# The Initial Clinical Trial of Nitrogen Mustard

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IT IS NOW almost twenty years since the first patient suffering from a malignant disease was treated with nitrogen mustard. Although the case history has been recorded in the scientific literature, Dr. Creech considers it of sufficient interest to warrant a personal narrative account of the events leading up to the therapeutic trial, or in his own words, "It will provide an interesting background for the material on the clinical use of chemotherapeutic agents." Having thus placed the responsibility where it belongs, I can write freely.

Early in 1942, largely due to the efforts of Milton C. Winternitz, a contract was signed between Yale University and the Office of Scientific Research and Development to investigate chemical warfare agents. The study of the nitrogen mustards was assigned to Louis S. Goodman and me. We were fortunate to be joined by Frederick S. Philips, who was then a N. R. C. Research Fellow in the Department of Biochemistry, and by Roberta P. Allen. Similar groups, representing various disciplines, were being formed at numerous research centers. Close contact was maintained between investigators by means of circulated research reports and frequent meetings. This accounted for the rapid elucidation of the unique and fascinating properties of the nitrogen mustards. Contrary to the present opinion of many, perhaps no compound had been more thoroughly studied prior to clinical trial than were the nitrogen mustards. The point to be emphasized is the collaborative nature of the basic investigations on the nitrogen mustards which led to their clinical trial. An attempt to give some concept of the enormity of this effort was made by Gilman and Philips [1] when they were given the privilege of presenting the first review in the open literature of the classified wartime investigations.

Early in the course of the study of the nitrogen mustards certain basic observations were made. It was immediately appreciated that in addition to a local vesicant action, the nitrogen mustards were cytotoxic following absorption. Death of experimental animals was the result of systemic effects even after topical application to the skin. The susceptible tissues were those with renewal cell populations, primarily lymphoid tissue, bone marrow and the epithelium of the gastrointestinal tract.

The chemists also made rapid progress. They described the sequence of events which led to the cyclization of β-chloroethyl amines and the formation of the highly reactive ethylenimmonium ring. In the case of the *bis* and *tris* β-chloroethyl amines this involved the formation and reaction of successive ethylenimmonium rings. It was possible to follow the kinetics of these reactions by means of suitable analytic technics. A major effort of the Yale group was devoted to a study of the relationship of the stage of transformation of a nitrogen mustard to its distribution, pharmacodynamic actions and toxicity. This study was later extended and published in full by Hunt and Philips [2].

One facet of this study was destined to have future clinical significance. The chemists had described the high reactivity of the ethylenimmonium ring with thiosulfate. This provided the pharmacologists with an effective antidote to the systemic effects of the nitrogen mustards. It was shown by the group at Yale that pretreatment of animals with thiosulfate could prevent the cytotoxic action of the nitrogen mustards, provided a rather high concentration of thiosulfate ion was present in body fluids at the time of formation of the ethylenimmonium ring. Under these circumstances the thiosulfate

ion could effectively compete with cell receptors. If the preformed ethylenimmonium compound was injected, it combined with cell receptors so rapidly that the subsequent injection of thiosulfate was without effect. A knowledge of the kinetics of transformation and the protective effects of highly reactive substances such as the thiosulfate ion provided fundamental information for the effective use of the nitrogen mustards by direct injection into the arterial circulation.

In the study of the comparative toxicity of the transformation products of the nitrogen mustards and the efficacy of various antidotes, one of the most sensitive tests was the rate of disappearance of lymphocytes and granulocytes in rabbits. Thus, a familiar scene at Yale early in 1942 was Philips with a stop watch timing the kinetics of cyclization and Goodman and Gilman with a battery of syringes and a hoard of rabbits prepared to inject the reaction products at the appropriate time. The jibes of our colleagues that the enemy did not intend to attack with hypodermic needles were ignored. The systemic effects of the nitrogen mustards were far more fascinating than the blisters they produced on the skin, although in retrospect both responses were the result of the same fundamental action.

The studies in rabbits provided a daily reminder of the remarkable sensitivity of normal lymphoid tissue to the cytotoxic action of the nitrogen mustards. It was only natural to turn to our colleague Thomas Dougherty in the Department of Anatomy and suggest studies of the susceptibility of experimental lymphoma in mice. The problem was fundamental and simple: could one destroy a tumor with this group of cytotoxic agents before destroying the host.

In anticipation of writing this narrative, a letter was sent to Dougherty for his recollections of the first experiments with nitrogen mustards in tumor-bearing animals. The story is best told in his own words.

> "The first indication that I had of the action of nitrogen mustards on lymphatic tissue occurred when you and Lou came around to the Anatomy Department and talked about what you had seen in rabbits. The first experiments that we did were on normal intact CBA mice. We gave a series of doses in order to ascertain the lethal dose and also the dose which could be given daily for a prolonged period without too drastic an effect on the bone marrow. At this time I was working on estrogen induced leukemia with Bill Gardner and we had several cases of leukemia which occurred following estradiol treatment. The problem was to find a tumor which would grow locally so that we would have a means of evaluating any effect of the compound. It so happened that I had transplanted a lymphoma which seemed to grow locally and had little, if any, tendency to metastasize until very late in the course of the disease. This tumor grew to an enormous size, often weighing as much as the mouse when it was excised at the time of death. The life span of the animal following transplantation was about three weeks.
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> It so happened that while we were attempting to establish a dose, I had one animal in which I had transplanted this tumor which was known as the $6C_3H$ EDT, so called because it had occurred originally in a $C_3H$ mouse treated with estradiol. T was used to indicate that the tumor was being transplanted. This tumor is now known as the Gardner tumor and is still in use in many laboratories studying chemotherapeutic agents. I make a point of the tumor because it relates to some thoughts which will be elaborated herein.
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> "Anyway, we could not wait to get a whole group of animals, so we gave the mustard to this one lone mouse which had a fairly advanced tumor. After just two administrations of the compound the tumor began to soften and regress. I cannot remember exactly how many doses we gave, but in any case, the tumor completely regressed to such an extent that we could no longer palpate it. This was quite a surprising event and I remember how exciting the next couple of weeks were. We stopped treating the animal and the regression remained for a period of a month or more before a very slight growth began to appear. We then treated the animal again and a regression occurred again, although it was not as complete as the first time. In any case, the tumor did decrease and finally began to

grow again, at which time further treatment brought about no inhibition of growth. This animal lived eighty-four days following implantation of the tumor, which was a very remarkable prolongation of survival time. Of course, during the time this animal was treated, other tumor bearing animals were added to the experiment and we also obtained regression in many of them, but not in all. Many animal experiments followed in which we varied the dose, number of administrations of the same dose, etc., in order to attempt to find a proper method of treatment during the course of lymphoma growth. We used not only the $C_3H$ EDT but several other mouse lymphomas, some in the AK strain, high leukemia, other estrogen induced tumors, etc. The results of these experiments, I think, have been confirmed and borne out many times since.

"Briefly, they were that the only one of the murine lymphomas in which we got complete regression was in the original tumor in which we tried the compound. That was in the $6C_3H$ ED tumor. Among these results was the interesting fact that the very first mouse treated turned out to give the best result. We never achieved an eighty day prolongation of life in any of the other animals. The best we did was some forty day prolongation which, of course, we now know is highly effective. However, in most of the murine leukemias, particularly those which metastasized readily, we frequently obtained no effect at all. I have often thought that if we had by accident chosen one of these leukemias, in which there was absolutely no therapeutic effect, we might possibly have dropped the whole project. Also, I think we made a point at that time that there is a considerable variation in lymphomas and I think we were among the first to think in these terms, that therapy may not be universal as far as tumors are concerned, but that if you find one agent that has an effect on any tumor it should be tried because there probably is not going to be any compound that is going to inhibit growth of all cancer cells. I actually think that we were among the first to think this way and, therefore, came up with a compound which has an effect on at least some cancers in the human being. I think this is a point which deserves some emphasis because in those days what research for treatment existed was usually considered in the sense of a general treatment either of all types of cancer or at least of a certain species."

It is unfortunate that these early observations were never published, because they provided the background for the first clinical trial and indicated the relationship between experimental observations and clinical application. By the time the secrecy restrictions on the nitrogen mustards were lifted several hundred patients had already been treated, and Dougherty in association with Abraham White was deeply engrossed with the effects of ACTH and adrenocorticosteroids on lymphoid tissue. However, these early experiments were not without their impact on Dougherty. I am sure he will forgive me if I quote from the final paragraph of his letter:

"You might be interested to know that I still have practically all the blood films, bone marrows and the sections of the organs, etc. of both mice and men treated at that time. I have thrown out a few blood films, but could not bring myself to throw this part of my life completely in the ash can."

The results of the experiments in mice were sufficiently encouraging to consider a therapeutic trial in man. Consequently, the animal data were presented to Gustav E. Lindskog, who was then Assistant Professor of Surgery. Lindskog agreed to supervise the clinical trial and not many days thereafter, early in December 1942, an x-ray resistant patient in the terminal stages of lymphosarcoma was selected as a suitable subject. The tumor masses involved the axilla, mediastinum, face and submental regions, with a resulting cyanosis, venous dilatation and edema of the face and the upper part of the chest. Chewing and swallowing had become almost impossible and a tracheotomy set was kept close at hand for immediate use. The blood picture was within normal limits.

The selection of a proper dose of a highly toxic chemical warfare agent for administration to man for the first time was made with unwar-

ranted confidence. We knew that the suppression of bone marrow function in animals was completely reversible. Moreover there was a fairly wide margin between the dose of a nitrogen mustard that was acutely lethal and that required to affect lymphoid tissue. Furthermore there was little species variation in susceptibility to the cytotoxic action. On this basis it was decided to administer a dose of 0.1 mg. per kilogram of tris β-chloroethylamine daily for ten days or for a shorter period if the total granulocyte count dropped below 5,000 per cu. mm. Apparently we thought it more appropriate to initiate clinical trial with a full therapeutic dose than to titrate slowly up to an effective dose in a moribund patient. The selection of the daily dose turned out to be a most fortunate guess, but our acumen on the duration of therapy left much to be desired.

The response of the first patient was as dramatic as that of the first mouse. It has been presented in detail by Goodman and associates [3]. The response of the investigators and that of their colleagues has not previously been reported. To put the reader in proper perspective it is necessary to think back to the early 1940's. As a result of the impact of the sulfonamides, medicine was beginning to emerge from a period of therapeutic nihilism, but new drugs were still regarded with suspicion. This was particularly true in the area of malignant disease. The brilliant work of Huggins and his associates on the effects of castration and estrogen therapy on prostatic carcinoma was beginning to have its impact, but in the minds of most physicians the administration of drugs, other than an analgesic, in the treatment of malignant disease was the act of a charlatan. Add to this the fact that the nitrogen mustards were classified at the time as "top secret" and that the entry on the patient's chart read, "0.1 mg. per kg. compound X given intravenously." Truly Lindskog had great faith in his pharmacologic colleagues, as the onus of the clinical trial was on him.

Within forty-eight hours after the initiation of therapy a softening of the tumor masses was detected. It soon became obvious that this was not wishful thinking. By the fourth day of treatment, obstructive signs and symptoms were relieved and by the tenth day, when the series of injections was terminated, cervical masses were no longer palpable and a few days later the axillary masses had completely receded. The excitement generated by the dramatic remission was heightened by the fact that the total white blood cell count remained above 5,000 per cu. mm. during the ten days of treatment, predominantly aging granulocytes. We were not unaware of the shift to the right in the granular series because the hematologic observations were in the capable hands of Jean Dougherty. Its true portent soon became very apparent. In an inexorable manner the geratic granulocytes slowly died off and between the third and fourth week following the inititation of therapy the total white blood cell count hovered around 200 cells per cu. mm. There was an attending severe thrombocytopenia.

As anticipated from animal studies, the bone marrow slowly recovered. As presaged by animal experiments, but nevertheless a great disappointment, the tumor regenerated with the bone marrow. A subsequent shorter course of therapy resulted in only transient improvement and a third course had very little effect. Twenty years later we can appreciate how accurately this first patient reflected the future trials and tribulations of therapy with alkylating agents.

The next incident represents a serious error in judgment but is reported because of the important principle it helps to emphasize. Stimulated by the dramatic clinical response of the first patient and before the initial series of injections had been completed, a second patient was started on a ten day course. By the time the extent of the bone marrow depression in the first patient was fully appreciated, the second course had been completed. The tumor of this patient was resistant and the tension during the period of leukopenia was not tempered by the satisfaction of a concomitant clinical response.

Five additional patients were treated at Yale with more conservative therapeutic regimens. The patients were in the terminal stages of a variety of malignant diseases and the observations merely served to point out the limitations of therapy with the nitrogen mustards. A study of a variety of tumors of animals yielded similar results.

In June 1943, the nitrogen mustard group at Yale dispersed to assume other responsibilities. However, clinical trial of the nitrogen mustards continued. Goodman, with collaborators in several institutions, pursued an extensive clinical study. Jacobson and his associates at the University of Chicago independently initiated

therapeutic trials early in 1943 and at a later date Rhoads started clinical studies at Memorial Hospital. Secrecy restrictions were lifted in 1946 and the reports of the clinical investigations conducted during the war years [1,3–5] provided the impetus for the extensive further investigation of a variety of nitrogen mustards, as well as other types of alkylating agents.

In 1946, Gilman and Philips [1] wrote as follows:

> "It is possible that the potential value of the nitrogen mustards in the treatment of neoplastic diseases will be fully realized only when the opportunity to explore the relationship between chemical constitution and pharmacodynamic action has been exhausted. At present only two of the nitrogen mustards have been investigated clinically, namely *tris* (β-chloroethyl) amine and methyl-*bis*(β-chloroethyl)amine. These have been the product of a screening program designed for the evaluation of toxic chemical warfare agents rather than of compounds of therapeutic interest. Literally hundreds of congeners remain to be synthesized and evaluated. Thus, a series of compounds which can reproduce in many ways the cellular effects of x-rays is available for chemical and biologic investigation. It may be hoped that the previous successes which have characterized the evolution of chemotherapeutic agents by chemical alteration of a parent compound may be duplicated in the case of the β-chloroethyl amines."

The chemists have not shirked their responsibilities and hundreds of congeners of the original nitrogen mustards, as well as other types of alkylating agents, have been made available to oncologists. Has the second part of the prediction been realized? The answer will be left to the objective readers of this symposium.

REFERENCES


1. Gilman, A. and Philips, F. S. The biological actions and therapeutic applications of the β-chloroethyl amines and sulphides. *Science*, 103: 409, 1946.
2. Hunt, C. C. and Philips, F. S. The acute pharmacology of methyl-*bis*(2-chloroethyl)amine (HN2). *J. Pharmacol. & Exper. Therap.*, 95: 131, 1949.
3. Goodman, L. S., Wintrobe, M. M., Dameshek, W., Goodman, M. J., Gilman, A. and McLennan, M. T. Nitrogen mustard therapy. *J. A. M. A.*, 132: 126, 1946.
4. Jacobson, L. O., Spurr, C. L., Barron, E. S. G., Smith, T., Lushbaugh, C. and Dick, C. F. Nitrogen mustard therapy. *J. A. M. A.*, 132: 263, 1946.
5. Karnofsky, D. A., Craver, L. F., Rhoads, C. P. and Abels, J. C. An evaluation of methyl bis(β-chloroethyl)amine hydrochloride and tris(β-chloroethyl)amine hydrochloride (nitrogen mustards) in the treatment of lymphomas, leukemia and allied diseases. In: Approaches to tumor chemotherapy, p. 319, 1947. American Association for the Advancement of Science, Washington, D. C.