periods of time (23 months) nor in swine urine kept at laboratory temperature for shorter periods of time (4 to 21 days).

In determinations 8, 9, and 10 (Table I) where twice as much preservative was used as in determinations 5, 6 and 7, the increase in chlorine content was slight (1.04%) being within the experimental error. These urines were taken from the same animals on successive days.

In one set only is there any evidence of a slight increase (3.6 to 7.1%) in the chlorine content and that occurred when the preserved acid urine was kept at laboratory temperature for 21 days. The slightly higher chlorine content in several of the preserved alkaline cow urines may be due to analytical variations rather than to actual increased chlorine content.

The authors wish to express their appreciation to Dr. E. B. Forbes, who made this work possible.

[CONTRIBUTION FROM THE DIVISION OF LABORATORIES AND RESEARCH OF THE NEW YORK STATE DEPARTMENT OF HEALTH, ALBANY.]

THE PREPARATION OF ARSPHENAMINE (SALVARSAN).[1]

BY PHILIP ADOLPH KOBER.

Received December 21, 1918.

I. Introduction.

The synthesis of an arsphenamine or salvarsan suitable for therapeutic purposes, in spite of the work of Ehrlich and Bertheim[2] and their collaborators, is still a vital problem. It is fairly well known that the toxicity of arsphenamine varies and that batches made by individual manufacturers vary more than can be accounted for by the differences in their procedures. Furthermore, since it seems fairly well proven that even Ehrlich's own manufacturers are unable to maintain a uniformly high standard,[3] it is evident that there are some factors which are not understood or not under control. I am informed by manufacturers of arsphenamine that about 50% of the arsphenamine made does not meet the Surgeon General's requirements[4] and therefore is not distributed.

In studying the subject, I came to the conclusion that the toxicity of arsphenamine or at least the variation of the toxicity is largely due to the use of methyl alcohol and ether in the final precipitation of the base as the dihydrochloride. While most chemists use Ehrlich and Bertheim's methyl alcohol and ether method or some modification of it for precip-

[1] Read in part before the Society of Experimental Biology and Medicine, New York City, November 20, 1918.

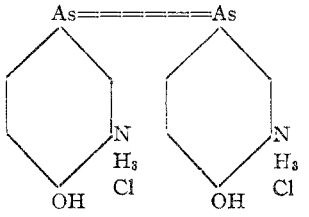
[2] *Ber.*, **45**, 756 (1912).

[3] Roth, *Hygienic Laboratory Bulletin* **113**, 7 (1918).

[4] The U. S. Public Health Service, *P. H. Reports*, **33**, 540 (1918), requires an M. L. D. of 0.060 g./kilo body weight, while the Surgeon General (recently) raised the requirement to 0.080 g./kilo, and to 0.100 g./kilo.

itating the dihydrochloride of salvarsan, not one will admit or is willing to believe, that the dry product contains a molecule of methyl alcohol.

The theoretical amount of arsenic in arsphenamine as shown by the formula is 34.2%, assuming that it is absolutely pure and contains no water or solvent in its crystal or solid form.



The German firm having a monopoly of the manufacture of the drug, tried at first to get the scientific public to accept its statement unchallenged of a 34% arsenic content. Analyses by others soon compelled them to abandon that claim and for seven years 31.6% (31.57%) has been and now is accepted as the arsenic content.

This different and smaller content of arsenic is in harmony with an assumption of two "molecules of water of crystallization" in the final dry substance.

This amount of water is given in the literature frequently and by the U. S. Health Service regulations[1] as an actual fact, and a higher content of arsenic, involving less solvent combined with the drug, is looked upon with suspicion.

I believe I have exhausted the references to the literature on this point, and I can find no justification for assuming two molecules of water in the drug, made by the usual and original directions.

The reference or work chiefly if not wholly relied upon for this assumption is that of Gaebel.[2] Myer[3] and DuMez quote him also in support of the arsenic regulations of the drug. While other authors make arsenic estimations, Gaebel is the only author mentioned or that I can find, beside Ehrlich and Bertheim, who has made any experiments as to the solvent and its amount in the dry substance.

Gaebel passed hydrogen gas, purified, over 0.07966 gram salvarsan, contained in a glass vessel called "Ente" (meaning goose-shaped), heated in an oven at 105° C.; the vapor coming off was tested only for hydrochloric acid with silver nitrate solution. He weighed the "Ente" and noted the loss of weight. After 6 hrs. its weight became constant and the loss corresponded to 7.2%. The theoretical loss for 2 molecules of water would be 7.6%.

[1] *Public Health Reports*, **33**, 540, 1004 (1918).
[2] *Apoth. Ztg.*, **26**, 215–216 (1911).
[3] *Public Health Reports*, **33**, 1004 (1918).

This one estimation apparently satisfied him for he records no duplicate estimation, and called the solvent thus lost water, apparently taking no account of the fact that arsphenamine was actually precipitated from methyl alcohol (undoubtedly anhydrous so as to mix with the ether) with non-aqueous ether. That Gaebel simply assumed it to be water, not knowing the method of Ehrlich and Bertheim used is indicated also by the fact that he published a year before Ehrlich and Bertheim published their method.

Against the statement that the solvent in the solid arsphenamine is water, are the words and experiments of Ehrlich and Bertheim.[1] They tested one preparation made with methyl alcohol and ether, in the same way all their preparations are supposed to be made, after drying the substance at 65° C. in a current of carbon dioxide. This temperature is about the boiling point of methyl alcohol. On dissolving this dried preparation in water and distilling over any volatile solvents they found methyl alcohol in appreciable quantities. They concluded upon this and other reliable analyses that it contained one molecule of methyl alcohol for every molecule of the arsphenamine.

In view of these facts, it seems fairly certain that arsphenamine made according to the original directions must contain methyl alcohol, unless, of course, it is removed by drying under more favorable conditions than Ehrlich and Bertheim used. The evidence in the literature gives one the strong impression that for commercial reasons its methyl alcohol content has been kept secret. The objections to methyl alcohol and other considerations connected therewith will be discussed on page 450. In order to eliminate the methyl alcohol or any other organic solvents in the process of preparation, the following method was developed:

II. Method of Preparation.

Finding that the dihydrochloride of the arsphenamine base was insoluble in an excess of chlorides, as might be expected from the Law of Mass Action,[2] an excess of hydrochloric acid was tried in "salting" out the drug. When first tried, by making a aqueous solution of the dihydrochloride directly from the base, by dissolving in twice normal sodium hydroxide and adding a slight excess of hydrochloric acid and then pouring the solution of hydrochloride into a strong solution of hydrochloric acid (1–1), a white precipitate was formed which, however, soon turned to a dark-colored gum. This transformation of the white precipitate into the black gum, was due simply to the coalescence of the particles. This coalescence was prevented when the precipitation was conducted, (1) at a low temperature, (2) under more dilute conditions, and (3) with vigorous stirring.

[1] *Ber.*, **45**, 756 (1912).

[2] Nernst, *Z. phys. Chem.*, **4**, 372 (1889); Noyes, *Ibid.*, **6**, 241 (1890).

As the purity of the hydrochloride depends also on the purity of the arsphenamine base, the details of this preparation will be briefly given; in other words, the process starting with the nitro-oxyphenyl-arsonic acid[1] will be described.

1. **Reduction of Nitro-Oxyphenyl-Arsonic Acid.**—Two hundred and twenty (220) grams of magnesium chloride are dissolved in 5500 cc. of distilled water and 1100 grams of sodium hydrosulfite are quickly added, while stirring or shaking, in an eight-liter bottle.

To this solution is then added, with stirring or shaking, 85 grams of crude nitro-oxyphenyl-arsonic acid, dissolved in 290 cc. of 2.0 *N* sodium hydroxide and diluted with 1700 cc. of water. The mixture[2] is then allowed to stand at room temperature or it is slowly warmed in a water bath at 40° C., until the suspension, first formed, seems to agglutinate and to be about to settle. The suspended matter seems to be impurities, mostly dark colored, from the nitro-oxyphenyl-arsonic acid; impurities from the hydrosulfite, also dark, and its reaction products; besides a little of the arsphenamine base. The total weight of the suspension, when filtered and dried, is rarely more than three to four grams, or four to five per cent. of the total yield.

When this stage is reached, the mixture is rapidly filtered, through hard paper, or alundum ware, and the clear, yellow filtrate digested, according to Ehrlich and Bertheim's directions, at 50° to 60° C. for two to two and a half hours, when the base—diaminodioxyarsenobenzene—separates out as a yellow precipitate.

Some chemists lay great stress on using only the purest nitro-oxyphenyl-arsonic acid and only to treat it subsequently with commercial hydrosulfite, which itself, in many cases gives, if not a muddy solution, at least a dark colored one; all this in spite of Ehrlich and Bertheim pointing out, that the reduction, even with the purest nitro-oxyphenyl-arsonic acid, produces its own by-products. These chemists then rely on bone-black to remove all such impurities from the hydrochloride in methyl-alcohol solution.

This preliminary digestion of ours and filtering removes the impurities to such an extent, that with the exception of a slight white, mineral residue found in some hydrosulfites, no further filtering or removal of extraneous coloring matter is necessary.

[1] Owing to the ease with which *p*-arsanilic acid can now be made, which is described in a separate communication (THIS JOURNAL, 41, 300 (1919) we prefer to make nitro-oxyphenyl-arsonic acid by nitrating the oxalic acid derivative of *p*-arsanilic acid (*Ber.*, 44, 3095 (1911)) and saponifying according to the directions of Ehrlich and his collaborators (*Ber.*, 44, 3451 (1911)).

[2] These amounts of material and solution practically fill an eight-liter bottle, so that little, if any, inert gas is needed nor do other anaerobic precautions have to be taken.

2. **Preparation of the Dihydrochloride.**—The crude diaminodioxyarsenobenzene, after filtering and washing with distilled water at 0° C., is transferred to a porcelain dish and suspended in 400 cc. of distilled water, likewise at 0° C., where it is dissolved in the least quantity of two normal sodium hydroxide, usually about 150 cc., which should also be at 0° C. We find it convenient to prepare all the necessary solutions two or three days ahead of time and to keep them in an ice-box or refrigerator, at the end of which time they will be cold enough for the purpose.

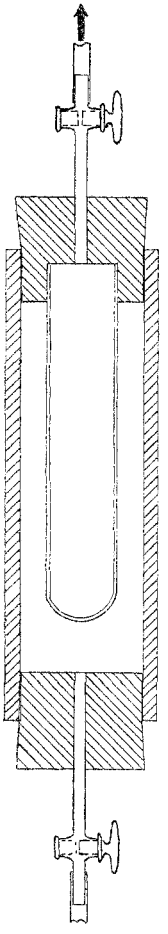


Fig. 1.

Laboratory Anaerobic Filter.

The outer cylinder may be made of glass, and the inner cone of "alundum" ware. When not completely filled with liquid, inert gas such as hydrogen or nitrogen is introduced and the position of the filter reversed.

The alkaline solution of the base is now filtered so as to remove any insoluble matter, like filter paper; any fibers of dust, and also, a small amount of the mineral precipitate, mentioned before, which is present when some hydrosulfites are used. For this purpose we have constructed a simple form of anaerobic filter, which is shown in Fig. 1.

To the perfectly clear alkaline solution of the arsphenamine, 150 cc. of strong hydrochloric acid (1–1) at 0° C. are added, which throws out and re-dissolves the yellow base. The clear solution, resulting from the addition of the hydrochloric acid, contains the dihydrochloride, and, for the purpose of getting a fine precipitate, the solution is further diluted with distilled water at 0° C. in order to bring the volume of the final solution up to 1700 cc.

Into a stirring apparatus, 3250 cc. of hydrochloric (acid 1–1, *i. e.*, 1 part concentrated acid plus 1 part water) at 0° C. are placed. While vigorously stirring, the cold aqueous solution of dihydrochloride of arsphenamine is run slowly into this hydrochloric acid. The grey-white precipitate, now formed, is allowed to settle for an hour. It is then filtered in thin layers and dried in a vacuum desiccator at a low pressure, fused calcium chloride and solid sodium hydroxide in sticks or flakes[1] being used as absorbents. It is preferable to place the solid alkali separately

[1] Flakes, containing 97% of sodium hydroxide can now be obtained for about 6 cents a pound.

in a dish surrounded by the calcium chloride. The heat of neutralization of the hydrochloric acid, undoubtedly, helps to a considerable degree to dry the substance quickly. After twelve or more hours hydrogen is introduced into the desiccator to equalize the pressure and the arsphenamine is ground and further dried until of constant weight. The yield is about 75%.

III. The Product.

Color.—The substance prepared by the method just described is usually a greyish powder sometimes having a slight yellowish tint. When very pure it seems to have little or no color and is practically white in a dry state, and is possibly analogous to the color changes of cupric sulfate when made anhydrous.

Analyses.—Analyses of preparations having about 1 molecule of water in the final product are as follows:

	% As.[1]		% N.		% Cl.	
Preparation.	Found.	Theory.	Found.	Theory.	Found.	Theory.
No. 2.....	32.67	32.85	..	..	...	...
No. 5.....	...	...	..	..	14.89	15.50
No. 12.....	32.89	32.85	6.37	6.13	14.09	15.50

Preparations with 2 molecules of water in the final product gave:

	% As.[1]		% N.		% Cl.	
Preparation.	Found.	Theory.	Found.	Theory.	Found.	Theory.
No. 9.....	31.83	31.57	5.60	5.90	16.79	14.90
No. 10.....	31.83	31.57	5.64	5.90	14.09	14.90
No. 11.....	30.38	31.57	5.83	5.90	14.67	14.90

The analyses that Ehrlich and Bertheim obtained on their product are given below for the purpose of comparison. One analysis made by us of arsenobenzene is also given. The theoretical values are calculated on the basis of 1 molecule of methyl alcohol to 1 molecule of arsphenamine.

	% As.		% N.		% Cl.	
Preparation	Found.	Theory.	Found.	Theory.	Found.	Theory.
Ehrlich and Bertheim's.....	31.99	31.85	6.06	5.95	14.51	15.07
Arsenobenzol No. 1449.....	30.89	31.85	6.20	5.95	15.24	15.07

Ehrlich and Bertheim found that in the same preparation just mentioned the carbon content was 32.63%. The theory for 1 molecule of methyl alcohol calls for 33.12% C., while the theory for 2 molecules of water requires only 30.32% C.

[1] The above results are in most cases the averages of the following analyses. The arsenic estimations were made according to the gravimetric method described in *Treadwell-Hall* (1914) and also in *Public Health Reports*, **33**, 1003–1018 (1918). The nitrogen was determined according to the Kjeldahl method (modified Gunning), U. S. Department of Agriculture, *Bull.* **107**, 8d. (1907). The chlorine was determined by fusing the product with two parts of potassium nitrate and one part of sodium carbonate

Qualitative Tests.—Of the qualitative tests suggested and compiled by Meyer[1] and DuMez, we have tried the following ones only, which we consider the most important, and obtained the reactions described in every respect:

Dilute sulfuric acid gave an insoluble hydrosulfate.

Nitric acid gave the characteristic color.

Bromine water gave the color described.

Phosphotungstic acid gave the color reaction for phenols.

Silver nitrate gave the beautiful red colored complex described by Danysz.[2]

Magnesium mixture, used as described, showed no inorganic arsenic.

Mayer's reagent for alkaloids, gave the characteristic test.

Mercuric chloride showed the precipitate described.

Carbon dioxide precipitates the base from its alkaline solution, as is

Preparation.	% As.	% N.	% Cl.	
No. 9	32.01	..	...	
	31.86	..	...	
	31.62	..	...	
	31.95	..	...	
	31.21	5.60	16.84	
	32.32	5.67	16.75	
Average	31.83	5.63	16.79	
No. 10	31.02	..	...	
	31.62	..	...	
	32.11	..	...	
	32.34	..	...	
	31.77	5.64	14.18	
	32.11	5.64	14.00	
Average	31.83	5.64	14.09	
No. 11	30.43	..	...	
	30.90	..	...	
	30.25	5.78	14.72	
	29.93	5.88	14.63	
Average	30.38	5.83	14.67	Sulphur 0.30%
No. 12	32.62	..	...	
	33.07	..	...	
	33.00	..	...	
	33.07	6.30	14.18	
	32.71	6.45	14.00	
Average	32.89	6.37	14.09	

and then proceeding according to the Drechel modification of Volhard's method. *Treadwell-Hall*, **2**, 707 (1914).

[1] *Public Health Reports*, **33**, 1004 (1918).

[2] *Compt. rend.*, **157**, 644 (1913); *Ibid.*, **158**, 199 (1914).

very characteristic for salvarsan, showing the presence of a very weakly acid substance.

Physical Properties.—The substance as soon as it is moistened with water becomes brownish yellow and dissolves in 5 parts of water to form a gel or gelatinous solution depending on the temperature. It is readily soluble in warm or hot water, slightly soluble in methyl alcohol, scarcely soluble in ethyl alcohol, insoluble in ether or benzene.

Melting Point.—It does not melt slowly when heated, but gradually darkens, beginning at 160° C.; when at about 180° C. it begins to char. Ehrlich and Bertheim state that their product decomposes, depending on the speed of heating at about 185–195° C. with charring.

Toxicity.—In general its other properties seem to be that of salvarsan in every respect. When tested by injection into rats according to the rules and standards recommended by the U. S. Public Health Service and adopted by the Federal Trade Commission,[1] it seems to have a relatively low grade of toxicity.

IV. General Discussion.

Our work on arsphenamine seems to show that the dihydrochloride, when pure and possessing only one or two molecules of water, is practically colorless. Samples of this colorless form can be shown and are easily prepared. Impure arsphenamine is tinted more or less, depending on the impurity and also on the physical form of the solid substance. Of the three commercial preparations made in America no two are alike in color. The Canadian Diarsenol is very yellow, the Metz preparation less yellow with a tint of green, while the Arsenobenzol has a light yellow tint.

The amount of yellow tint may be due to the sulfur content, as our grey substances have about $^1/_2$ to $^1/_4$ the amount of sulfur that is present in the ordinary yellow material.

The analyses of arsphenamine indicate that the amount of colored impurity in most preparations must be negligibly small, at most 1% or less. The toxicological studies also show that the amount of colored impurities is small and negligible. In fact Ehrlich,[2] G. T. Morgan[3] and we in this laboratory have found that a very light-colored arsphenamine is sometimes very toxic compared to a darker preparation. Since it is possible also to alter the color of the solid form by changing its physical state, comparison of the color of different arsphenamines should be based on the color of the substance in standard solutions.

To sum up: The color of the solid arsphenamine seems to be due to a small amount of highly colored impurities in an otherwise colorless substance.

[1] *Public Health Reports*, **33**, 540 (1918).

[2] *Loc. cit.*

[3] Longmans, "Organic Compounds of Arsenic and Antimony," **1918**, p. 156.

Our experience with the methyl alcohol and ether method has brought to our attention four possible objections against the use of these substances: These solvents are highly inflammable; they are expensive even in peace times; they are difficult to make pure, and finally methyl alcohol, ether and other solvents are easily oxidized to easily reduceable substances. As a concomitant with arsphenamine, a substance easily oxidized to very toxic and therefore dangerous products, methyl alcohol and similar substances, are *a priori* not safe to use or have present.

The advantages of the hydrochloric acid method are:

(*a*) The medium of precipitation, both the water and the hydrochloric acid can be absorbed by common and inexpensive absorbents; they are not easily oxidized or reduced.

(*b*) It is an inexpensive method as the excess hydrochloric acid can be recovered ready for use by simple distillation.

(*c*) It requires no inflammable material.

(*d*) The reagents used are pharmacologically suitable and raise no question as to toxicity, such as does the use of methyl alcohol and ether.

(*e*) The product, being less hygroscopic, is less liable to oxidation and other chemical change, when exposed to the air, and is therefore more stable.

(*f*) The method, as some preliminary experiments show, can be used for reprecipitation, and from a chemical standpoint seems better calculated to eliminate impurities.

The same method is used to obtain sodium chloride of the highest purity for atomic weight work.

My thanks are due to Mr Leonard M. Wachter for making the special tests and for supervising the analytical work, and to Mr. F. W. Gilcreas for careful and painstaking analytical work.

V. Summary.

(1) It is shown that there is no justification for Gaebel's assuming two molecules of "water of crystallization" in salvarsan made according to the directions of Ehrlich and Bertheim. While Gaebel made his assumption apparently in ignorance of Ehrlich's and Bertheim's method published a year later, no valid reason can be given for the general acceptance of that assumption since Ehrlich and Bertheim pointed out in 1912 that their preparation contained one molecule of methyl alcohol.

(2) A new method which is much less expensive and simpler than Ehrlich and Bertheim's method has been developed for the preparation of the dihydrochloride of arsphenamine base in pure aqueous solution by means of hydrochloric acid, being salted out similarly as in the precipitation and purification of sodium chloride with hydrochloric acid.

(3) The final product of the new method may have one or two molecules of water depending upon the drying. This seems to be the first

time the dihydrochloride of the arsphenamine base has been prepared without organic or other solvents in combination or present in the final product.

[CONTRIBUTION FROM THE DIVISION OF LABORATORIES AND RESEARCH OF THE NEW YORK STATE DEPARTMENT OF HEALTH, ALBANY.]

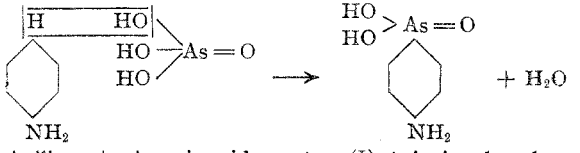
THE PREPARATION OF PRIMARY AND SECONDARY ARSANILIC ACIDS.[1]

BY PHILIP ADOLPH KOBER AND WALTER S. DAVIS.

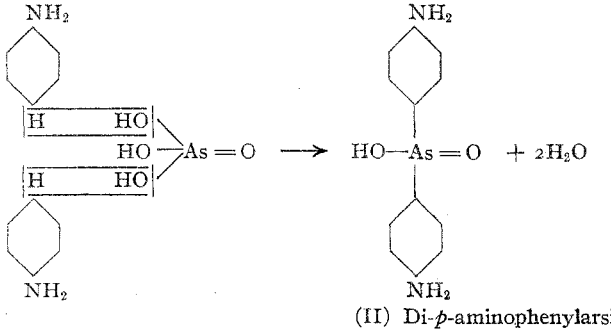
Received December 21, 1918.

I. Introduction.

The preparation of what is now known as primary arsanilic acid, the starting point and the basis of Ehrlich's synthesis of arsphenamine or salvarsan, was first mentioned by Béchamp[2] in 1863. That Béchamp and others considered the substance an anilide until Ehrlich[3] and Bertheim proved it to be *p*-aminophenyl-arsonic acid is well known. Ehrlich,[4] however, started his work with a commercial source of arsanilic acid, namely, atoxyl, the sodium salt of the acid, and although his collaborators studied the Béchamp synthesis, the fact remains that the literature does not contain definite directions for making the primary arsanilic acid. Many laboratories have spent much time and money in trying to evolve



Aniline + Arsenic acid ⟶ (I) *p*-Aminophenylarsonic acid.



(II) Di-*p*-aminophenylarsinic acid.

[1] A description of the preparation of the primary arsanilic acid was read before the Society of Experimental Biology and Medicine, New York City, November 20, 1918.

[2] *Compt. rend. soc. biol.*, **56**, 1172 (1863).

[3] *Ber.*, **40**, 3292 (1907).

[4] *Ibid.*