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Mechanism of action of the anti-syphilitic arsenical compounds and their use in early syphilis

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THE MECHANISM OF ACTION OF THE ANTISYPHILITIC ARSENICAL COMPOUNDS AND THEIR USE IN EARLY SYPHILIS

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INTRODUCTION

The object of this paper is to review the literature pertaining to the mechanism of action of the arsenical preparations now being used in the treatment of syphilis with the idea in mind of attempting to evaluate the effectiveness of the compounds with reference to their therapeutic uses. This seems to be a timely subject in view of the fact that with the present war time conditions the incidence of syphilis is almost certainly bound to increase in spite of public health attempts at its control. With this inevitable increase in the incidence of early syphilis, primarily the problem of adequate therapy becomes more and more important. Recently new drugs have been added to the armamentarium of the syphilologists, in particular mapharsen, and with the introduction of this drug, modern syphilologists have again begun to think of Ehrlichs dream of "therapia sterilisans magna" in the form of continuous intravenous medication for a few days and massive intravenous injections over a ten day period in the treatment of syphilis particularly the primary and secondary stages. This principle of one sterilizing course of treatment would naturally be of inestimable value since the present day mode of therapy is definitely too long a process for adequate management of all cases. In short, it can be said the present form of treatment

is too long, too painful, too dangerous, and too expensive. Our present system of antisyphilitic treatment has much to be asked for in the proper control of syphilis.

Naturally, any therapeutic agent must be evaluated both clinically and experimentally to determine its effectiveness, and far better insight into a disease process and means for its control can be obtained if the clinician understands the mechanism whereby his drugs exert their effect rather than using them empirically. It is with this purpose in mind that the following pages have been written, i.e. to review the literature with the intent of reaching some conclusion regarding the mechanism of action of the arsenicals and a rationale for their use in the treatment of syphilis. With this knowledge of the mechanism of action we can then perhaps better evaluate the present day therapeutic methods which are being used, especially in the treatment of early syphilis, and it is in early syphilis that we stand our best chance of eradicating syphilis both from the public health standpoint and from the late ravages to the individual victim.

HISTORY

A history of the development of the arsenical preparations used in the therapy of syphilis is almost a history of syphilis. Ever since the clinical recognition of syphilis man has sought after means for combating this dread disease. The origin of syphilis is still problematical and only of academic interest in the first place; although, the disease was apparently not recognized in Europe until after the discovery of the New World which has led historians to presume that syphilis was introduced to Europe by the sailors of Columbus and other explorers who contracted the disease in American and brought it home to Europe whence it spread like wild fire, until at present, it is known among all peoples of the world.

Although syphilis was recognized as disease entity in Europe for a long time, it is of interest to note that there was little or no progress in the development of therapy following its introduction to Europe for over three hundred years. It is somewhat paradoxical to note that while amazingly good clinical observations and descriptions of the disease were made by many workers ever since the discovery of the disease, literally no progress was made in the treatment of the disease. The treatment which was used in past centuries also seems somewhat unjustified to Kemp (1) since, in spite of the treatments used, no clinical arrests of the disease were made.

However, it must be remembered that during these times no laboratory procedures were available to check upon the efficacy of different preparations such as we have today in the form of dark-field examinations and sensitive serological tests.

Many preparations (1) were used in the treatment of syphilis and among these might be listed guaicum, China root, sarsaparilla, and sassafras, all of which enjoyed their respective periods of popularity. It was not, however, until approximately 1600 that any antisyphilitic agent of therapeutic potency as we see it today was introduced. Paracelsus in 1568 is usually credited with the introduction of mercury as an antisyphilitic agent. .It is unfortunate that the advent of mercury should meet with so many disastrous reactions due to its misuse. Following its introduction (1) many cases of hemorrhagic gastroenteritis and nephritis were noted in patients being treated with the drug. These reactions were so serious at one time that medical students of Heidleberg were required to take an oath, from 1580 to 1655, to the effect that they would, at no time, use mercury in the treatment of syphilis. The next drug of valuable therapeutic power to be introduced was potassium iodide, which is supposed to have been introduced by Wallace in 1835.

Following the introduction of mercury and potassium iodide

to the armamentarium of the syphilologist, little or no progress was made in the treatment of syphilis until the turn of the present century and the momentous work of Ehrlich and his contemporaries--with one exception. In 1887, Von Jauregg (1) noted that there was often an improvement in cases of general paresis following an acute febrile attack, and in 1917 he used malarial fever therapy (tertian) in the treatment of paresis and this therapy was admitted of good therapeutic efficiency. Since then, it has been in vogue until the advent of modern artificial hyperpyrexia cabinets, et cetera.

With the turn of the present century and the discovery of the Treponema pallida as the etiological agent of syphilis by Schaudin in 1906, and the development of serological diagnostic tests by Bordet, Wassermann, and subsequent workers the field was indeed ripe for some concrete advances in the therapy of syphilis. It had long been known that arsenic at times produced certain effects in syphilis (2), but the results were unreliable and consequently arsenic had never played any great part in the therapy of syphilis. Occasionally however, it had been used in those cases which were resistant to mercury therapy. It was the experimental work of Uhlenhuth, Gross, and Bickel in 1907 with atoxyl in experimental hen spirillosis which precipitated all the further work of Ehrlich and his colleagues. The hen spirillosis (Sp. gallinarum) was

found to be favorably treated with atoxyl by these workers and they found that doses (single) of 0.05 gm. per kilo. were both curative and prophylactically sound. Naturally, this suggested the use of atoxyl in the treatment of syphilis which had recently been shown to be a spirochetal disease. However, treatment of syphilis with organic arsenical preparations including atoxyl was soon abandoned because of the detrimental effects to the host, especially to the optic nerve.

At this time Ehrlich attacked the problem from the viewpoint of determining the mechanism whereby the chemotherapeutic agents killed the parasites. He also advanced his "tropism" theory as explained later in this paper and began a series of investigations into the organotropic and parasitotropic effects of many organic arsenical compounds. He first employed atoxyl (2) and with the assistance of Bertheim showed it to be a sodium salt of paraminophenyl acid, a very stable and at the same time strongly reacting substance. By transforming and attacking the amido group Ehrlich succeded in obtaining an infinite variety of compounds, all of which contained the radical of an organically fixed arsenic acid. Out of hundreds of substances experimented with on animals, only a few were found to be available particularly arsacetin, arsenophenylglycin, and salvarsan. Salvarsan or arsphenamine was prepared by Dr. Bertheim in Ehrlichs laboratory and the animal

experimentation was done by Hata. When the therapeutic potency of salvarsan had been determined by them; and when they had noticed the lack of gross toxicity in experimental rabbit syphilis, Ehrlich sent the preparation of Professor Alt at Uchtpringe, Germany (4) for investigation of its toxic and therapeutic properties as related to men. After a number of investigations upon dogs with reference to toxicity, two physicians permitted themselves to be injected with salvarsan and they experienced nothing but pain and swelling at the site of injection with no untoward after effects whatsoever. From here on Alt performed his work upon paralytics and noted that while pain was severe at the point of intramuscular injection and there was a temporary rise in temperature to about 102° F. neither infiltration or abscesses were noted and the patients showed rather marked clinical improvement and reversal of the Wassermann reaction in a large percentage of cases. Salvarsan was originally given intramuscularly because it was believed that its therapeutic effect was enhanced because of delayed elimination. These experiments and clinical trials of Professor Alts were accomplished in 1909, and in 1910 the drug was placed on the market as salvarsan, so named because it was believed that the preparation would be the salvation of mankind.

Ehrlich's aim had been to produce an antisyphilitic agent

of such potency that would kill every spirochaete in the body and thus realize his dream of "therapia sterilsans magna". As a result of this aim of Ehrlich's many syphilitics were grossly undertreated with disasterous late results. In 1911 (4) the intravenous method of treatment was adopted and Ehrlich's "therapia sterilisans magna" became only a cherished dream. Arsphenamine and neoarsphenamine were introduced to the syphilologists armamentarium from 1910 to 1911 and in rapid succession since that time until the present several anti-syphilitic agents of high therapeutic efficiency have been introduced, namely; silver arsphenamine in 1918, tryparsamide and sulpharsphenamine in 1919, stovarsol in 1921, and mapharsen in 1934. It is of interest to note that all of these preparations except silver arsphenamine and sulpharsphenamine were studied by Ehrlich but were discarded because of real or supposed toxicity.

The next big advance in the chemotherapy of syphilis was made by Sazerac and Leviditii in 1921 (1) with the introduction of bismuth compounds to the list of antisyphilitic agents.

MECHANISM OF ACTION OF ANTISYPHILITIC ARSENICAL COMPOUNDS

Kolmer (3) summarizes the possible mechanisms by which chemotherapeutic agents exert their parsiticidal action in the human body by listing the following possibilities:

1. A direct chemical interaction between the compound or drug administered, or after some transformation of the compound within the body, with some protoplasmic constituent of the parasite resulting in the death or crippling of the parasite by interference with its vital processes of internal respiration;

2. physico-chemical interaction between the compound and and the protoplasmic colloids of the parasite involving precipitation, coagulation, and changes in electrical charge sufficient for destruction of the parasite;

3. production of new compounds in tissues capable of chemical or physico chemical protoplasmic action on parasites producing effects different from those of the original compound;

4. production of antibodies by releasing antigenic substance from the parasites;

5. stimulation of oxidation, production of hyperemia, stimulation of reparative processes, production of leukocytosis phagocytosis, and mobilization of proteolytic and lipolytic enzymes capable of crippling parasites.

With these five possible means whereby the chemotherapeutic agents may exert their action it is the purpose of this section to critically review and discuss the literature with the purpose in mind of trying to arrive at some conclusion as to the nature of action of the organic arsenical compounds used in the treatment of syphilis. It appears to the author that such a review is especially timely in view of the modern introduction of new drugs as mapharsen into the treatment of syphilis and the new intensive massive dose therapy and continuous intravenous therapy. Since all chemotherapeutic agents must be less toxic to the host than to the parasite in order to be of any therapeutic value, it seems that a thorough and critical analysis of the pharmacological actions of these drugs is needed especially as pertains to their mechanism of action upon the parasites, since unless the drugs can be shown to have relatively quick action upon the parasites themselves, the massive continuous form of therapy is not reasonable.

Ehrlich (5) postulated the "parasitotropic" and "organotropic" views of chemotherapy early in the present century when he stated that the mechanism whereby parasites were killed by chemotherapeutic agents was such that these agents became fixed to the parasites thereby killing them. He also stated that the cells of the individual being treated with these agents were capable of having the drug fixed to

them. This action between drug and parsite was the "parasitotropic" action of the drug and that between drug and body cell was the "organotropic" action of the drug. Ehrlich was fully conscious of the fact that a proper balance between these two actions was essential before success could be attained with any chemotherapeutic agent. Ehrlich was also aware of the fact that there were exceptions to this rule, but he used it as a guiding principle in his work. Ehrlich believed that living cells had "chemoreceptors" of a similar nature to those which he postulates in his famous antigen-antibody side chain theory of immunity. He believed that these chemoreceptors were specific for certain drugs as evidenced by his experiments which have shown that animals may be drug fast to a certain preparation so that this preparation has no therapeutic effect after numerous small doses of the preparation, but after becoming drug fast the therapeutic effect of a similar drug is at once evidenced with its administration.

As further evidence for the principle of fixation Ehrlich cites the work of Hata (5) who first showed that parasites when mixed alone with salvarsan did not reduce their motility. However, when the treated parasites were injected into laboratory animals these animals showed no signs of infection, which he believed cast out the theory of antibody stimulation as the mechanism of salvarasan action and also served as evidence for linkage of drug to parasite.

Lee (6) has an interesting idea as to the mechanism of action of the organic arsenical antisyphilitic agents. He states that the Treponema derives its nourishment from tissue cellular protein, and that arsenical drugs as they pass through the capillary system in a fine crystalloid state pass through the endothelial membranes by diffusion and osmosis. The arsenobenzol group, the group of greatest affinity, attaches itself to the cellular protein, producing an arsenoprotein substance which is not only an unfavorable culture medium for the parasite, but is directly poisonous to the parasite. Lee cites two facts as evidence for this theory, one clinical and one experimental. Clinically he states an area of redness is often seen around a syphilitic lesion twenty-four to forty-eight hours after treatment and the patient will often complain of heat, pain, and tenderness in these areas. Experimentally he states that excised syphilitic tissue is spirocheticidal in vitro after treatment with arsphenamines as further evidence in support of his theory as well as the fact that Noguchi has shown that fresh tissue from an animal to which the Treponema is adapted is necessary for cultivation of the organism.

McDonagh in 1917 (7) advanced a theory concerning the chemotherapy of syphilis which is of only passing interest in view of more modern concepts of the treatment of the disease.

He observed the rise in central nervous system syphilis following what was at the time believed to be adequate therapy consisting of eleven intravenous salvarsan injections followed by eleven intramuscular bismuth or mercury injections and pronouncement of cure. He blamed these recurrences upon the use of arsenic compounds and condemmed their use at this time. He believed that the combined use of oxidizing and reducing agents was much better than the use of one alone. He postulated that salvarsan does not attack the parasites directly, but only indirectly by increasing the oxidizing action of protein particles in serum and in plasma cells. Metals are in general oxidizing agents and non-metals reducing. He stated that the chemotherapeutic agents are more potent in protozoal diseases and especially syphilis, because of the increased size and number of protein particles circulating in serum, forming resistance substance of the host or antibody. He introduced intramine as a substitute for arsenic compounds and believed his clinical results were much better. Intramine contains sulphur, and he believed it less toxic than the arsenical compounds and by using this substance with mercury in form of combined therapy he had his treatment of alternating reducing and oxidizing agents.

In the days when Ehrlich was making his revolutionary studies of organic arsenical compounds in search of his "therapia sterilisans magna", Hata in conjunction with

Bertheim (5) first showed that when spirochaetes were mixed with salvarsan, the parasites did not lose their motility in vitro. From this, it was concluded that tissue was essential for the therapeutic effect to become active. Bronfennbrenner and Noguchi in 1913 (8) showed that arsphenamine and neoarsphenamine were non-toxic to spirochaetes and trypanosomes in the test tube. They showed that neoarsphenamine is broken up by living tissue and the derivatives of neoarsphenamine are especially toxic for spirochaetes. Experiments with neoarsphenamine showed that the toxicity to spirochaetes was greatly increased by the presence of living tissue in test tube experiments, but when this tissue was boiled the toxicity was markedly descreased. These investigators used liver extract and defibrinated whole rabbits blood as their experimental tissues.

The first experimental spirochaetal infection to be treated with an organic arsenical compound was hen spirillosis (3) (Sp. Gallinarum) with atoxyl (Sodium arsanilate) by Uhlenhuth, Gross and and Bickel in 1907 and these workers found that single doses of 0.05 gm. per kilo. was both curative and prophylactically sound. However, atoxyl was found to have no action in vitro which could justify its pharmacological action and this fact in addition to the in vitro action of salvarsan, et cetera, immediately started speculation as to the mode of action of these interesting compounds. Levaditi (9) postulated the mechanism of action of atoxyl as a

reduction by liver substance and a combination of the reduction product with protein, the latter acting as the link for anchorage to the parasite. These views of some chemical change occurring to the drug being used within the body were strongly held by many workers, not the least of whom was Ehrlich, who had long before noted that the trivalent arsenical compounds were more toxic both to the host and to the parasite than the pentavalent forms, and from these observations, it was concluded that oxidation-reduction reactions took place in the body before the compounds became therapeutically effective.

It was not until the important work of Voegtlin and Smith (10) in 1920, however, that the nature of the altered compound was thought to be known. These workers showed that the sodium salt of arsphenamine is first oxidized to the corresponding oxide and that this compound is simultaneously oxidized to the pentavalent arsenical. They have shown that in experimental trypanosomiasis in rats arsphenamine injections showed a latent period of from two to three hours before the trypanosomes began to disappear from the peripheral blood, but when arsenoxide or the pentavalent form was administered in exactly the same manner no such latent period elapsed. They also found that when neoarsphenamine was partially oxidized in the air the latent period as described above was also considerably reduced. This work seems to show rather

conclusively that the parasiticidal action of the arsphenamines is due to conversion in the body of the host of the drug to a compound of the arsenoxide type.

This work of Voegtlin and Smith received considerable impetus and confirmation by a subsequent work of Rosenthal (11) who found that arsenoxide was ten times more toxic to organisms than arsphenamine and twenty times more toxic than neoarsphenamine. Rosenthal developed a color test for determining the presence of arsenoxide by means of napthaquinone and by means of this color test found considerable amounts of arsenoxide in the liver and kidneys of rats following the injection of arsphenamine and neoarsphenamine, thereby confirming the theory of Voegtlin.

With this theory that the trivalent arsenical compounds as arsphenamine and neoarsphenamine are oxidized to arsenoxide before the therapeutic effect is apparent, the next problem at hand was the mechanism where the arsenoxide compound was therapeutically effective. It was noted by Voegtlin, Dyer, and Leonard in 1923 (12) that reduced glutathione and related compounds counteracted the action of arsenoxide compounds on trypanosomes both in vitro and in the circulating blood of experimentally infected rats. This substance, glutathione, is a complex protein with a mercaptan SH radical which has been isolated from liver, muscle and yeast, and is believed to be an important component of living

tissue by Voegtlin (13). Hopkins and Kendall (14) have more recently shown that the SH glutathione is a tripeptide composed of cystein, glutamic acid and glycine.

Following this discovery that glutathione counteracted the action of arsenoxide compounds upon spirochaetes and trypanosomes in vitro and apparently in vivo a mass of work has been done in an effort to determine just what the action of this glutathione (if any) is upon the parasites or upon the drugs in question. With the work of Voegtlin and his associates in 1923 (12) this work was started and these workers showed that while the reduced glutathione counteracted the toxic actions of the arsenoxide in vitro and in vivo such compounds as amino acids with no SH group, glucose, lecithin, and inorganic salts did not show this effect and these workers therefore concluded that the antitoxic effect of SH compounds is due specifically to the SH group. These workers also found that trypanosomes as well as all cells with an active metabolism contain an SH group probably in glutathione as indicated by a characteristic nitroprusside reaction. These workers have also postulated that SH compounds injected intravenously are partly oxidized within the blood and diffuse in part into the tissues. In conclusion, they believe that arsenic in the form of arsenoxide R-As= o is a specific poison for the SH group of glutathione and possibly other SH groups

which occur in protoplasm.

Along this same line of work Rosenthal working with Voegtlin (14) showed that rats will easily survive a minimum lethal dose of arsenoxide if they have previously received an injection of SH glutathione in the ratio of 10 moles of SH glutathione to 1 mole of arsenic and they have also showed that a similar protection is afforded to trypanosomes in vitro in that if the organisms and arsenoxide are mixed together with SH glutathione the organisms lose neither their motility or infectivity powers. These workers also believe that local inflammatory lesions which arise from arsenoxide injections are also lost if protection is afforded by the addition of SH glutathione to the arsenoxide solution prior to its injection as shown by tests in the ears of rabbits.

These experiments involving the organisms and living tissues are further confirmed by an excellant work of Rosenthal (15) in which he mixed arsenoxide (trivalent arsenical) with egg albumin, blood serum, and casein and then ultra filtered the mixture. None of these compounds contains an SH groups. He concluded from these experiments that there was no combination between the arsenic and these proteins since the arsenic was present in the filtrate in the same concentration as it was in the mixture. He also showed that when the proteins were coagulated to bring out the SH groups there was marked combination between the arsenoxide and the proteins

which was directly proportional to the concentration of the SH groups as evidenced by the nitroprusside test. Interestingly also he showed that fresh tissue which was supposedly free of glutathione but which contained fixed SH groups was shown to combine with arsenoxide as shown by his ultrafiltration experiments and he also believed and showed by similar experiments that there was no combination between the pentavalent arsenicals and proteins containing the SH group. In conclusion, he believes that the presence of SH groups gives protection to trypanosomes from arsenical compounds.

These experiments upon the sulfhydril combination of arsenicals have been summarized by Voegtlin and his associates (16) by their statement of the theory that tissue asphyxia and death results when the oxidized $\rightleftharpoons$ reduced glutathione equilibrium is upset by the addition and combination of arsenical compounds with the SH group of this protein. They also postulate that the sulfhydril group in tissue may well be the "arseno-receptor" of Ehrlich for mammalian tissue although it must be born in mind that there may well be other arseno-receptors than the sulfhydril group.

Eagle (17) in a series of in vitro experiments with experimental syphilis found that cysteine, glutathione, thioglycollic acid and presumably any compound containing a reactive sulfhydril group when added in sufficient excess to

compounds of arsenic, mercury, or bismuth almost completely abolishes their antispirocheticidal powers in vitro. In connection with this finding the fact that a considerable excess of the SH substance is necessary suggests that there may be a hydrolysis of the addition reaction product. The important finding with reference to his work seems to be that arsenoxide, arsphenamine, neoarsphenamine, mercuric chloride, and many proprietary bismuth compounds were tested, and all proved alike in their action with SH groups. This seems good evidence for the fact that the chemotherapeutic agents in syphilis combine with the SH groups in the protoplasm of the Treponema pallida. He also showed that thiamin chloride and methionine which contain an — S— group but no — SH group did not show any such inhibitory action.

SUMMARY

1. It appears from the evidence as presented that salvarsan, neosalvarsan, et cetera, are not directly spirocheticidal as such, since this cannot be demonstrated in vitro, and the evidence all points to some change in the compound within the human body before spirocheticidal properties are evidenced.

2. It is now believed that this substance is arsenoxide which is formed from the neosalvarsan or salvarsan as shown by the work of Voegtlin and Smith, and since confirmed by many experiments particularly the work of Rosenthal in which he demonstrates arsenoxide in the tissues of animals previously injected with arsphenamine.

3. Evidence has been shown that points to the excellent possibility of the connecting link between drug and parasite being the mercaptan sulfhydril radical not only with the organic arsenical compounds, but also with the other antisyphilitic preparations as mercury and bismuth.

4. The evidence points to arsenoxide being directly toxic to spirochetes rather than stimulating antibody production or some other such mechanism.

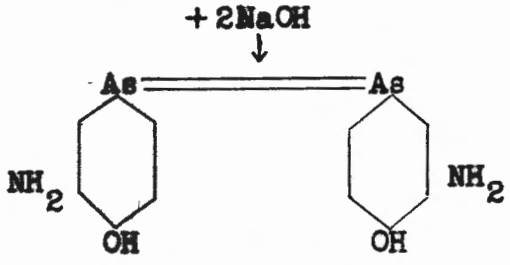
In conclusion, it may be said that the organic arsenical preparations exert their action directly upon the spirochaetes rather than by antibody stimulation, et cetera, and that the active principle is probably arsenoxide or the oxidizidation product of salvarsan, et cetera. The salvarsan may be oxidized in the body or prior to injection but it is this arsenoxide which is spirocheticidal. There is evidence which points to sulfhydril radicals within the spirochetes as being the linkage bond with antisyphilitic drugs; not only the organic arsenicals, but mercury and bismuth also.

ESSENTIAL PHYSICO-CHEMICAL PROPERTIES OF ARSENICAL ANTISYPHILITIC AGENTS

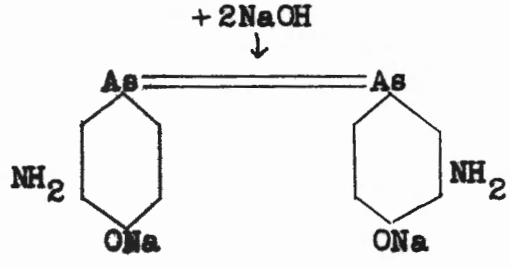
The following formulae from Goodman and Gilman's text (18) are now given for guidance in reading the following pages:

Alkalinization of Arsphenamine

Arsphenamine hydrochloride
(Arsphenamine U.S.P.)

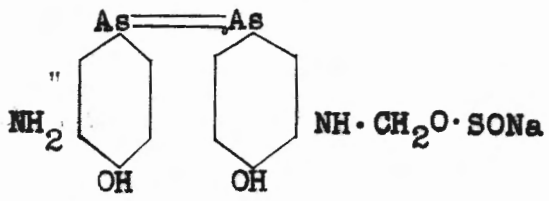


Arsphenamine base

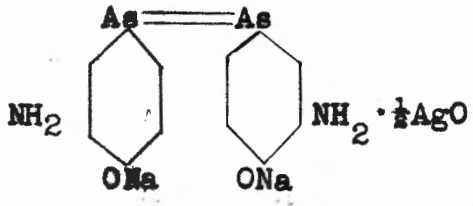


Na Salt of Arsphenamine

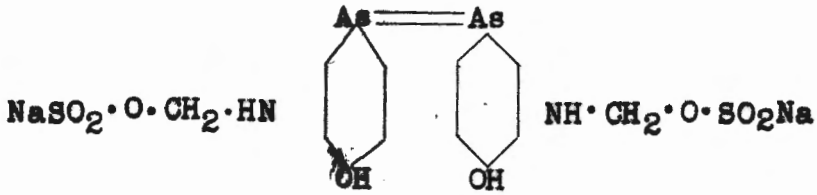
Neoarsphenamine:



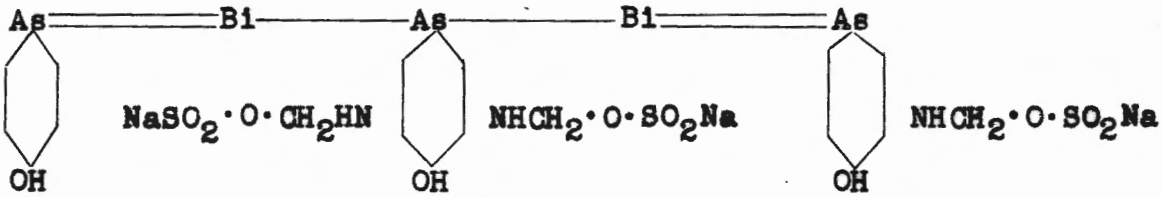
Silver Arsphenamine:



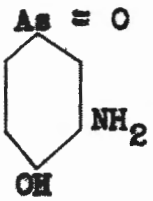
Sulfarsphenamine:



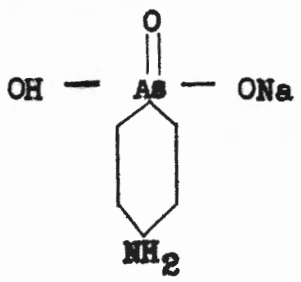
Bismarsen:



Mapharsen:



Atoxyl: (Ehrlich and Bertheim)



The arsphenamines all contain arsenic in the trivalent form and this particular valence seems to be essential for their therapeutic action. The important feature of the arsphenamine structure is the double bond linkage between two atoms of arsenic as R·As= As·R in which R is the benzene ring with an amino group attached in the meta and a hydroxy group in the para position to the arsenic. Isomers of arsenic are less spirocheticidal and it must be therefore concluded that the amino and hydroxy groups are optimally placed in the ring for proper physical and chemical properties in their parasiticidal action.

The arsphenamines, as all chemotherapeutic agents, owe their action to their physical and chemical properties. The physical properties may be classed as colloidal properties, electrical properties, crystalloid properties, et cetera, while the chemical properties are classed as those chemical reactions between the compound and tissue constituents. Arsphenamines behave as semi-colloids, since

the drug dialyses very slowly through parchment and somewhat more rapidly through collodium membranes, and it has both colloid and crystalloid fractions, (19). Arsphenamine base, on account of its basic amino groups and acidic phenolic group, may be considered a complex ampholyte whose conduct is markedly influenced by the pH of the solution (20). In view of these ampholytic properties and colloidal properties of the arsphenamines they may be likened to proteins and show many similarities to proteins. The isoelectric point of arsphenamine lies in the neighborhood of blood pH and hence only a small degree of ionization takes place at blood pH.

As stated before arsphenamine and its derivatives are trivalent arsenicals and reduction leads to arsine; oxidation to trivalent arsenious oxide which in turn can be oxidized to the pentavalent arsenical as shown by the following formulae (20):

$$R\cdot As\!=\!O \begin{matrix} \nearrow OH \\ \searrow OH \end{matrix} \longleftarrow R\cdot As\!=\!O \longleftarrow R\cdot As\!=\!As\cdot R \longrightarrow R\cdot AsH_2$$

(arsenoxide) (Arsphenamine) (arsine)

R represents substituted benzene ring.

None of the arsenicals used in practice are chemically pure with the exception of mapharsen or arsenoxide, which has made biological testing of the compounds imperative and it has been shown by Voegtlin that the physical properties

of the arsphenamines are probably responsible for the untoward toxic reactions following intravenous injection, i.e. the colloidal, protein, and viscosity properties (13). The colloidal properties are probably the factor in producing the nitritoid crises and shock-like responses and the pH of the solution probably is also a factor. In this respect neoarsphenamine (13) is more dispersed in solution, only slightly alkaline and readily soluble in range of physiological pH with consequent fewer nitritoid reactions and anaphlactoid reactions. Wright (21) and his co-workers have shown in conjunction with this work that the crystalloid fraction is of higher therapeutic index and lower toxic index than the colloid fraction.

In summarizing the essential chemical properties of the arsphenamines, we then have:

1. The arsphenamines all contain trivalent arsenic which is essential for their therapeutic activity.

2. The arsphenamines have great similarity to proteins in being ampholytic and having colloidal and crystalloid fractions, and it is believed that these physical properties account for many of the toxic reactions to the host.

3. The arsphenamines are all readily oxidized both in vitro and in vivo, and the role of these oxidation products in the spirocheticidal activity of the drug has been discussed at more length earlier in this paper.

4. Mapharsen, or arsenoxide, is a chemically pure compound thereby not necessitating biological assay prior to its use.

EVALUATION OF DRUGS AND METHODS OF THERAPY

With the foregoing in mind, it now remains to be seen if any logical evaluation of the therapeutic uses of the organic arsenicals can be made. We do know that arsphenamines bring about clinical cure and arrests of syphilis, but we still do not know any optimum treatment for all cases. The reasons for this are many, as stated by many authors but a few can possibly be enumerated. (1) We know very little concerning the characteristics of the etiological agent especially its metabolism, "life cycle", chemistry, and relationship to human tissue. (2) We do not know exactly how our drugs exert their therapeutic effect. (3) We have no satisfactory means for pronouncing a patient cured. In spite of these drawbacks, however, we do have effective means for controlling the infectiousness of the disease and fairly reliable prophylactic measures. As stated in the introduction to this paper, our management of syphilis, while it has progressed amazingly, still lacks a great deal. The great need, now as always, has been for an <u>early diagnosis</u>, followed by a course of treatment which can be terminated in a few days to weeks with positive assurance to the patient that he is cured. We now have at our disposal excellent means for the establishment of early diagnosis but adequate and

convenient therapy is still lacking. Because of this, many syphilitics are grossly mismanaged, either because of inconvenience to the patient, or ignorance on his part; or because of lack of information or diligence on the part of the physician. It is the evaluation of the treatment of early syphilis which will now be undertaken with the idea in mind that what we are really after is the dream of Ehrlich--to cure every syphilitic with from one to several injections of the therapeutic agent and to kill every spirochaete in the body.

Before the use of mapharsen in 1934, the majority of early syphilitics were treated with salvarsan, neoarsphenamine, or silver arsphenamine. A small number were treated with sulpharsphenamine or bismarsen. The intermittent form of treatment was used to a large extent with rest periods during the course of treatment and the arsenical drugs were supplemented with mercury and/or bismuth injections. In 1931 Cannon and Karelitz (22) reviewed the cases of 436 syphilitics whose histories showed that their disease was of no more than six months duration before treatment was started and who remained under observation and treatment for at least six months. All of the patients received supplemental bismuth and/or mercury injections. The three common antisyphilitics which they studied were

salvarsan, neoarsphenamine, and silver arsphenamine. The criteria which they based their evaluation upon were: (1) time involved in effecting disappearance of the lesions; (2) reversal of the Wassermann reaction; (3) complications arising from the administration of the drug, and; (4) the number of relapses. After detailed evaluation of the figures they concluded that salvarsan was superior to both neoarsphenamine and silver arsphenamine in all respects. They showed that salvarsan requires fewer injections and a smaller quantity of the drug to produce the desired results. They also showed that a shorter period is necessary for reversal of the Wassermann reaction with salvarsan than with the other preparations. They concluded that salvarsan is the drug of choice and should be used in spite of technical difficulties encountered in its administration since its effect on the patient certainly warranted its use.

Stokes and Beerman in 1941 (23) gave the following criteria for the clinical testing of antisyphilitic drugs: (1) rapidity of surface spirillicidal activity; (2) reversal of serological reactions; (3) low incidence of relapses; (4) low incidence of central nervous system involvement; (5) good effect on resistant syphilitic manifestations and late syphilis; (6) ultimate curative action, and; (7) low incidence of drug reactions. With these criteria in mind

a great mass of literature has cumulated evaluating all of the antisyphilitic preparations with the result that practically the only arsenical preparations now in use in the treatment of early acquired syphilis are salvarsan, neoarsphenamine, and mapharsen. Most of the literature evaluating these drugs consists of little more than a tabulation of their toxic reactions to the patient; and it is not the purpose of this paper to repeat these tabulations, but rather to see if there is any logical reason for the use of arsenoxide or mapharsen, and whether the new short term massive dose therapy in early syphilis is practical. The results with arsphenamine and neoarsphenamine in the treatment of early syphilis with respect to the above mentioned therapeutic criteria are quite well known to every physician and will not be repeated. In recent years the continuous form of treatment has come to be accepted as the standard procedure in early syphilis as compared to the form of treatment used in the past in which rest periods were believed to be beneficial. Excellent evidence to support the continuous form of treatment is given by Padget (24), who in 1940 by a statistical study of 551 patients treated for early syphilis showed the intermittent form far inferior to the continuous form. These 551 patients were followed for from five to ten years after their initial treatment and

the conclusions were based upon all the criteria for clinical testing of therapeutic agents as stated earlier. Padget (24) also believes as many syphilologists, that the incidence of neurosyphilis is higher among patients treated intermittently or inadequately than it is among patients who receive no treatment at all. This evidence shows plainly that the present trend is for early continuous rigorous treatment without rest periods so as to hit the spirochaetes hard before they have time for entrenchment and multiplication.

Similarly hyperpyrexia has been shown to be inadequate when used alone in the treatment of early acute syphilis. Boak and her co-workers (25) in 1942 gave eight patients with primary and secondary syphilis from nine to fifteen hours of artificial fever at from 41.0° C. to 41.5° C. There was prompt resolution of the early lesions in all cases, but in four out of five patients who received no chemotherapy whatsoever there were mucocutaneous relapses and the fifth patient continued to have positive serology, but no obvious infectious relapse. She therefore concluded, as does Leifer (26), that hyperpyrexia alone is not suitable in the treatment of early syphilis because human tissue cannot tolerate the thermal death point of the treponema pallida.

With these seemingly well established principles in mind; namely, early treatment is essential, treatment must

be continuous and rigorous, and chemotherapy is essential for proper treatment, we can now proceed to attempt to evaluate the best possible means for treating early acquired syphilis. As has been stated earlier the evidence points to an arsenoxide compound being the active antispirochetal agent, and salvarsan and neosalvarsan are not directly spirocheticidal. We now have at our disposal arsenoxide or mapharsen which has been used clincially and evaluated in the literature as much as possible. Tatum and Cooper (27), in a study of forty-four cases of experimental rabbit syphilis treated with mapharsen showed that mapharsen has certain definite advantages over salvarsan and neoarsphenamine. They state that mapharsen is a chemically pure compound recquiring no biological assay as arsphenamine; it is less toxic upon oxidation than arsphenamines; the therapeutic dose is from 1/50th to 1/30th that of neoarsphenamine; the therapeutic index is greater than for the arsphenamines; and the preparation may be ampouled with $NaCO_3$ and NaCl for neutralization and isotonicity. Leifer (26) in a clincial study of one hundred and eighty early cases treated with mapharsen and one hundred and eighty early cases treated with neoarsphenamine showed that the percentage of "cures" was about equal or slightly greater with mapharsen. However, the number of reactions were much less with mapharsen than with neoarsphenamine and there were

no nitritoid reactions with mapharsen. Stokes and Beerman (23) in an evaluation of mapharsen have shown that the drug is rapidly spirillicidal and effectively heals lesions. The drug also gives an early reversal of serology and good symptomatic response. Naturally the big question at present concerning mapharsen is whether or not its apparently excellent effects will be permanent. Stokes and Beerman, however, believe that any deficiencies in this respect will be compensated for by heavy metal therapy.

From a theoretical standpoint mapharsen seems to be the drug of choice in the treatment of early syphilis. This conclusion is arrived at from the fact that the evidence points to its being actively spirocheticidal and also because it is easy to administer and has a relative lack of toxicity. Naturally its staying power from a therapeutic standpoint cannot be very well evaluated as yet. This theoretical viewpoint is born out by clinical evidence as outlined above.

In 1935 and again in 1939 Chargin, Leifer and Hyman (28) described the treatment and clinical cure of early syphilis with five day intravenous continuous drip methods for twelve hours daily. They first used salvarsan and neoarsphenamine but these drugs were soon discarded because of central nervous system damage and peripheral neuritis.

Since that time a large number of patients have been treated by this method and also by massive doses over a

slightly longer period with or without hyperpyrexia. Following the abandonment of arsphenamine and neoarsphenamine in massive dose therapy, mapharsen has been used. The usual dosage is from 80 to 240 mg. daily with a total dosage of about 1200 mg. (28) This is the average amount used either with continuous drip or massive syringe method regardless of whether or not hyperpyrexia is used in conjunction.

The following chart (29) is a compilation of recent reports on massive dose arsenotherapy.

SUMMARY OF RECENT REPORTS ON MASSIVE DOSE ARSENOTHERAPY

	Reactions				
Failures including possible reinfection	Toxic encephalopathy	Toxicoderma	Peripheral neuritis	Exfoliative dermatitis	Jaundice
6	2	50	39	1	4
24	3	35	5	0	2
8	--	--	--	--	--
5 to 15%	--	11%	--	0	2
8	2	--	--	0	0
--	--	--	--	--	--
--	No serious reactions				
10%	1	--	--	--	--
0	No serious reactions				
8	1	5	--	--	--
--	1	--	--	--	--
--	1	60	4	2	2
7	1	7	7	--	2
5	4	8	2	--	1
3	0	7	18	0	1

1. Arsenosan--A hydrochloride of metaaminoparahydroxyphenylarsine similar to mapharsen.
2. Oxiarsolan--Arsenoxide hydrochloride.

		← Treatment →					
Author	No. of Patients	Arsenical Administered	Method	Total Dose (mg.)	Duration (days)	Deaths	
erry	50	mapharsen	I.V. Drip	1200	5	0	
sher and ill	36	mapharsen	I.V. Drip	1200	5	0	
run, Ramirez nd Roman	270	mapharsen	I.V. Drip	360 to 440	10	1	
runes and evia	60	neo	I.V. Drip and one injection one week later	5400	3 to 7	0	

	← Reactions →				
Failures including possible reinfection	Toxic encephalopathy	Toxicoderma	Peripheral neuritis	Exfoliative dermatitis	Jaundice
1	0	1	0	0	1
2	1	5	3	0	0
2 to 4%	1	0	15%	--	3.5%
--	--	--	1	--	--

Summarization of Mapharsen Treatment

Total cases	3300	
Failures	291	— 8.8% of total
Deaths	12	— 0.36% of total
Reactions	400	— 12.1% of total
Toxicoderma	291	
Peripheral neuritis	72	
Encephalopathy	15	
Exfoliative dermatitis	2	
Jaundice	15	

From a summarization of the preceding chart, it is at once apparent that the toxic effects of neoarsphenamine are prohibitive of its use with this method. In the case of mapharsen, however, the results are considerably better. A sample of 3300 cases seems sufficient for some fairly definite statements. The percentage of deaths (0.36%) and the percentage of serious toxic reactions (12.1%) are both too high for conservative therapeutic standards. However, the number of failures, including possible reinfections (8.8%), is very encouraging although naturally this figure is open to dispute since sufficient time has not elapsed in many instances for proper evaluation of the curative powers of the procedure.

To summarize, we may then say that a shorter, more intensive form of therapy is needed since our present long term therapy is not suited for proper treatment of all patients and the evidence points to continuous rigorous treatment being the method of choice in early syphilis. Mapharsen seems to be the theoretical drug of choice and this is born out to a certain extent by clinical evidence although the preparation is too new for a complete evaluation therapeutically. Massive arsenotherapy is theoretically sound and a step toward the ideal treatment although at present it must be considered in the experimental stages.

CONCLUSIONS

1. The evidence at present is that the arsphenamines exert their spirocheticidal properties only after oxidation; and the oxidation product is directly spirocheticidal. The spirocheticidal agent is believed to be arsenoxide and there is evidence which points to sulfhydril groups in the protoplasm of the Treponema pallida being the "chemoreceptor" between drug and parasite.

2. At the present time, the theoretical drug of choice in the treatment of early syphilis seems to be mapharsen or arsenoxide. This opinion is advanced in view of the fact that the evidence points to its being the active spirocheticidal agent. Also the drug is relatively non-toxic, is easy to administer, and is a chemically pure compound not requiring biological assay.

3. The evidence points to the use of continuous rigorous, massive doses of the therapeutic agent as the method of choice in the treatment of early syphilis.

4. Arsenoxide apparently acts directly on the spirochaetes and its toxic action upon the spirochaetes is relatively quick. It therefore seems that if ways and means of getting a sufficient concentration of the drug into the body for its sterilization can be attained, we will have answered the question of the ideal treatment of

early syphilis. At present, however, we have not found these means since the drug is too toxic to the patient when used in these concentrations. The new methods of massive arsenotherapy are a step in this direction, however, but at present they must be considered in the experimental stages because of technical difficulties and the high mortality and morbidity associated with their use.

5. The method of choice for all but the expert in the treatment of early syphilis at the present time is a form of continuous arsenical and bismuth injections over a period of months to years as outlined in all texts on the treatment of syphilis.

6. There is a great need for more knowledge of the physico chemical relationships between the drugs and parasites and between the drugs and tissues since herein lies the secret of producing the therapeutic agent which will chemically combine with the protoplasm of the parasite with a toxic result, but which will not toxically combine with the tissues of the host.

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