

SOME SALIENT FACTS REGARDING THE TOXICITY OF ARSPHENAMIN AND NEO-ARSPHENAMIN *

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INTRODUCTION

The introduction of the newer arsenicals, arsphenamin and neo-arsphenamin,¹ in the treatment of syphilis brought into prominence the intravenous method of administration, formerly a less extensively used method. The intravenous method is generally considered the most effective method to use in administering these compounds, but by many it is considered more hazardous to the patient than either the subcutaneous or intramuscular methods. For these reasons, especially, the compounds should be made as free from objectionable impurities as possible; furthermore, the limitations and dangers of the intravenous method of administration should be of prime importance to syphilographers. Studies now in progress at the Hygienic Laboratory, dealing directly with the routine conduct or modifications of the official biologic tests for arsphenamin and neo-arsphenamin intended for shipment from one state to another, indicate some of the factors that may be responsible for untoward results when these drugs are administered intravenously.

OFFICIAL METHOD FOR TESTING ARSPHENAMIN AND NEO-ARSPHENAMIN

In the official method for testing arsphenamin, it is required that white rats weighing from 100 to 150 gm. shall tolerate 100 mg. per kilogram of the drug for forty-eight hours when given intravenously as a 2 per cent. alkaline solution, 0.9 c.c. of normal sodium hydroxid being used for 100 mg. of arsphenamin. White rats are required to tolerate 200 mg. per kilogram of neo-arsphenamin for seven days when given intravenously as a 4 per cent. aqueous solution. It is further required that for both compounds the rate of injection shall be 12 to 15 seconds for every 0.1 c.c. of solution.

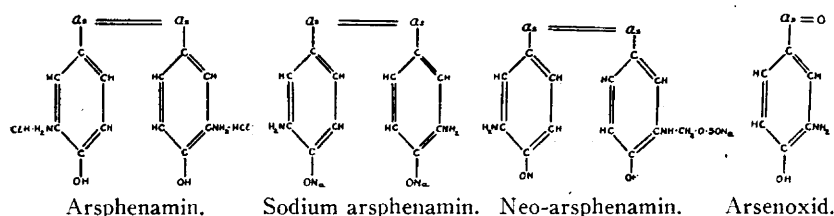
CHEMICAL NATURE OF ARSPHENAMIN AND NEO-ARSPHENAMIN

Arsphenamin is the dihydrochlorid of 3,3'-diamino-4,4'-dihydroxy-arsenobenzene, while neo-arsphenamin is sodium 3,3'-diamino-4,4'-

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1. These refer to the chemical equivalent of 606 and 914, formerly known exclusively as salvarsan and neosalvarsan.

dihydroxyarsenobenzene-N-methylene sulphinate. When arsphenamin is properly made alkaline with sodium hydroxid, as is required before its administration, the disodium salt of 3,3'-diamino-4,4'-dihydroxyarsenobenzene is formed. If oxidation occurs in any of these compounds, the more toxic substance 3-amino-4-hydroxyphenylarsenoxid may be formed. This more toxic compound is frequently referred to as "arsenoxid," and is a cleavage product in which only one arsenic atom exists in the molecule. The structures of these compounds are illustrated by the following formulae:



BEHAVIOR OF ARSPHENAMIN AND NEO-ARSPHENAMIN IN THE BODY

Neo-arsphenamin is regarded by many simply as arsphenamin in a convenient form for administration. From the formula alone it is seen that neo-arsphenamin is a modified arsphenamin and, as will be shown later, neo-arsphenamin is distinctly unlike arsphenamin as regards its behavior in the animal organism.

A statistical study of the death rate and time of death of the rats used in the official testing of arsphenamin examined during September and October, 1919, showed that a little over 80 per cent. of the animals that died within fourteen days did so within twenty-four hours, and that almost 90 per cent. of the deaths occurred within the first forty-eight hours (Chart 1). On the other hand, similar computations for neo-arsphenamin gave figures which contrasted markedly with those obtained for arsphenamin. Approximately 5 per cent. of the deaths from neo-arsphenamin occurred within the first day, 15 per cent. within the first two days, 30 per cent. within the first three days and 60 per cent. within the first four days. The highest death rate from neo-arsphenamin occurred on the fourth day. Within the fifth day the death rate was about 75 per cent. After the fifth day the death rate closely approached the curve for the death rate in "normal" animals (Chart 2).

The death rate for the entire fourteen day period is given in Table 1.

TABLE 1.—DEATH RATE AND TIME OF DEATH IN RATS AFTER THE ADMINISTRATION OF ARSPHENAMIN AND NEO-ARSPHENAMIN

	Number of Days after Injection						
	1	2	3	4	5	6	7
Rats treated with arspenamin: Number of deaths	139	9	4	3	0	2	0
Rats treated with neo-arsphenamin: Number of deaths	5	10	19	37	11	6	5
							8th to 14th inclusive 12
							19

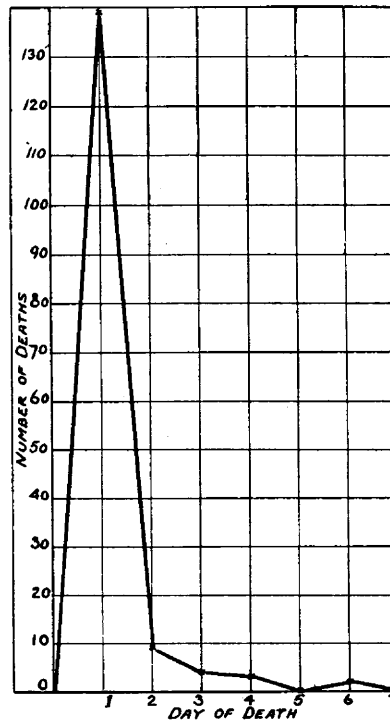


Chart 1.—Death rate and time of death of rats treated with arspenamin.

The total number of rats injected with arspenamin was 475, with neo-arsphenamin, 515.

Comparison of the death rate in treated and nontreated rats shows that of the 1,470 rats purchased by the laboratory from Oct. 14, 1919, to Jan. 14, 1920, only seventy-four, or about 5 per cent., of the nontreated animals died before being injected. The death rate after the second day in rats treated with arspenamin when based on the total number of rats injected, was about 4 per cent; similarly after the

seventh day in rats treated with neo-arsphenamin the death rate was about 4 per cent.

From the data given above it is evident that the two compounds behave differently in the rat, arsphenamin producing death only a short period after its administration, whereas neo-arsphenamin kills relatively slowly. An acute death after neo-arsphenamin in rats is relatively rare when tested at the official dosage, whereas after arsphenamin it is the rule. Furthermore, neo-arsphenamin rarely causes immediate symptoms in the rat, while arsphenamin usually causes rather pronounced depression when these compounds are given intravenously in the standard test doses.²

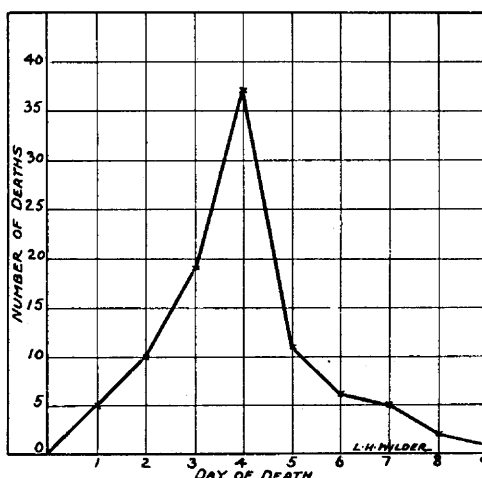


Chart 2.—Death rate and time of death of rats treated with neo-arsphenamin.

EFFECT OF CONCENTRATION OF THE SOLUTION ON THE TOXICITY OF ARSPHENAMIN

It was previously noted that the concentration of the solution of arsphenamin seemed to modify the toxicity to some extent.³

Quite recently Dr. G. W. McCoy, director of the Hygienic Laboratory of the United States Public Health Service⁴ called attention to the fact that on investigation of the untoward results that have been reported to the Hygienic Laboratory from the use of arsphenamin made by the various American producers, it was almost invariably

2. Rats injected intravenously with neo-arsphenamin bleed profusely while those injected with arsphenamin bleed but little. The effects of the two compounds on the coagulation of blood in vivo, therefore, are strikingly unlike.

3. Roth, George B.: Hygienic Lab. Bull., No. 113, July, 1918.

4. McCoy, G. W.: The Administration of Arsphenamin. J. A. M. A. **72**:1386, 1919.

found that the drug had been administered in either too concentrated solution or at a too rapid rate. He therefore directed attention to these points and warned against the use of too concentrated solutions and urged that due attention should be paid to rate of administration.

On the other hand, Nelken⁵ considers that concentrated solutions given rapidly are as safe as the more dilute solutions given slowly.

Pusey⁶ states that while it is true that animal experiments have shown arsphenamin to be less toxic when well diluted, the necessity for highly dilute solutions arises only when arsphenamin is contaminated by toxic substances.

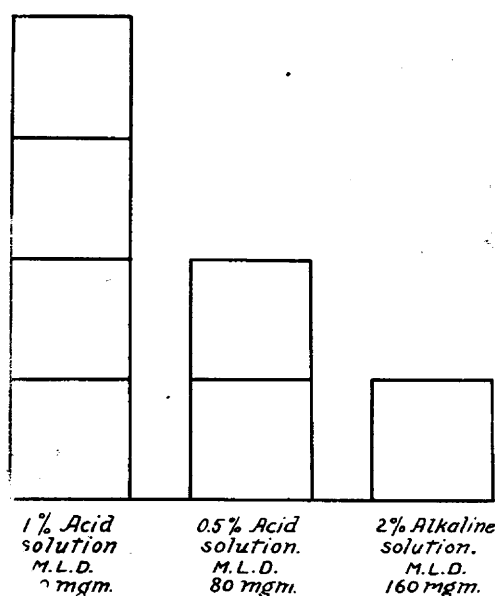


Chart 3.—Toxicity of arsphenamin before and after alkalization.

Experiments have lately been carried out on white rats in which the official rate of administration was used, which demonstrate that the toxicity of arsphenamin under certain conditions increases as the concentration increases. The increase in toxicity is strikingly demonstrated by the more toxic acid solutions of arsphenamin. When acid solutions of arsphenamin were given intravenously to white rats, it was found that the minimal lethal dose of a high grade preparation⁷ was 80 mg. per kilo when given as a 0.5 per cent. solution, and 40 mg. per kilo when administered as a 1 per cent. solution.

5. Nelken, A.: Administration of Arsphenamin, J. A. M. A. **72**:1695, 1919.

6. Pusey, William Allen: Administration of Arsphenamin, J. A. M. A. **72**:1786, 1919.

7. This arsphenamin passed the official test at 140 mg. per kilo, the minimal lethal dose being 160 mg. per kilo.

These findings not only indicate that concentration is a factor in modifying the toxicity of acid solutions, but in addition show that dilute acid solutions of arspenamin may be given intravenously if properly administered, the above preparation in dilute acid solution having complied with the standard required for alkaline solutions of arspenamin made by the American producers during the early period of their existence. However, it should not be inferred from this that acid solutions should be used clinically.

In this connection it is interesting to compare the toxicity of the same arspenamin as an acid solution and as an alkaline solution. As a 2 per cent. alkaline solution the minimal lethal dose was found to be 160 mgm. per kilo, hence it is seen that as a 1 per cent. acid solution the toxicity is four times, and as a 0.5 per cent. acid solution, twice as great as a 2 per cent. alkaline solution (Chart 3).

Using a different brand of arspenamin than the one used for determining the toxicity of acid solutions, comparative experiments were made to determine the relative toxicity of 2 and 0.5 per cent. solutions which were made alkaline with 1.06 c.c. normal sodium hydroxid per 100 mg. of arspenamin. These solutions were administered to white rats intravenously at the same rate. The animals in each group, except in groups A and AB, received the drug alternately as a 2 and 0.5 per cent. solution. The animals in each group were from the same stock and varied but little in their weight and condition.

Except in groups A and AB, the 0.5 per cent. solution was made from the 2 per cent. solution by diluting with distilled water. The experiments appear in detail in Table 2.

TABLE 2.—TOXICITY OF ARSPHENAMIN IN WHITE RATS WHEN GIVEN INTRAVENOUSLY EITHER AS A 2 OR 0.5 PER CENT. ALKALINE AQUEOUS SOLUTION

Group	2 per Cent. Solution					Group	0.5 per Cent. Solution				
	Dose Mg.	No. In- ject- ed	No. Died in 48 Hrs.	No. Died in 14 Days	No. Lived 14 Days		Dose, Mg.	No. In- ject- ed	No. Died in 48 Hrs.	No. Died in 14 Days	No. Lived 14 Days
A.....	140	5	5	5	0	AB	140	5	2	2	3
B.....	140	5	5	5	0	B-1	140	5	3	3	2
C.....	140	5	3	3	2	C-1	140	5	4	4	1
D.....	140	5	4	5	0	D-1	140	5	3	4	1
Totals 140 mg. dose.....	...	20	17	18	2	20	12	13	7
Per cent.	85	90	10	60	65	35
E.....	120	5	4	4	1	E-1	120	5	5	5	0
F.....	120	5	1	1	4	F-1	120	5	2	2	3
G.....	120	5	2	3	2	G-1	120	5	0	0	5
H.....	120	5	0	2	3	H-1	120	5	0	0	5
Totals 120 mg. dose.....	...	20	7	10	10	20	7	7	13
Per cent.	35	50	50	35	35	65

These experiments with alkaline arsphenamin indicate that the toxicity is increased but slightly as the concentration is increased from 0.5 to 2 per cent., provided the solutions are given slowly.

INFLUENCE OF THE RATE OF INJECTION OF ARSPHENAMIN ON TOXICITY

The influence of the rate of injection of arsphenamin solutions on their toxicity was determined in guinea-pigs. The variation inherent in the animals themselves, when obtained from different sources, was eliminated by using animals obtained from the same source of supply in each series of experiments.

These experiments were carried out in two ways. In groups M and N, using guinea-pigs of practically the same weight and condition, the ten animals of each group were given 40 mg. of arsphenamin per kilo as a 2 per cent. alkaline solution intravenously; the first five received the drug rapidly, that is, in from 6 to 10 seconds, while the second five received the same solution slowly, that is, in from 95 to 128 seconds.

In groups O, P, Q and R, the 10 guinea-pigs of each group received the drug alternately rapidly and slowly; that is, animal number one received the drug rapidly, while animal number two received it slowly, and so on through the set, the dosage being 50 mg. per kilo. The summarized results are given in Table 3.

TABLE 3.—THE INFLUENCE OF RATE OF INJECTION OF ARSPHENAMIN IN GUINEA-PIGS WHEN GIVEN INTRAVENOUSLY AS A 2 PER CENT. ALKALINE AQUEOUS SOLUTION PER LEG VEIN

Group	Rapid Injection				Slow Injection			
	Number Injected	Number Died in 48 Hours	Number Died in 14 Days	Number Lived 14 Days	Number Injected	Number Died in 48 Hours	Number Died in 14 Days	Number Lived 14 Days
M.....	5	2	2	3	5	0	0	5
N.....	5	0	0	5	5	0	0	5
O.....	5	5	5	0	5	0	3	2
P.....	5	1	4	1	5	1	4	1
Q.....	5	5	5	0	5	0	0	5
R.....	5	4	4	1	5	1	3	2
Totals.....	30	17	20	10	30	2	10	20
Per cent.	57	67	33	..	7	33	67

Table 3 shows clearly that the slow injection of arsphenamin into guinea-pigs is much better tolerated than rapid injection, the mortality within forty-eight hours after rapid injection being many times greater than after slow injection. From these experiments we conclude that the rate of administration is a potent factor in modifying the toxicity of arsphenamin, the toxicity increasing as the rate is

increased. Furthermore, the depression which occurs subsequent to the injection is more pronounced after rapid than after slow injection.

RELATION OF SOLUBILITY OF NEO-ARSPHENAMIN TO TOXICITY

The degree and ease of solubility of neo-arsphenamin have been found to be intimately associated with its toxicity. Preparations which were found to be incompletely soluble within 10 to 15 minutes when made up as 4 per cent. aqueous solutions, were found to kill one or more rats in every official test. In twenty-two tests which represented seventeen lots of incompletely soluble neo-arsphenamins in which 110 rats, or twenty-two sets of five each, were used, only thirty-one rats survived. In eighteen of these sets three or more animals died, in two sets two in each set died, while in the remaining two sets but one rat in each set died. The amount of insoluble matter contained in these samples was usually slight and consisted of a small amount of flocculent material which would settle in a few minutes, leaving only a film on the bottom of the mixing cylinder, while in other cases the amount was considerable. In most of these cases only the supernatant clear liquid was injected; in other cases the solutions were filtered through a soft paper filter. Filtration, however, did not cause any marked decrease in toxicity. Tests were made with two samples to determine the effect of filtration. In one sample the filtered solution killed eight of ten rats at the official dose, while the unfiltered solution killed all of ten rats injected in the same manner. In the second sample the result was practically the same as the preceding.

All of these preparations were reported by the manufacturer to be readily soluble in his tests so that the decrease in solubility mentioned above probably occurred after the products left the factory. As it cannot be definitely stated whether these preparations would have passed the official toxicity tests conducted by the Hygienic Laboratory before the preparations became less soluble, positive conclusions are unwarranted from the above findings. In the manufacturers' tests, however, all of these preparations were of satisfactory toxicity.

From these observations we have come to regard incompletely or slowly soluble products as dangerous, even if filtered. The marked toxicity noted above may have been due either to an increase in the amount of shaking of the solution or to the physical state of the product itself. The latter explanation seems the more probable as the amount of shaking in the above experiments was no more than was done when completely soluble preparations of satisfactory toxicity were examined.

THE EFFECT ON TOXICITY OF SHAKING A SOLUTION OF NEO-
ARSPHENAMIN OR ARSPHENAMIN IN THE
PRESENCE OF AIR

Neo-arsphenamin.—When neo-arsphenamin is dissolved in freshly glass distilled water as a 4 per cent. solution and shaken vigorously⁸ by hand with about twice its volume of air, it rapidly increases in toxicity, presumably from the formation of "arsenoxid." In most of the tests a domestic neo-arsphenamin (P — 4) was used to determine the effect of shaking. This preparation was an unusually high grade preparation passing the official tests at 420 and failing at 500 mg. per kilogram of rat. When this preparation was made up as a 4 per cent. aqueous solution and shaken vigorously by hand in a glass-stoppered 25 c.c. cylinder at room temperature (about 20 degrees C.), its toxicity was over four times as great as that of the unshaken solution. When shaken for 5 minutes, it failed to pass at the official dosage of 200 mgm. and when shaken for 1 minute it failed to pass at a dosage of 300 mg. and gave only a 60 per cent. pass at both 200 mg. and 140 mg. dosages.

Vigorous shaking for one minute in the presence of air will therefore increase the toxicity of high grade neo-arsphenamin considerably and thereby render it a dangerous borderline product. There was no color change in this product (P — 4) even after shaking for 10 minutes in the manner described above.

By using a lower grade domestic neo-arsphenamin (B — 4), it was found that vigorous shaking for one minute in the presence of air resulted in a complete failure when tested at the standard test dose of 200 mg. When tested in the official manner as an unshaken solution, this preparation passed at 200 mg., but failed at 240 mg. per kilogram. It was unlike P — 4 in that a slight deepening of the light canary yellow solution to a deep golden yellow occurred after shaking for ten minutes. However, after shaking for one minute no color change was detected in this preparation (B — 4).

Arsphenamin.—The effect on toxicity of shaking a solution of arsphenamin was determined by using an alkaline solution of arsphenamin, the amount of alkali added being a trifle greater than was required to form the disodium salt, that is, 1 c.c. of normal sodium hydroxid was added for every 100 mg. of arsphenamin.

For these experiments a domestic arsphenamin (B — 2) was used. This lot passed the official tests at 140 and failed at 160 mg. per kilo (see Table 4).

After making a 2 per cent. aqueous alkaline solution of disodium arsphenamin as described above, it was shaken vigorously in a 25 c.c.

8. Approximately 230 excursions per minute.

glass-stoppered cylinder at room temperature in the presence of about twice its volume of air for a period of ten minutes. When tested on rats in the official manner, eight of ten rats died at a dosage of 100 mg. per kilo, while only two of ten rats died when given a similar dose of the unshaken solution.

The symptoms after the administration of the shaken solution were especially severe, the animals developing marked dyspnea and extreme weakness immediately after the injection. These symptoms lasted for about an hour. The control animals which received the unshaken solution became slightly drowsy, but were able to walk immediately after the injection.

These experiments conclusively show that the toxicity of a solution of arspnenamin as prepared for administration to man is materially increased by shaking for ten minutes in the presence of air, and that vigorous shaking should be avoided after the alkali is added. Unlike neo-arsphenamin, the solution was distinctly darkened after shaking for several minutes, and decidedly darkened after shaking for ten minutes.

COMPARISON OF THE TOXICITY OF ARSPHENAMINS AND NEO-ARSPHENAMINS MADE IN THE UNITED STATES WITH THOSE OF FOREIGN MANUFACTURE

It is generally recognized that the standards set by the Hygienic Laboratory for the purity of arspnenamin and neo-arsphenamin are sufficiently rigid to insure products of high quality. In order to ascertain whether United States products compared favorably with foreign products a toxicologic study was made of five arspnenamin and five neo-arsphenamin preparations made in the United States, also

TABLE 4.—COMPARATIVE TOXICITY OF ARSPHENAMIN AND NEO-ARSPHENAMIN PREPARATIONS MADE IN THE UNITED STATES WITH THOSE OF FOREIGN MANUFACTURE

Sample of Arsphenamin	Maxi- mal Toler- ated Dose, Mg. per Kilo.	Mini- mal Lethal Dose, Mg. per Kilo.	Arsenic Con- tent, per Cent.	Sample of Neo-Arsphenamin	Maxi- mal Toler- ated Dose, Mg. per Kilo.	Mini- mal Lethal Dose, Mg. per Kilo.	Arsenic Con- tent, per Cent.
U. S. Products:				U. S. Products:			
B-1.....	100	120	31.3	B-3.....	200	240	19.0
B-2.....	140	160	29.8	B-4.....	200	240	18.4
P-1.....	180	220	31.3	P-3.....	280	320	18.8
P-2.....	120	140	30.5	P-4.....	420	500	19.5
C-1.....	160	180	31.5	C-2.....	240	280	18.6
Foreign Products:				Foreign Products:			
German H-1.....	80	100	32.1	German H-4.....	260	300	19.7
German H-2.....	120	140	32.0	German H-5.....	240	300	18.8
German H-3.....	60	80	31.0	French B-1.....	240	280	19.5
French S-1.....	60	80	30.0	French S-2.....	120	150	19.8
Canadian M-1.....	80	100	24.9	Canadian T-1.....	200	240	18.8
Japanese T-1.....	140	160	31.8	Japanese T-2.....	360+	?	17.7

of six arsphenamin and six neo-arsphenamin preparations of foreign manufacture. These preparations were all administered intravenously to white rats in the official manner, the minimal lethal dose for arsphenamin being the minimal dose which killed 60 per cent. or more of the animals within forty-eight hours, whereas for neo-arsphenamin the minimal lethal dose refers to the minimal dose which killed 60 per cent. or more of the animals within seven days. The results of this study are summarized in Table 4.

It may be concluded from this study that the arsphenamins made in the United States are generally less toxic than those of foreign manufacture, while the domestic neo-arsphenamins compare favorably with the foreign neo-arsphenamins examined and in certain instances are decidedly less toxic than most of the foreign products.

CONCLUSIONS

The results of the foregoing experiments warrant the following conclusions:

1. Neo-arsphenamin behaves differently in the animal organism from arsphenamin and should not be regarded simply as arsphenamin in a convenient form for administration.
2. When administered intravenously and at a constant rate, acid solutions of arsphenamin are much more toxic than the corresponding alkaline solutions, the toxicity of the acid solutions increasing with the concentration.
3. A properly alkalinized 2 per cent. arsphenamin solution when administered intravenously and in high dosage is slightly more toxic than a 0.5 per cent. solution.
4. The toxicity of properly alkalinized arsphenamin increases greatly as the rate of its intravenous administration is increased. Rate of administration is, therefore, an important factor in determining toxicity.
5. When neo-arsphenamin is found to dissolve with comparative difficulty, it is generally highly toxic and should be discarded.
6. Shaking aqueous solutions of neo-arsphenamin or alkalinized arsphenamin in the presence of air increases their toxicity markedly. When a 4 per cent. solution of neo-arsphenamin is shaken vigorously for ten minutes its toxicity is more than quadrupled.
7. Arsphenamin preparations made in the United States are generally less toxic than those of foreign manufacture.
8. Neo-arsphenamin preparations made in the United States compare favorably, and in certain instances are decidedly less toxic than most of the foreign products.