(26) who investigated the systemic effects and the mechanism of action of mustard gas. Sollman(27) studied the influences of various solvents, adsorbents and chemical antidotes on the severity of the lesions in human skin, rather dramatically utilizing himself and volunteer students as "research victims". Krumbhaar and Krumbhaar(28) described the changes in the bone marrow of fatal cases and also described briefly the peripheral blood picture for several days prior to death. Warthin and Weller(29,30) described the general pathology and the medical aspects of mustard gas poisoning as well as the more specific lesions of the respi-

ratory and gastro-intestinal tracts. The interested reader is referred to these articles by Warthin and Weller in which detailed descriptions and numerous illustrations appear of both animal and human specimens. Pappenheimer and Vance(31) did work with dichlorethylsulfide on rabbits with special reference to the leucotoxic action. In addition various German investigators including Flury and Wieland(32), Freundlich(33), and Muntsch(34) reported on the subject.

During the period following World War I there was some work done on the adverse effects of mustard gas on leucopoietic tissues and on the growth of experimental tumors, but in general the biological research on chemical warfare agents remained quiescent. Berenblum(35) reported on experimental inhibition of tumor induction by mustard gas; and Marvel and Cowan(36) reported on some purely biochemical experiments dealing with nitrogen mustards.

With the advent of World War II the study of war gases was resumed with a new vigor for all of the advances made by science during the period between wars could now be applied. New compounds were proposed as potential offensive agents among which mustard, bis (beta chloroethyl) sulfide and a series

of nitrogenous analogues, bis- and tris (beta-chloroethyl) amines were included. Early it was appreciated that the sulfur and nitrogen mustards were not only contact vesicants but following absorption could exert cytotoxic actions on a variety of tissues. In addition it was early found that, in general, cellular susceptibility was related to the degree of proliferative activity of the tissues. The importance of these discoveries emphasized the necessity for systematized planning in efforts to carry out extensive studies in the research field. As a solution for this need the Army created the Medical Division of the Chemical Warfare Service and a presidential directive originated the Office of Scientific Research and Development. Under the latter a Committee on Medical Research was set up, one division of which was a Committee on Treatment of Gas Casualties.

Later, through the cooperation of the Chemical Warfare Service, United States Army, the National Defense Research Committee, the Committee on Medical Research of the Office of Scientific Research and Development, the National Research Council and with aid from the Jane Coffin Childs Memorial Fund for Medical Research studies were undertaken to ascertain

the value of the clinical use of the nitrogen mustards in the treatment of neoplastic diseases.

Thus far the only compounds studied through the above association have been tris (beta-chloroethyl) amine hydrochloride and methyl bis (beta-chloroethyl) amine hydrochloride. The studies were begun under a contract between the Office of Scientific Research and Development and Yale University, and the authority to carry out the work was given by the Committee on Atypical Growth of the National Research Council to the University of Chicago Medical School, the University of Utah Medical School and Memorial Hospital in New York.

The bulk of the information found in this paper regarding the clinical trial of these compounds has been derived from the reports of the above authorized groups. The agents are now available for experimental purposes only through the Committee on Growth, National Research Council, in cooperation with the Chemical Warfare Service. Many groups have subsequently obtained materials for research programs but as yet only a few of these groups have reported formally.

THE BIOLOGICAL, PHARMACOLOGICAL AND BIOCHEMICAL PROPERTIES OF THE NITROGEN MUSTARDS

In the preceding brief historical resume' of the progress made in the therapeutic utilization of the nitrogen mustards, some brief mention was made of early studies of the biochemical and pharmacological properties of these drugs. From these early investigations arose some of the first concepts of the mechanism of actions of these drugs. As stated before it was first thought that mustard gas exerted its vesicant action by the intracellular release of hydrogen chloride. Some mention was also made of the more important reports concerning the systemic effects of mustard gas on the hematopoietic tissues, gastrointestinal tract, and electrolyte and fluid balance. The work of Berenblum(35) reporting on his applications of mustard gas in the experimental inhibition of tumors was included in the foregoing resume'. It was also told how the new compounds including the nitrogen mustards came into being. It is these last mentioned compounds- the nitrogen mustards- with which this paper is primarily concerned, hence, the remainder of this paper shall be more or less limited to this particular series of compounds.

From the standpoint of the treatment of vesicant war-gas casualties it was early seen that an understanding of the basic mechanisms of cellular action must be obtained. Subsequent studies of this factor revealed a type of action for the nitrogen mustards unlike any other chemical agent but closely resembling the action of X-ray. With this in mind it is interesting to note that investigators have reported that the additive lethal effects in mice of $CH_3N(C_2H_4CL)_2$ and X-rays varies with the sequence of administration. If 80% of a lethal X-ray dose is given first and followed by 80% of a lethal dose of $CH_3N(C_2H_4CL)_2$ an hour later the lethal action is only slightly additive. If the sequence is reversed much greater lethal effect results (37).

By far the most basic and the most complete report on the biological actions of beta chloroethyl amines and sulfides is that resulting from the work of Gilman and Philips(38). It is largely from this source that the following information has been obtained and the interested reader is referred to their article for a more detailed presentation.

According to the above mentioned investigators the nitrogen and sulfur mustards owe their physiological activity to a basic chemical reaction which they share in common, namely, intramolecular cyclization in a polar solvent to form a cyclic onium cation with liberation of Cl. The reaction is depicted as follows, Z representing the sulfur or nitrogen atom:

$$R\text{-}Z\text{-}CH_2CH_2Cl \longrightarrow R\overset{+}{Z}\text{-}CH_2\overset{-}{C}H_2 \text{ plus } Cl^-$$

The onium cations -ethylenimmonium in the case of beta chloroethyl amine, ethylenesulfonium in the case of beta chloroethylsulfide - react readily with anions and various charged nucleophilic molecules. It is the great reactivity of the cyclic onium cation which impart to the group of vesicants their varied action.

So important are the basic chemical findings for an understanding and explanation of the physiological action of the nitrogen mustards that pertinent facts are reviewed here. The beta chloroethyl amines do not undergo cyclization when a proton becomes coordinated with the N atom, thus solutions are stable in strong acid. This is not true for the sulfur mustards. Similarly the various ethylenimmonium compounds are less reactive as

a group than are the ethylenesulfonium compounds but the reactivity is ample to allow the compounds to produce toxic action in general similar to those of sulfur compounds.

The majority of the nitrogen mustards are bis (beta-chloroethyl)amines, the third valence of the nitrogen being occupied by a variety of alkyl groups. The activity of the ethylenimmonium cation and the rate of cyclization are influenced by the various groups that may be present in the molecule. From this it may be surmised that a large number of nitrogen mustards of different physicochemical and pharmacological properties may be available providing a wide scope for future investigation. In pure aqueous solutions at physiological hydrion concentration the ethylenimmonium cation reacts with water. If other substances are present, however, they tend to react competitively some to the extent that the reaction with water is negligible. Using this property as a basis hundreds of compounds have been studied in the search for an effective antidote for the local and systemic action of the mustards.

More pertinent to this paper is the ability of the ethylenimmonium compounds to alkylate the functional groups of biologically important compounds.

Among these are the alpha-amino, imidazole, sulfhydryl sulfide, phenolic, alpha-amino and imino groups of amino acids and peptides; inorganic phosphates as well as glycerophosphates and hexose phosphates; the amino groups of adenosine and thiamine; and the pyridine-N of nicotinic acid amide and pyridoxine. The carboxyl and amino group of numerous proteins appear vulnerable to alkylation, although other groups are also involved. The likelihood that the basic mechanism of the cytotoxic action of the mustards involve a similar reaction with a vital cellular constituent, probably as yet unknown, is great.

Systemic pharmacology and pathology of the mustards

The nitrogen mustards elicit a variety of systemic pharmacological actions which for the most part are prominent only after the administration of acutely lethal doses. These will be described in the following paragraphs. Again the bulk of the material used in this discussion has been gleaned from the excellent work of Gilman and Philips(38). Later in the discussion of the therapeutic administration of these chemical compounds, the toxic effects likely to be encountered when non-lethal dosages are employed will be discussed more fully.

Perhaps the most outstanding systemic action of the nitrogen mustards is their cytotoxic action. In a manner yet unexplained these compounds cause the death of cells and, generally speaking, it is those cells with the greatest proliferative activity which are the more susceptible. This action is thus evidenced early in the gastrointestinal mucosa and in the formed elements of the blood. The effect on the blood-forming organs is reflected in the peripheral blood resulting in a lymphopenia, granulocytopenia, thrombocytopenia and moderate anemia. The severity of the response is in direct relationship to the dose, and marked hemopoietic effects can be obtained with sub-lethal doses.

Nausea and vomiting within a few hours after drug administration evidence the effects on the gastro-intestinal tract. The mechanism of this response has been postulated to be either a reflex response from the gastro-intestinal mucosa or a result of direct medullary stimulation. Within twenty-four hours diarrhea may become evident and become progressively more severe. Both vomitus and feces may contain blood. As a result of the fluid and electrolyte loss accompanying the above reactions marked body fluid changes occur. The action of lethal

doses on the kidney may add to these changes since polyuria and renal wastage of extracellular electrolytes may ensue. A loss of intra-cellular potassium and water may occur too, and eventually circulatory failure and typical shock occurs and the experimental animals die in respiratory failure.

With the administration of supralethal doses central nervous system excitation results in convulsion and sudden death. Parasympathomimetic effects are evidenced by salivation and mioses. Subconvulsive doses cause a progressive muscular paralysis eventually resulting in death as a result of paralysis of the muscles of respiration.

Several investigators point to the fact that only those beta-chloroethyl compounds which can form a cyclic cation are capable of exciting the aforedescribed actions. The toxicity appears to be related to the chemical characteristics of the onium cation and the rate of cyclization of the parent compound.

From the viewpoint of the pathologists we may describe the intestinal lesion as progressing from vacuolization and nuclear swelling of the epithelial cells to eventual necrosis and desquamation with hemorrhage. The lymphoid tissue of the body is

rather uniformly involved with fragmentation evident within ten hours leading to a persistent lymphatic atrophy for a number of days. Early bone marrow changes include swelling and alteration in staining reaction of hematopoietic cells and a disappearance of mitotic activity. Progressive marrow depletion follows and eventually almost complete aplasia is found.

In vivo and in vitro inactivation of enzymes by the mustards

The widespread systemic intoxication resulting from nitrogen mustard administration to experimental animals led to the concept that the agents inhibited certain basic metabolic functions vital to maintenance of normal cellular activities. Further experimentation has shown that the oxygen consumption and glycolysis of a large number of cells and tissues are inhibited to varying degrees following exposures to the agents *in vitro* and in some cases *in vivo*. Some of the tissues utilized experimentally included brain, chick embryo, minced tumor tissue, avian erythrocytes, lymph nodes, bone marrow, liver and kidney and others. The above work has led to the theory that the primary mechanism of the action of the vesicants was the inactivation of essential cellular

enzymes. In addition it has been postulated that primary inactivation occurs only in a special class of essential cellular enzymes, the phosphokinases which are concerned with phosphate transfer to or from adenylic compounds.

The above theory led to the study of the in vitro sensitivity of a large number of enzyme systems including proteins containing copper, iron, zinc and flavin prosthetic groups: dehydrogenases: hydrolytic enzymes as fumarase, urease, and invertase: catalysts involved in glucose metabolism and so on. The majority proved resistant or only moderately sensitive. Some, however, were highly sensitive. The latter includes hexokinase, creatine and pyruvate phosphokinases, inorganic pyrophosphatase, adenylic acid deaminase, chick pepsin, kidney pepsinase, and peptidase of serum, skin and lung, and choline oxidase and acetylcholine esterase isolated from the brain.

At present the "enzyme-inactivation" theory is still in question. That some enzymes possess a high order of sensitivity in vitro is certain but that the same is true in vivo is questionable. However, this general trend of investigation is so in keeping with the late investigations of the cancer problem that it presents a most interesting

aspect of study. It is this phase of the nitrogen mustard studies which the writer referred to early in this paper when he emphasized the importance of the nitrogen mustards as "tools" to be used in the investigation of fundamental cell metabolism. Although not strictly a component of the topic of this thesis there is enough correlation between Hodgkin's disease and the known malignancies to justify the inclusion of the following interesting digression.

A theoretical possibility of the relationship between nitrogen mustards and abnormal cell metabolism

Current trends in cancer research indicate a growing realization that cancer tissues are abnormal in respect to one or more enzymes which go to make up the total mosaic of interlocking biocatalysts responsible for the metabolism of living tissue. More and more agreement has been developing in the field of energy transformations which involve the phosphorylative, glycolytic, and respiratory enzyme systems. In Potter's report(39) on this phase of research there is an interesting discussion limited to these three enzyme systems. This investigator also presents an extensive review of the literature pertaining to the enzymological aspect of cell metabolism.

Potter presents an interesting theory of cancer that is of interest in its relationship to the anti-carcinogenic substances such as the nitrogen mustards. This theory considers cancer to result from an abnormal protein which is assumed to rise spontaneously in certain cases, to be formed by the continuous action of carcinogenic agents in other cases, and to be introduced into the cell from other affected cells in the case of tumors of known virus origin. The cancer protein is assumed to be almost identical with an enzyme "X" except that it lacks the specific catalytic potency of this enzyme. Enzyme "X" is thought to be a constituent of normal differentiated cells and possibly a complex of respiratory enzymes. Both the cancer protein and enzyme "X" are assumed to require essentially the same building blocks for their synthesis and hence compete for them. The synthesis of both enzyme "X" and the cancer protein is assumed to proceed autosynthetically and the results of the competition between the two depend upon their relative concentrations. When the cancer protein attains proportions where the restraining enzyme protein can no longer compete with or restrain it, the cell containing them becomes a "cancer cell".

It is assumed in this theory that both enzyme "X" and "cancer protein" are broken down in time with

antigen-antibody reaction playing an important role in the case of the latter. If the disposal of the "cancer protein" is rapid enough the competition with the normal enzyme is unsuccessful and no cancer cells result.

The nucleotoxic actions of the mustards

The systemic effects of toxic doses of the nitrogen mustards have been described. However, threshold doses evoke pathological changes only in cells and tissues with relatively high rates of proliferation and growth. The mitotic activity of a variety of cells is peculiarly sensitive and inhibition of this activity is readily attained. This inhibition occurs at the resting stage of mitosis apparently for cells in active mitosis at the time of exposure complete their division so that soon the tissue is depleted of mitotic figures. More severe exposure of certain cells has caused multiple chromosome breaks and nuclear fragmentation, pyknosis and ultimate cell death. Milder exposure caused chromosome breaks which if not too numerous were transmitted to daughter cells in subsequent mitosis as heritable chromosome abnormalities. It has been demonstrated that in threshold doses the nitrogen mustards act directly on the structure of the chromosome without apparent influences on

other cellular entities to produce an inheritable chromosomal abnormality which can be reproduced indefinitely by the normal process of cell division. Thus it can be transmitted from ovum to ovum through successive generations(40,41). The exact mechanism of this action is not clear, and investigators caution against the assumption that it is solely that of enzyme inactivation and disturbances of nuclear function. Some studies have suggested cytoplasmic changes and cell membrane changes which require additional study.

METHODS OF THERAPEUTIC ADMINISTRATION OF THE NITROGEN MUSTARDS

The knowledge of the marked effect that the nitrogen mustards have on lymphoid tissue plus the fact that actively proliferating cells are especially vulnerable to their action has led to the usage of the compounds for the treatment of lymphoid neoplasms. Fortunately the nitrogen mustards in the form of their hydrochloric salts are water soluble, crystalline compounds readily soluble in sterile saline thus abetting intravenous administration.

The events leading to the experimental trial of the nitrogen mustards in various neoplastic diseases, especially those of the hematopoietic system, have already been discussed. Naturally, the early therapy required a great deal of trial and error and many adjustments were necessitated as regards dosage, methods of administration and even interpretation of results. The remaining sections of this paper are to treat in detail of the reports resulting from the study of clinical administration, and clinical results as applied to Hodgkin's disease specifically. Most of these studies, however, have included patients with leukemias and lymphosarcoma as well and the interested reader is referred to the origin-

al articles for information concerning these other pathological conditions.

Goodman, Wintrobe, Damashek, Goodman, Gilman and McLennan(42) utilized the water soluble hydrochloride salts of both tris- and bis (beta-chloroethyl) amine. Standard single doses of 0.1 mg. kilogram of body weight (but not exceeding 8 mg. in a single dose) were given daily or every other day until three to six doses were administered. Subsequent treatment was varied with the response of the individual patient, but usually was not given more often than every six to eight weeks with but 2-4 doses being administered. This group prepared the agents by adding normal saline to exactly 10 milligrams of the dry salt and then administered them within five minutes to prevent hydrolysis. In most of the twenty patients receiving tris (beta-chloroethyl) amine hydrochloride, the dose was dissolved in 25-50 cc. of saline solution and injected directly into the vein. Pain and thrombophlebitis were frequent complications. In all the other cases 10 milligrams of the drug was dissolved in 10 cubic centimeters of saline solution and the calculated dose was injected into the rubber tubing during the course of an intravenous infusion of glucose or saline solution.

Jacobsen, Spurr, Barron, Smith, Lushbaugh, and Dick(43) used methyl bis (beta-chloroethyl) amine hydrochloride and also used daily doses of 0.1 milligram/kilogram of body weight. They prepared the agent in the same manner as the above group and injected it into an infusion set-up with normal saline. Their patients were given courses of treatment varying from one to six consecutive daily injections at first, but later they limited the number of injections per course to four.

Wintrobe, Huguley, McLennan, and Lima(44) utilized the hydrochloride salt of di (beta-chloroethyl) methyl amine and prepared the agent by adding 10 cc. of sterile physiological saline to 10 milligrams of the crystalline salt. They also injected the material into the tubing of a previously set-up saline infusion administering a usual dosage of 0.1 milligram per kilogram of body weight. The infusions were given at intervals of every 1-2 days until a total of four to six infusions had been given. This routine was varied depending on the presence or absence of toxic symptoms in the particular patient concerned. In several cases as many as eight daily infusions were given. In patients with chronic Hodgkin's disease (or chronic leukemias) who had already responded to nitrogen mustard therapy, a

maintenance routine was carried out by giving two to three infusions on successive days at two to four week intervals without hospitalizing the patient.

Bortz and Haden(45) utilized the nitrogen mustards on a total of sixteen patients, five of whom had Hodgkin's disease. The same method of preparation and of determining dosage was used as above. The calculated dosage was injected directly and rapidly into the antecubital vein daily on four consecutive days. It has already been pointed out that the direct injection method is more likely to produce phlebothrombosis, burning of the skin and extravasation than is injection into the tubing of an infusion set-up.

ApThomas and Cullumbine(46), English investigators, utilized methods described by American reports in the treatment of twenty cases of Hodgkin's disease. Using bis(beta-chloroethyl) amine, they dissolved the drug in 10-20 cc. of saline and injected it intravenously. The usual dose in the first treatment was 0.4 milligrams per kilogram of body weight. The first patients treated were given an injection of 0.1 milligram per kilogram of body weight on four consecutive days. Later, two injections each of 0.2 milligram per kilogram of body weight at twenty-four hour intervals were given. With the

latter technique the period of vomiting following administration was found to be shorter. A few patients were treated by the intravenous drip method (each dose being dissolved first in 500 cc. of saline) with no obvious advantage. The courses were repeated after an interval of 6-8 weeks, at a larger dose of 0.3 milligram per kilogram of body weight on each day. Two of the patients received four daily injections of 0.2 milligram per kilogram of body weight, but it was found that this dose caused severe leucopenia.

Alpert and Petersen(47) failed to give their method of administration in their brief report.

THE TOXIC EFFECTS OF NITROGEN MUSTARD THERAPY

Since the earliest studies of mustard gas and the related nitrogen mustards were made in an effort to better utilize their toxic effects as an offensive war weapon, it follows that this aspect was investigated widely at an early date. References of these early studies of toxicity have been cited in that earlier portion of this paper which treated of the historical background and development of the nitrogen mustards. Since these studies were based largely on animal experimentation and usually were made in view of the effect of lethal doses they will not be discussed in this section. Instead it is the writer's desire to review the toxic effects observed clinically as the result of the therapeutic administration of non-lethal doses of the drugs.

The reports of the various groups which have investigated the drugs show close agreement as regards the toxic effects observed upon their administration. If direct injection into the vein is the method of administration there may result immediate pain, especially if there is extravasation of the material into the surrounding tissues. Somewhat later thrombosis or thrombophlebitis is prone to occur at

the injection site(42,43,46). The incidence of these undesirable effects is reduced by utilizing the procedure of injecting the material into the tubing of an infusion set-up as previously mentioned.

Nausea and vomiting are common reactions usually appearing within 1-3 hours after injection and persisting for 3-48 hours(42,43,46). The severity varies with the individual, and the duration seems to be somewhat longer after the tris form than after the bis form of the agent(46).

Anorexia and resultant weight loss was noted on some patients(43). Preliminary sedation with barbiturates and over-night fasting, however, reportedly diminished the gastro-intestinal symptoms(42).

The most important late toxic effect is a leucopenia which progresses from a lymphopenia to a neutropenia(42,43,46). It may begin as a lymphopenia within twenty-four hours after the first injection and progress for 6-8 days. The lymphocyte values tend toward normal in some cases between the second and third week but in others the depression persists longer. The total leucocyte count may decline progressively for 15-21 days(43).

A normocytic anemia similar to that following X-ray may occur in the first week or two, the degree varying with the dosage of nitrogen mustard and the

number of injections(42,43). Evidence of regeneration may be noted in the second to third week following injection and normal values may be reached in 4-5 weeks(43).

Thrombocytopenic and bleeding tendencies are rare after the ordinary course of treatment but may occur(48).

THE THERAPEUTIC

EFFECTS OF NITROGEN MUSTARD THERAPY

An analysis of 123 cases of Hodgkin's disease treated with the nitrogen mustards

In the literature read for the preparation of this paper there has been found a total of 123 cases of Hodgkin's disease treated by means of the nitrogen mustards. It must be remembered that most of the groups of investigators carrying out clinical research did not limit their studies to any one disease entity. Nearly every group carried out simultaneous therapy on patients suffering from lymphosarcoma, the leukemias, Hodgkin's disease and other forms of malignant growths. Many of the reports have not included a clear-cut detailed report of each individual pathological process, hence it is sometimes difficult to abstract the true evaluation of the results in Hodgkin's disease alone. Then, too, there is a great variation in the completeness of the reports as well as in the degree of detail.

Wintrobe, Huguley, McLennan, and Lima(44) reported on 27 cases of Hodgkin's disease. Prior to treatment the patients presented the general characteristics as summarized in the following tabulations:

Sex : Male -16 ; Female -11
Age : Range - 14 to 67 years;Average-36.3 years
Duration of symptoms : Range-3 to 60 months;
Average-23.8 months.
Number previously treated with X-ray : 22.

Utilizing the dosage and methods of administration already discussed the above group gave their individual patients total dosages ranging from 24 to 193 milligrams of nitrogen mustards in separate courses ranging from 4 to 40 in number. As a result of this therapy 61% of their total patients were able to return to their usual occupation for several months or more and were free of troublesome symptoms; 18% showed definite improvement but less striking or more shortlived freedom from symptoms than the first cited group. Also included in this percentage group are those with striking relief from some particularly distressing symptoms such as intractable pain; 21% showed little or no improvement, or improvement of only a few weeks. At the time of their report 15 of the patients were still living representing a range of 1-26 months survival following their first treatment. The 12 patients succumbing to their disease died within a time range of from one week to sixteen months after their first treatment.

In the above series one of the most striking effects was upon fever. With the first course of nitro-

gen mustard therapy the temperature of all but two of the patients returned to normal. This effect was usually repeated with subsequent courses of treatment. Symptomatic improvement was evidenced by an increase in appetite, weight, and general well-being. In general large lymph nodes decreased moderately in size with a diminution of symptoms caused by the enlarged glands. If the spleen had previously been enlarged, it usually decreased in size with therapy. In three patients with back pains (two of whom showed bone involvement) all obtained striking relief. Of nine extremely ill patients who were "roentgen ray resistant", five showed marked improvement. Several of the patients, seemingly terminal, responded well. However, some of the patients early in the disease showed only a brief response to this form of therapy.

Goodman and Gilman at New Haven Hospital; Wintrobe and McLennan at Salt Lake City General Hospital; Damashek at Boston; and Goodman of Portland published their reports simultaneously although their studies were carried out separately(42). The methods of administration used have already been discussed. The group treated a total of 27 patients with Hodgkin's disease verified pathologically by biopsy. Of this total 22 were treated with methyl

bis (beta-chloroethyl) amine hydrochloride, and 5 were treated with tris (beta-chloroethyl) amine hydrochloride. All but three of their patients had previously been treated with radiation therapy. Most of these patients were in the advanced or terminal stage of their disease and were considered resistant to radiation therapy, yet nearly all of them derived some benefit from the chemo-therapy. In three patients the sensitivity to radiation seemed to be restored, and in one patient, who had Hodgkin's disease for seven years and was still responsive to radiation therapy, a more satisfactory remission was obtained from nitrogen mustard therapy than from any previous course of X-ray. In another patient who did not respond adequately to either the halogenated alkyl amines or radiation therapy alone, good results were obtained by combining the two.

This group also reported that in addition to the rapid partial or complete disappearance of Hodgkin's tumor masses, most patients experienced improvement in appetite, weight, strength and sense of well-being. Fever, if present, disappeared. Symptom free remissions varying from two weeks to at least seven months were observed.

Another of the original groups authorized to carry out the therapeutic trial of the nitrogen mustards by the Office of Scientific Research is that composed of Jacobsen, Spurr, Barron, Smith, Lushbaugh and Dick(43). This group utilized methyl bis (beta-chloroethyl) amine hydrochloride with the methods of administration and the doses already described. The patients studied were those taken from the regular admissions to their hematology clinic with no attempt to make selections on the basis of the duration or status of the disease. For the first two years of study all patients were routinely hospitalized for treatment for periods of 2-8 weeks to facilitate study. After this first period of study only new or selected cases were hospitalized for a course of treatment. This illustrates the feasibility of ambulatory therapy with the nitrogen mustards, and emphasizes the eventual economy of this method of therapy.

This group also studied a total of 27 patients with a thoroughness that warrants a detailed discussion of the features included in the analysis. Clinical evaluation of each patient included a careful history, a study of biopsies taken prior to admission to the clinic, if available, and the amount and response to previous roentgen therapy. In the

treatment special attention was given to abnormal masses such as an enlarged spleen, liver or lymph nodes. All such abnormalities were recorded whenever possible by direct measurement. A lymph node biopsy was performed in all cases. Studies of the peripheral blood routinely included: hemoglobin in grams per 100 cc., erythrocyte, leucocyte, platelet, and reticulocyte counts and a differential made before and after treatment. Similarly urea clearance, plasma protein, albumin and globulin ratio and cholesterol were determined. X-ray studies of the chest in all patients and of the skeleton and abdominal areas where indicated were made.

In this study remissions varied from 0-33 months. Eight patients died from extension of the disease process, but in four of the radio-resistant patients significant remissions were obtained. Notable effects were again resolution of the lymphadenopathy, a decrease in spleen and liver size, and the prompt alleviation of fever, malaise, osseous pain and pruritus.

ApThomas and Cullumbine(46) in their report on 21 cases described all of the general improvements already described as a decrease in temperature to normal in twenty-four hours, an increase in weight,

and a disappearance of lassitude. All of their patients improved after the first course but many of them required further treatment for recurrences of symptoms. Of thirteen patients requiring a second course, twelve showed marked improvement after the second course. In comparison with radiotherapy these investigators state that the improvement in general condition, nodes, and other signs were produced more quickly with the nitrogen mustards than with X-ray. They believe, however, the effect to be of shorter duration for their series of X-ray cases showed remissions in 9.5 months while the cases treated with nitrogen mustards showed remissions in as little as 2 months with a few cases going 4-6 months without remission.

Alpert and Peterson(47) have reviewed the action of the nitrogen mustards, and have presented a series of cases including 15 cases of Hodgkin's disease. This review includes a brief case report on eight of the patients with Hodgkin's disease selected as typical of the results of their therapy. Virtually all showed the same beneficial results previously noted, and the reader is referred to the original article for a more detailed clinical report on the eight patients.

Bortz and Haden(45) treated a group of patients with the nitrogen mustards among whom were 5 cases of

Hodgkin's disease. Of the five, two died within 6-8 weeks after the first course of treatment though their disease had previously been treated with irradiation for seven and thirteen months respectively. Two progressed exceedingly well, and no follow-up was available in the remaining one patient.

CONCLUSION

It is thus seen that from a clinical standpoint the halogenated alkyl amines would appear to be able to produce the same qualitative clinical effect in Hodgkin's disease as does radiation therapy. In general the effect on the lymphoid cells, the cells of the bone marrow, and on the hyperplasia of the reticulum cells is the same. It is fairly well established that the beta-chloroethyl amines are capable of producing therapeutic remissions in some patients who have become "resistant" to roentgen therapy. In some patients more benefit is derived from a combination of X-ray and nitrogen mustards than from either form of therapy alone. It would seem that local and systemic reactions are less severe after chemotherapy than after radiation, and of course there is not the danger of radiation dermatitis.

Therapy would undoubtedly become less expensive with the nitrogen mustards once an ambulatory routine is feasible since no expensive equipment is required for their administration. The margin of safety at present is narrow but with the establishment of optimal dosage and administrative routines and methods this feature may be eliminated as a hazard.

One must not overlook the important fact that hundreds of variants of the two original nitrogen mustards await synthesis and evaluation. From this group may come a drug with a wide range of safety and even more efficacy than the two already studied have shown.

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