ERLICH, BERTHEIM, AND ATOXYL: THE ORIGINS OF MODERN CHEMOTHERAPY *

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"Statistics teach that one-seventh of all human beings die of tuberculosis, and that, if one considers only the productive middle-age groups, tuberculosis carries away one-third and often more of these" (1). So stated Robert Koch (1843-1910) when he presented his paper on the etiology of tuberculosis to the Physiological Society in Berlin in 1882. It is difficult for us, at the end of the twentieth century, to understand the impact of infectious disease on humanity at the end of the nineteenth century. At that time the number of effective drugs was very small, digitalis for heart problems and morphine for pain but for infectious diseases, with the exception of quinine for malaria, there were basically none. The treatment and control of infectious diseases is, arguably, the most outstanding scientific and humanitarian achievement of the twentieth century. The untold death, pain and misery caused by just two, tuberculosis and syphilis, is difficult for us to comprehend today. Perhaps nothing in the history of mankind has so affected the quality and quantity of life for all people as have chemical therapeutics. The genesis for this great humanitarian and scientific achievement has its roots in nineteenth century chemistry.

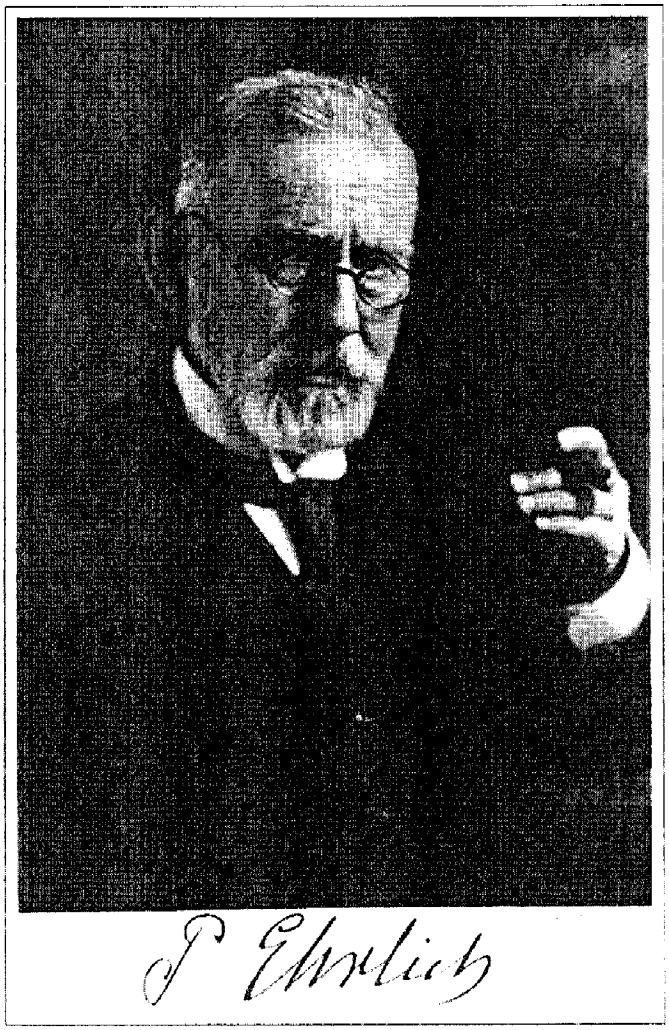


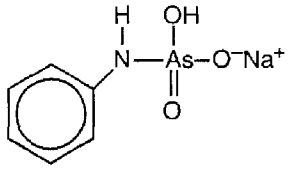
Figure 1. Paul Ehrlich (1854-1915). (Courtesy of the Rockefeller Archive Center.)

Paul Ehrlich (1854-1915), (Fig. 1), is the father of chemotherapy; in fact he coined the word and defined it as "the use of drugs to injure an invading organism without injury to the host(2)." Ehrlich was born in Strehlen, in what was then the Prussian state of Silesia. He studied medicine and pathology in Breslau and earned his MD degree from the University of Leipzig in 1878. His MD thesis was entitled "Contributions to the Theory and Practice of Histological Staining," indicative of his

early and lifelong interest in the use of dyes to stain bacteria. Carl Weigert (1845-1910), Ehrlich's cousin and noted pathologist, had interested Ehrlich in bacteriology and the use of aniline and other dyes to stain bacteria (3). The idea of staining and perhaps killing bacteria with aniline or other dyes was a seed planted early in Ehrlich's professional life. After receiving his MD degree he worked for ten years in Berlin's Charite_ hospital and between the years of 1891-1899 he worked with Robert Koch in his Institute for Infectious Diseases also in Berlin. It was during this time that he developed methods for evaluating and standardizing the diphtheria antitoxin which led to his sharing the Nobel Prize in Medicine with Metchnikoff in 1908.

In 1899 Ehrlich moved to Frankfurt, where he would spend the rest of his life, and to the Royal Institute for Experimental Therapy. He was to remain at the Institute until 1906. During this period several key discoveries, which would play a fundamental role in the genesis of chemotherapy, were made. In 1903 Bruce discovered that a trypanosome was the cause of African sleeping sickness, in 1905 Schaudinn discovered that a spirochete caused syphilis. Also in 1905 Thomas discovered that an organic arsenic compound, "atoxyl," was effective against trypanosomes. This compound had been synthesized some forty years earlier by the French physician/chemist Béchamp.

Pierre J. A. Béchamp (1816-1908), (Fig. 2), was an interesting and controversial scientist. Born in the Moselle region of France, he held a doctorate in both medicine and the physical sciences. In 1852 he developed a cheap method of making aniline from the reduction of nitrobenzene and in 1863, while teaching medical chemistry at the University of Montpellier, he synthesized a compound from aniline and arsenic acid (4). This organic arsenical became



Atoxyl, according to Béchamp

known later on, because of its decreased toxicity to animal forms of life, as "atoxyl." Béchamp characterized this compound as an anilide and described some of its chemistry; however, interest in his *anilide de l'acide arsenique* languished until the turn of the twentieth century.

The use of arsenic as a medicinal drug had been known for centuries. As early as the fifth century B.C.,

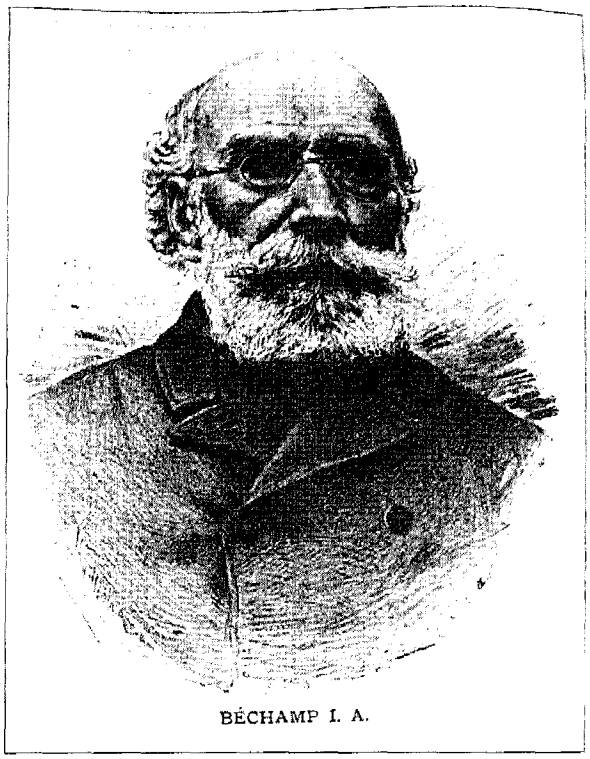


Figure 2. Antoine Béchamp (1816-1908). (Courtesy of Archives de l'Academie des Sciences, Paris.)

Hippocrates recommended
using arsenic trisulfide for abscesses and when syphilis first appeared in Europe in the end of the fifteenth century it was only natural to try some of these inorganic compounds of arsenic. The high toxicity of these inorganic preparations precluded widespread usage (5). In the eighteenth and nineteenth century the work of de Gassicourt (6) and Bunsen (7) led to the discovery of organic arsenic compounds. Forty two years after Beuchamp first announced his aniline and arsenic preparation, Thomas showed that "atoxyl" was effective in the treatment of trypanosomiasis (8). *Trypanosoma brucei gambiense*, is the cause of African sleeping sickness and was at one time Africa's number one health problem. In the years around 1900 an epidemic in the Belgian Congo killed half a million people (9).

Thomas's paper apparently peaked Ehrlich's interest for he wrote to Karl Herxheimer (1861-1942), Professor of Dermatology at the University of Frankfurt(10):

> August 29, 1905, Please be good enough to send me the paper on atoxyl, since I would like to read it in the original.

Thus began, with this simple inquiry, modern chemotherapy. Ehrlich became the first person to elucidate the correct chemical structure of a therapeutically ac-

tive compound and then set out to modify this structure to improve its medicinal effectiveness. There had been earlier attempts to correlate structure to effectiveness (11) but Ehrlich was the first to understand and exploit this idea. There is very little mention of atoxyl again until Ehrlich wrote to Julius von Braun, a Privatdocent specializing in organic chemistry at Göttingen(12):

> November 7, 1905, Dr. Bertheim, who has given up his position at the factory, has in the meantime arrived here, and since he is free, he can start to work and be of help to me, which is very welcomed.

This is the first mention of Alfred Bertheim (1879-1914), Ph.D., Berlin in 1901. He worked as a manufacturing chemist until joining Ehrlich. After joining Ehrlich in 1905 he remained with him until the outbreak of World War I; joining the German army he was killed in an accident in Berlin on August 17, 1914 (13). Bertheim, either on his own or under Ehrlich's direction, discovered the true nature of atoxyl. This seminal discovery led to the first truly effective therapeutic agent in the fight against syphilis. In this same letter to von Braun (12), Ehrlich discussed the chemistry of atoxyl and specifically referred to it as "arsenic acid anilide." He goes on to say (12):

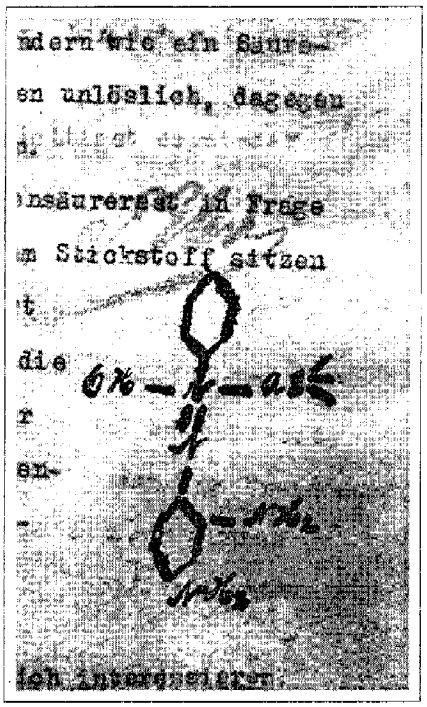


Figure 3. Sketch in a letter from Ehrlich to von Braun, November 7, 1905, Copirbuch XVIII, 186-188, box 24, Ehrlich Collection. (Courtesy of the Rockefeller Archive Center.)

> I have found that if one treats atoxyl with nitrous acid one gets a diazo compound that must contain the arsenic residue. This compound couples easily to produce dyes that still must contain an acid residue. Thus, for example, the coupling product with toluylaminediamine does not behave like the diamidoazobenzol but like an acid derivative. It is insoluble in weak acids, but easily soluble in alkalies.

He then sketched the structural formula in his copy book as he believed it to be (Fig. 3). So, on the day that he wrote of the arrival of Alfred Bertheim, Ehrlich still tried to rationalize the structure of atoxyl as that of an anilide. Since diazotization should only be possible for primary aromatic amines and not for anilides it is difficult to understand how Ehrlich could have continued to persist in this idea. Perhaps it was because he was described as a "self taught chemical investigator(14)." Scarcely one week later and only one week after Bertheim had been on the job, he again wrote to von Braun (15):

> November 14, 1905, one must consider if the constitution of the atoxyl is really correct, or if perhaps it is not in fact a paraamino derivative of benzol arsenic acid.

Almost three weeks after the arrival of Alfred Bertheim, Ehrlich wrote to Ludwig Darmstaedter, a trained chemist and one who helped Ehrlich raise research funds (16):

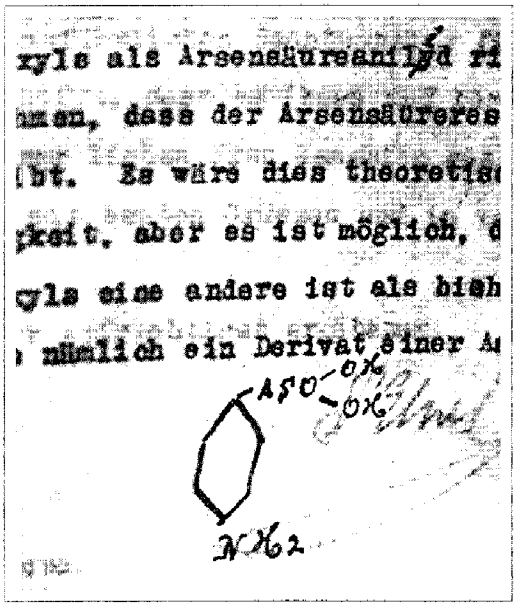
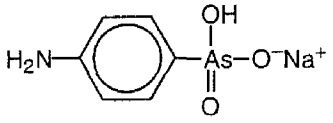


Figure 4. Sketch in a letter from Ehrlich to Darmstaedter, November 25, 1905, Copirbuch XVIII, 303-305, Ehrlich Collection. (Courtesy of the rockefeller Archive Center.)

November 25, 1905, either the structure of atoxyl as an arsenic acid anilide is correct, in which case one would need to propose that the arsenic acid residue remains attached to the azo group. Theoretically this would be of the greatest importance, but it is possible that the structure of atoxyl is different than assumed until now, and that it is really a derivative of aminophenyl arsenic acid.

Figure 5. Copirbuch XVIII, box 24, Ehrlich Collection. (Courtesy of the Rockefeller Archive Center.)

He then sketched the proposed structure in his copy book as an amino phenylarsenic acid. (Fig. 4). Ehrlich dictated some of his voluminous correspondence to his secretary, which she typed and placed in copy books (Copirbuch in German). The cover of copy book number XVIII is shown in Fig. 5. From that time on there seemed to be no question that atoxyl was not an anilide but an arsenic acid, and he and Bertheim published a paper to that effect in 1907 (17).



Atoxyl, according to
Ehrlich and Bertheim

Interestingly, this point, i.e. who discovered the true nature of atoxyl, is a most important one and was made into a major bone of contention both in the film (18) made about Ehrlich and the biography written about Ehrlich by his former secretary, Martha Marquardt (19). In one scene in the movie Ehrlich is talking to his three chemists (von Braun, Schmitz, and Bertheim) and telling them to proceed with their work as if atoxyl is an amino acid and not an anilide. Two of the three quit then and there and only Bertheim, meekly, says he will stay. The Marquardt biography describes an even more contentious confrontation (20):

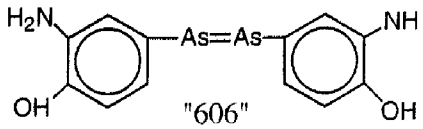
Ehrlich declared firmly to the three chemical workers at the Georg Speyer Haus: 'Atoxyl is *not* an anilide of arsenic acid. On the contrary, it contains a free amino group; I have worked on the arsenic azo-dyes, which can be made in consequence of this, for some considerable time'.

She then continues to describe the scene between Ehrlich and his three chemists which results in von Braun and Schmitz's resigning with only Bertheim willing to remain. If, as Marquardt says, Ehrlich knew that atoxyl was not an anilide he did not describe it as such, according to his own copy books, until several weeks after Bertheim's appearance in his laboratory. The facts, as revealed in Ehrlich's copy books, are on November 7, 1905, Ehrlich thinks atoxyl is an anilide and Bertheim joins him and begins working on the problem. On November 14, 1905, Ehrlich questions whether atoxyl is an anilide and finally on November 25, 1905, almost three weeks after Bertheim's arrival he draws the correct structure of atoxyl. This is well before the summer of 1906 and the famous confrontation described by Marquardt. Is this just a coincidence or did Bertheim, after hearing the facts, suggest to Ehrlich in November, 1905 that if atoxyl could be diazotized it could not be an anilide and therefore was probably an amino phenylarsenic acid? We shall never know but the elucidation of this critical fact, i.e. that atoxyl is indeed an phenylamino arsenic acid and not an anilide allowed Ehrlich and Bertheim to proceed with the synthesis of many variants of the atoxyl structure. That this was the critical discovery in the synthesis of therapeutic arsenicals was evidenced in a commemorative address made later by Bertheim (21):

> A readily cleavable anilide held out no promise chemically, although a stable p-amino-phenylarsenic acid could be expected to combine in itself the numerous reactions of aniline with that of the arsenic acids and would also possess special properties deriving from the combination of both. Probably for the first time, therefore, a biologically effective substance existed whose structure was not only known precisely but also-unlike the alkaloids-was of a simple composition and extraordinary reactivity, which permitted a wide variety of modifications.

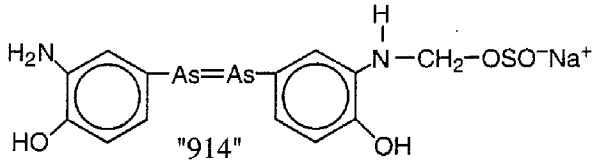
The last sentence in this statement, underlined for emphasis, is particularly important in that it points out that the genesis of modern chemical therapeutics began with Ehrlich and Bertheim. After this Ehrlich and Bertheim began a program of synthesizing various modifications of the atoxyl molecule. Eventually with the 606th compound of this series, they came upon a compound which was very effective against syphilis and was the first major triumph in the synthesis of chemotherapeutic agents.

This compound, arsphenamine or Salvarsan, was introduced into general usage in 1910.



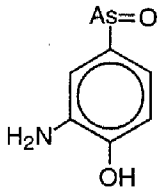
Salvarsan, arsphenamine

This compound brought world wide fame and recognition to Ehrlich. However, problems with making an injectable solution of it caused Ehrlich to seek an improved version. This resulted in the water-soluble variant Neosalvarsan.



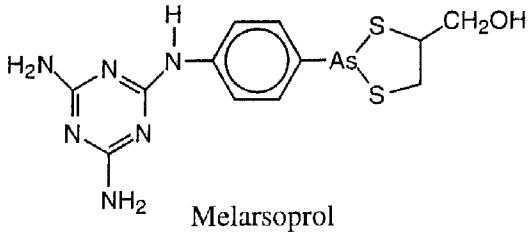
Neoalvarsan

Interestingly, doubt about the proposed As=As bond in these molecules arose as early as 1921, when it was found that the per cent arsenic did not conform to the structure. Later work convincingly showed that neither Salvarsan nor Neosalvarsan was a pure substance and suggested that they were mixtures of polymeric materials containing only arsenic-arsenic single bonds. There is no evidence that an As=As bond exists in either of these compounds (22). By the 1930s it was recognized that the arsenoso relative:



Oxophenarsine, mapharsen

was really the active ingredient and, under the trade name of Mapharsen, was the drug of choice in the treatment of syphilis until the advent of penicillin in the early 1940s. Ironically, this compound had been synthesized in Ehrlich's laboratory early on and listed as compound number 5 but was thought to be too toxic to be of use (23). Even though research into arsenicals has ceased, Paul Ehrlich and Alfred Bertheim's legacy of chemical therapeutic arsenic drugs lives on. Recently an article detailed how Melarsoprol, a compound closely related to atoxyl:



Melarsoprol

is still being used to treat sleeping sickness (9).

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