CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

BIOLOGICAL SERIES. I.

THE TOXIC ACTION OF N-PHENYLGLYCINEAMIDE-*p*-ARSONIC ACID.

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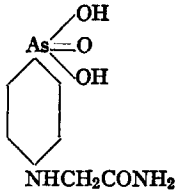
The chemotherapeutic investigations which have been in progress in these laboratories for several years have yielded a number of substances of striking activity in the treatment of experimental infections such as those produced in laboratory animals by various species of trypanosomes, the spirochetes of relapsing fever, and *Treponema pallidum*. Among the first of these substances was the amide of *N*-phenylglycine-*p*-arsonic acid, a description of which is given by Dr. Jacobs and Dr. Heidelberger, in the chemical series of these papers.[1] It may be said that while *N*-phenylglycine-*p*-arsonic acid, described in German Patent, No. 204,664, is a substance of practically no importance in the treatment of these infections, arsenophenylglycine, produced from it by reduction, was among the earliest of the highly active trypanocidal agents. The amide of this acid which has the structural formula



was first made and studied in the fall of 1915 with results which at once opened the way to the development of a number of important substances which will be dealt with in subsequent papers.

[1] Jacobs, W. A., and Heidelberger, M., *J. Exp. Med.*, 1919, xxx, 411.

Methods of Employing N-Phenylglycineamide-p-Arsonic Acid.

The amide of *N*-phenylglycine-*p*-arsonic acid, or A 63 as it was designated on our lists, presents no difficulty in the way of its use for purposes of animal experimentation. In the form of the monosodium salt, the drug is readily soluble in water in concentrations as high as 50 per cent and when injected directly into the tissues or into the body cavities of animals, it is readily absorbed and produces but slight irritation or local injury.

Preparation of Solutions.—Solutions of A 63 may be prepared by dissolving the monosodium salt in sterile distilled water, or in case the acid is used, the requisite amount of sodium hydroxide (0.37 cc. of N sodium hydroxide per 0.100 gm. of drug) to form the monosodium salt should be added slowly with stirring. The product then dissolves without difficulty.

Administration.—This drug may be administered to animals by almost any route which is convenient and has been used subcutaneously, intramuscularly, intraperitoneally, intravenously, and *per os* with no especial disadvantage following its use by any except the last named route.

Measurement of Doses.—The measurement of doses was accomplished in one of three ways. With the smaller animals, a stock solution of the drug was prepared from which individual doses for mice were measured by the use of a Fornier tuberculin syringe graduated in hundredths of a cubic centimeter; in the case of rats and guinea pigs, doses were measured with standardized pipettes. Finally, doses for larger animals such as rabbits and monkeys were always weighed and prepared separately. The values given in all cases refer to amounts of the monosodium salt which was used almost exclusively. These figures may be transposed into equivalents of the acid by the use of the factor 0.9 if desired.

Minimum Lethal Dose.

Although A 63 contains 24.57 per cent of arsenic, its toxicity for laboratory animals is comparatively low. The lethal dose of the drug varies between the extremes of 0.75 and 2.75 gm. per kilo of body weight for the different animal species in which it has been tested and for different routes of administration.

Mice.—The tolerance of mice is particularly good, as is indicated by the figures given in Table I which represents the combined results of several experiments carried out with mice. From the data at our disposal, we would place the minimum lethal dose for mice at 2.5 to 2.75 gm. per kilo of mouse when given subcutaneously, 2 to 2.25 gm. given intraperitoneally, and 2 gm. when given intravenously. These figures show a reasonably close agreement in the toxicity of the drug when administered by these routes, and the results obtained from successive experiments were comparatively uniform. Some mice survive even higher doses than those shown in the table, and conversely an occasional mouse may succumb to lower doses than those given as the lethal or minimum lethal dose; but we have had no

TABLE I.

Lethal Effects Obtained from the Administration of a 5 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Mice in Doses Equivalent to 1.75 to 2.5 Gm. per Kilo of Mouse.

Dose per kilo.	Subcutaneous injection.		Intraperitoneal injection.		Intravenous injection.	
	No. of mice used.	No. died.	No. of mice used.	No. died.	No. of mice used.	No. died.
gm.						
2.5	2	0	5	1	2	2
2.25	4	0	21	5	4	1
2.0	4	0	25	2	4	1
1.75	4	0	8	0	4	0

deaths resulting from doses of the drug below 2 gm. per kilo of mouse.

Rats.—The resistance of white rats to the toxic action is lower and more irregular than that of any other animal with which we have worked. When the drug is administered intraperitoneally, doses as small as 0.75 gm. per kilo are sufficient to cause death in a fair percentage of animals and yet some rats will survive as much as 1.75 gm. per kilo. The results obtained from subcutaneous administration are distinctly better; the minimum lethal dose rises to 1 gm. per kilo and the action of the drug is definitely more constant, as may be seen by an examination of the results from the two experiments incorporated in Table II.

TABLE II.

Lethal Effects Obtained from the Subcutaneous Administration of a 10 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Rats and from the Intraperitoneal Administration of a 3 Per Cent Solution.

Dose per kilo.	Subcutaneous injection.		Intraperitoneal injection.	
	No. of rats used.	No. died.	No. of rats used.	No. died.
gm.				
1.50	4	4	5	4
1.25	4	1	5	3
1.00	9	6	9	7
0.90	5	0	5	2
0.75	5	0	5	3
0.60			5	0
0.50			5	0

Guinea Pigs.—Contrary to the opinion which is generally held as to the tolerance of guinea pigs for arsenicals, these animals withstand relatively large doses of A 63 and the resistance of individual animals appears to be fairly uniform. The lethal dose found for the drug was 1.5 gm. per kilo of body weight whether given subcutaneously or intraperitoneally (Table III).

TABLE III.

Lethal Effects Obtained from the Administration of a 20 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Guinea Pigs.

Dose per kilo.	Subcutaneous injection.		Intraperitoneal injection.	
	No. of guinea pigs used.	No. died.	No. of guinea pigs used.	No. died.
gm.				
1.75			4	1
1.50	4	3	9	3
1.40	4	0	4	0
1.25	4	0	4	0

Rabbits.—A great deal of time has been devoted to the study of the toxic action of A 63 in rabbits. The major part of this work was carried out by intravenous administration of the drug but the effects

of subcutaneous, intramuscular, and *per os* administrations have all been investigated to some extent. The solutions used in the experiments varied between concentrations of 5 and 50 per cent, partly for the purpose of studying the effects of the use of different volume doses and partly for the purpose of determining the influence of such factors as rate of administration and concentration of solutions upon the action of the drug.

It was found that as much as 20 cc. per kilo of a 5 to 10 per cent solution could be injected intravenously into rabbits about as rapidly as one wished, and that in general, solutions of low concentration (5 to 10 per cent) were better borne than those of a higher concentration. The effects of concentration were less evident, however, when the drug was administered by other routes, and more concentrated solutions were employed as a means of reducing the volume of fluid which had to be used.

Under the conditions described, the minimum lethal dose for rabbits was found to be 0.75 to 0.9 gm. per kilo of body weight when given intravenously and 1.1 gm. given either subcutaneously or intramuscularly, with lethal effects as indicated by the experiments recorded in Table IV.

TABLE IV.

Lethal Effects Obtained from the Intravenous Administration of a 5 to 10 Per Cent Solution of N-Phenylglycineamide-p-Arsonic Acid to Rabbits and from the Subcutaneous or Intramuscular Administration of 50 Per Cent Solutions.

Dose per kilo.	Subcutaneous injection.		Intramuscular injection.		Intravenous injection.	
	No. of rabbits used.	No. died.	No. of rabbits used.	No. died.	No. of rabbits used.	No. died.
gm.						
1.25	8	6	4	3		
1.1	4	4	4	2		
1.0	3	0	3	0	6	3
0.9					12	4
0.75					10	1

Our experience in giving this drug to rabbits by mouth may be recited very briefly. The drug was given in solution by means of

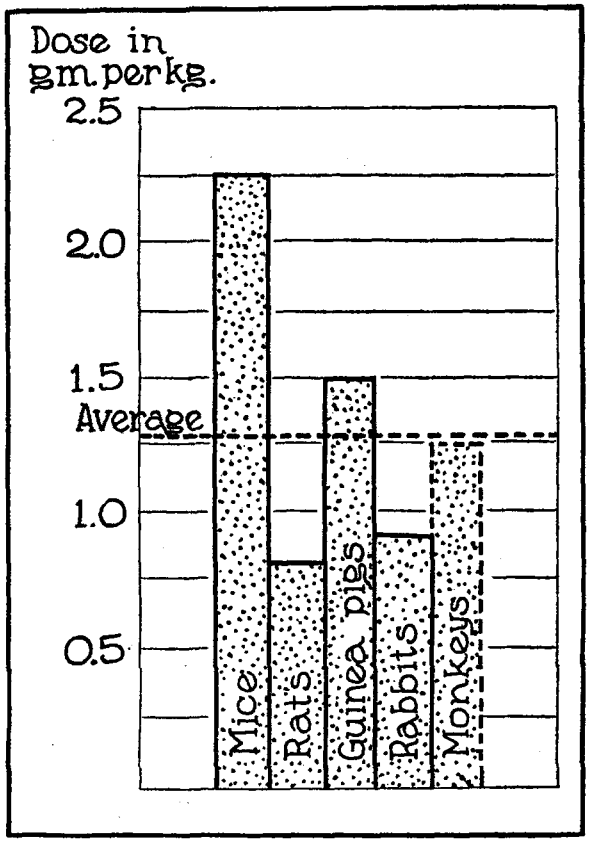
a stomach tube following the administration of a small dose of bicarbonate of soda. In all, there were only seven rabbits which received the drug in this way. Two of them were given doses of 1.25 gm. per kilo of body weight, four were given 1 gm., and one was given 0.75 gm. The last of these animals showed no ill effects from the drug and only two of the others actually succumbed to its action, both of which received a dose of 1 gm. per kilo. One of these rabbits lived 4 days and the other 24 days after the administration of the drug. It appeared certain, however, that none of the other animals would recover completely and they were killed at different times for purposes of pathological examination. As the matter stands, therefore, we are hardly justified in attempting to fix the toxic limits of A 63 when given *per os*.

Monkeys.—Lastly, we have used two monkeys (*Macacus rhesus*) in studying the toxic action of A 63. The first monkey, a female weighing 2,325 gm., was given an initial dose of 0.75 gm. per kilo of body weight. The animal was observed for 5 days during which time no ill effect from the drug could be detected, and a second dose of 1 gm. per kilo was given with a like result. 4 days later, the animal received its third dose which was 1.25 gm. per kilo. Following this dose, the only evidence of intoxication noted was possibly a slight loss of weight (75 gm.). At the end of 3 weeks, the weight of the animal was 2,450 gm., and a fourth dose of the drug was given—this time 1.5 gm. per kilo, or double the initial dose. The monkey showed slight signs of intoxication lasting for a few days, followed by rapid recovery. The animal was kept under observation for 3 months and was then killed for pathological purposes.

The second monkey used was likewise a female *rhesus* weighing 2,650 gm. This monkey was given an initial dose of 1.25 gm. per kilo intravenously. There was a loss of weight in this animal amounting to 175 gm., but no other evidence of intoxication developed, and after waiting 1 week, a second dose of 1.5 gm. per kilo was given; death followed within 24 hours.

These two experiments are cited merely to show that monkeys possess a degree of tolerance for A 63 comparable with that of other animals and that they react to the drug in much the same way.

Represented graphically, the values obtained for the minimum lethal dose of this drug in the five species of animals studied would form a curve such as that given in Text-fig. 1. While these values are not strictly comparable on account of differences in the mode of



TEXT-FIG. 1. Comparative magnitudes of the minimum lethal dose of *N*-phenylglycineamide-*p*-arsonic acid for different animal species. The dose given for monkeys is an approximate estimate.

administration used, they will serve to indicate the relative magnitude of the toxic dose for different animal species. The mean toxic dose for this group of animals is found to be approximately 1.28 gm. per kilo; the maximum variation from this mean is as 1: 1.75 and the extremes compare as 1: 2.8.

Symptoms and Course of the Intoxication.

The symptom-complex of the intoxication produced by *N*-phenylglycineamide-*p*-arsonic acid in laboratory animals is characterized by two groups of phenomena, one nervous and the other nutritional in character. The most prominent of these symptoms appear at an early period of the intoxication, as pronounced tremors with incoordination of movements, or in extreme cases, as clonic spasm, usually associated with some weakness or complete prostration. With remission of these early symptoms in mice, a tic develops which is characterized by peculiar jerky movements of the head and occasionally by the continuous circling movements of dancing mice. Nutritional disturbances are indicated chiefly by a loss of appetite, more or less weakness, loss of weight, and occasionally by diarrhea. Altogether the picture is one previously recognized and described as characteristic of the toxic action of a number of pentavalent arsenicals, particularly of arsacetin, dichlorophenolarsonic acid, and aminohydroxyphenylarsonic acid.

These symptoms are by no means constant either as to the frequency or the intensity of their occurrence in different animal species or in individual animals of the same species.

Mice.—In mice symptoms of intoxication occur only following relatively large doses of the drug (1.75 gm. per kilo and above) and even then are not of constant occurrence. In the most marked cases, mice show violent muscular tremors on being disturbed and marked locomotor incoordination. After 2 or 3 days, these symptoms disappear entirely or gradually give place to the tic described, which in turn rarely persists for more than a week. Disturbances of this general character appear to be more pronounced following subcutaneous and intravenous administrations of the drug than after intraperitoneal administration.

Mice also show some nutritional disturbances for 2 or 3 days following toxic doses of the drug but make a rapid recovery with an increase in weight above their normal level and remain subsequently in excellent condition.

Rats.—Rats are, on the whole, the most sensitive animals to this drug and individual idiosyncrasy is quite pronounced among them.

Nevertheless, toxic symptoms in these animals are fairly well confined to doses within what we regard as the lethal range. While some rats show no toxic symptoms at all, in others, tremors, incoordination, convulsions, weakness, and emaciation reach a most extreme grade and progress to a lethal termination, recovery under such conditions being very rare.

Guinea Pigs.—Guinea pigs on the other hand are quite resistant to A 63. They show very slight evidence of intoxication either of a nervous or of a nutritional character from doses below the lethal level. The dose that is survived is usually well borne and even lethal doses produce comparatively slight tremors, unsteadiness, weakness, or loss of weight.

Rabbits.—The reaction of rabbits to A 63 is rather irregular. By whatever route the drug is administered, doses above 0.5 to 0.6 gm. per kilo of body weight cause some loss of appetite with an initial loss of weight which is in proportion to the size of the dose used. With doses of 0.75 to 0.9 gm. per kilo of body weight, the loss of weight in surviving animals may reach as much as 200 to 300 gm. and is associated with some degree of weakness. This loss of weight, however, is quickly regained, leaving no symptomatic evidence of intoxication. Nervous phenomena of the exact type described in mice and rats do not occur in rabbits. Instead, rabbits manifest some hypersensitiveness in the milder cases of intoxication, while in extreme cases incoordination develops with a tendency to clonic spasms, and there is eventual loss of muscular control or even paralysis. As in the case of the rat, pronounced nervous symptoms in rabbits nearly always portend a lethal outcome so that these phenomena cannot be said to occur as symptoms of a sublethal intoxication.

When toxic doses are given to rabbits *per os*, the drug produces symptoms which are not observed when it is given by other routes, the characteristic feature of which is a marked abdominal distention with flatulence as a result of atony of the colon. Associated with this condition, there is a loss of appetite, diarrhea, progressive loss of weight, and weakness. The condition is extremely persistent and recovery, if it occurs at all, is problematical.

Monkeys.—In our limited experience with monkeys, we have observed no symptoms of intoxication other than slight loss of weight and some weakness as previously recorded.

Having described the symptoms of intoxication produced by A 63, we shall now take up the question of the time element in these toxic reactions. In mice symptoms of intoxication are usually apparent within 24 hours after the administration of the drug but may be delayed until the 2nd day. Death also occurs relatively early in these animals and is rare after 48 to 72 hours. Symptomatic recovery is relatively prompt.

Rats, on the other hand, usually show no symptoms of intoxication until the 2nd or 3rd day except with very large doses of the drug. Out of twenty-eight rats that died as a result of intoxication with doses below 1.5 gm. per kilo of body weight, the earliest death occurred 3 days after the administration of the drug, the latest deaths 10, 11, and 14 days, while the average period was 6 days. Recovery in rats is also a slow and uncertain process.

Symptoms of intoxication in guinea pigs usually make their appearance on the 2nd day following the administration of the drug. Among the deaths recorded in our series from doses below 2 gm. per kilo, the earliest occurred 2 days and the latest 7 days after the administration of the drug with an average period of 4 days survival. Recovery in guinea pigs is usually prompt and complete.

Of the rabbits which died after intravenous injections of a single dose of A 63, one with the smallest dose (0.75 gm.) died in almost exactly 24 hours, while the longest survival was 6 days and the average 2.6 days. The minor symptoms of intoxication in rabbits are not apparent as a rule until the 2nd day, while the graver symptoms occur more promptly. As previously indicated, symptomatic recovery in the rabbit is usually prompt and complete.

Pathology of the Intoxication.

The pathological changes produced in the animal organism by toxic doses of A 63 constitute an important phase of its toxicologic action. Viewed from the standpoint of the bearing of these changes upon the possible usefulness of the drug as a therapeutic agent, this phase of the subject resolves itself largely into a consideration of organic injury and recovery therefrom.

Local Effects.

As we have already indicated, the injury produced by the drug at the site of injection is almost negligible. Full toxic doses may be injected into the veins of animals even in saturated solution, with practically no local reaction. When a 50 per cent solution of the drug is injected into the subcutaneous tissues of animals, a slight edema develops at the site of the injection but clears up almost immediately, leaving such slight evidences of tissue reaction as are barely recognized from external examination. If given intramuscularly in amounts small enough to obviate mechanical laceration of the tissues, the reaction which follows is likewise very mild and is of essentially the same character as that which follows subcutaneous administration.

Systemic Effects.

The effects produced by the drug upon the organism as a whole are of much more importance than those of a purely local character. These effects are divisible into major and minor phases of pathological action, the details of which differ somewhat in different animal species as well as with the amount of the drug used and the route of administration employed. In general, however, the changes seen during the early stages of the intoxication consist in moderate vascular dilatation and congestion with a few scattered petechial hemorrhages, occasional effusions into the serous cavities, and widespread cellular degenerations or even necrosis in some organs. The organs which share most prominently in these changes are the kidneys and adrenals, and the cardiovascular system, with an uncertain involvement of the central nervous system, the gastrointestinal tract, the blood, and blood-formative organs; the changes produced in other organs appear to be of minor importance. Animals which survive the intoxication rarely show lesions in organs other than the kidneys and myocardium.

Kidneys.—The central feature of the pathological action in all animals is the injury to the kidneys. In acute poisoning, the kidneys are somewhat enlarged and tend to be pale except for the presence of more or less congestion or even hemorrhage in the boundary zone

which may extend outward along the medullary rays to the capsular surface. The glomerular tufts are usually swollen, the capillaries congested, and the covering epithelium is degenerated. Degeneration or even necrosis of the tubular epithelium is rather widespread but occurs chiefly in the ascending loops of Henle and the convoluted tubules, particularly those located along the medullary rays and the outer portion of the cortex. The interstitial elements of the kidney are, as a rule, much less affected except when overwhelming doses of the drug are given.

As serious as these injuries appear to be in the acute stages, recovery is remarkably satisfactory in most animals. Reaction of the tissue elements sets in promptly and restoration of the injured parts is accomplished within a short period of time and with comparatively slight scarring or distortion of the architecture except where the initial destruction was unusually extensive. In these cases, the marks of injury are found in a growth of connective tissue and in round celled infiltrations extending along the medullary rays and through the outer portion of the cortex, while a few glomeruli show shrunken tufts and thickened capsules, all of which gives a granular surface to the kidneys.

Adrenals.—Evidences of injury to the adrenals consist in swelling, with some congestion of the cortical vessels and the occasional presence of focal hemorrhages in the cortex. In guinea pigs, the later changes are manifested by a decrease in the cortical pigmentation with well marked degenerative changes in the cortex and medulla or even necroses in the midcortical zone. Here again recovery appears to take place fairly promptly.

Cardiovascular System.—The most pronounced effects of A 63 upon the cardiovascular system appear during the early stages of the intoxication. There is a moderate vascular dilatation and congestion throughout the body and a few petechial hemorrhages may occur in any of the organs. The sites of chief importance are the kidneys, the adrenals, and heart in which occasional foci of hemorrhage are found beneath the epicardium and the endocardium as well as in the myocardium itself. In several instances, hemorrhages from the meningeal vessels have been observed, either in the region of the torcular or in the retroorbital tissues.

Animals which survive the acute poisoning show little evidence of either congestion or hemorrhage, but in their stead one finds an accumulation of more or less fluid in the serous cavities, perirenal edema, and in rare instances an increased tension of the cerebrospinal fluid. These conditions we regard as at least indicative of vascular injury. In addition to such changes as these, the myocardium shows a moderate fatty degeneration with occasional areas of fibrosis. On the whole, the pathological effects of A 63 upon the cardiovascular system are not pronounced and the conspicuous congestion, hemorrhage, and degeneration so often observed with arsenicals are largely absent from the changes which characterize the action of this drug.

Central Nervous System.—From the symptoms of the toxic action of A 63, one might expect the central nervous system to share the position of chief pathological importance with the kidneys and cardiovascular system. Such changes as occur, however, are rather obscure and difficult of determination in laboratory animals. We have already mentioned the occurrence of congestion of the meningeal vessels and occasional hemorrhages together with an increased tension of the cerebrospinal fluid. Histologically, the chief evidences of injury are associated with the choroid plexus and with the small and medium vessels of the meninges and brain. The plexus itself shows definite degeneration in some animals and the tissues immediately surrounding the vessels are somewhat edematous. Apart from these changes, however, we have been able to demonstrate only minor degrees of cellular degeneration of a rather indefinite character and of uncertain extent.

Blood and Blood-Formative Organs.—While the action of A 63 upon the blood and blood-formative organs forms an important feature of the effect of the drug, this action is perhaps more physiological than pathological and hence there is little that can be said in the present connection.

Poisoning with the drug produces an initial destruction of red blood cells and an accumulation of blood pigments in the spleen, liver, and bone marrow. The formative centers, especially those of the spleen, show degeneration or even necrosis after the administration of very large doses of the drug, but following these changes, there is an extremely active hyperplasia in which all elements participate.

This is most strikingly shown by a myeloid metaplasia in the spleen of rats and mice which is characterized by the presence of large numbers of megacaryocytes. These changes alone, however, might mean very little, since such reactions are easily excited in mice and rats.

The effect of A 63 upon other organs of the body is relatively inconsiderable. In the liver, parenchymatous and fatty degeneration are usually but not constantly present, and in exceptional instances isolated cells or groups of cells here and there show necrosis. As a rule, these changes clear up very quickly leaving nothing to indicate the existence of any previous injury.

Lesions of the gastrointestinal tract are likewise inconstant and of uncertain significance. During the early stages of the intoxication, a catarrhal condition of the entire tract is not infrequently observed but is rarely of serious degree. An exception to this rule is found in animals which have been given the drug by mouth. These animals always show a pronounced and persistent colitis with a lesser degree of involvement of the stomach and small intestine. This, however, is clearly referable to the mode of administration of the drug.

Apart from a knowledge of the character of the lesions which may be induced by this drug, the features of chief interest from the pathological point of view are the promptness with which repair is accomplished and the fact that, as the dose of the drug used falls below the level of the lethal dose, the extent of the resulting injury rapidly diminishes until, with doses only slightly below the level of the lethal dose, organic injury is barely demonstrable. These features of the pathological action of A 63 are both unusual and of considerable practical importance in their bearing upon the use of the drug for therapeutic purposes.

Tolerance of Repeated Doses.

The facts presented thus far have dealt entirely with effects produced by the administration of single doses of the drug, which brings us to a consideration of the reaction of the animal organism to the use of repeated large doses. This phase of drug action is bound up to a large extent with two conditions, first, the time during which the drug remains in the animal body in a form capable of exercising a

toxic effect, and second, the character and duration of the effect produced, whether functional or organic.

In attempting to arrive at some idea of the time during which A 63 remained biologically active after being administered to animals, we made use of two types of experiments—one based upon protection against infection and the other upon superposition of fractional parts of toxic doses. In general, it was found by experiments of the first class, that the protection afforded such animals as mice against infection with *Trypanosoma brucei* was practically nil 24 hours after the administration of as much as twice the dose of the drug which was capable of curing a 24 hour infection of the same organism (0.5 gm. per kilo). With rats and with rabbits on the other hand, some degree of protection still existed at the end of 48 hours. In the case of the rabbit, four animals which were inoculated with *Trypanosoma brucei*, 48 hours after having received an intravenous injection of 0.5 gm. of A 63 per kilo, gave the following results: One rabbit showed no protection, two were permanently protected, and the fourth showed an incubation period of 21 days as contrasted with an incubation period of 9 days in controls which received the same dose of organisms at the same time.

From these experiments it appeared that retention of the drug in a biologically active form was a variable condition both specifically and individually, but that in animals such as the rat and the rabbit, there was the possibility of the drug's remaining in the body for 48 hours or even longer in a condition in which it might still be capable of exerting toxic effects. To test this further, we carried out a few experiments on the effects of the administration of fractional parts of a toxic dose at various intervals of time. Without going into the details of these experiments, we may say that it was found that when the toxic dose of A 63 for rabbits (0.75 gm. per kilo) was divided into three equal parts administered at intervals of 24 hours, the toxic effect approached that produced by the administration of the entire amount of drug at one time. This, of course, might be interpreted either as evidence of an accumulation of drug or as evidence of superposition of effects notwithstanding the fact that no toxic effect could be recognized from the administration of a single such fractional part of the toxic dose.

Combining these facts with what we had already learned in regard to the general reaction of animals to single doses of A 63, we undertook some experiments intended to determine the tolerance of mice and of rabbits to prolonged repetition of large doses of the drug and something of the interval at which such repetitions might be carried out successfully. The results obtained with one series of mice are summarized in Table V.

These mice were given an initial dose of 2 gm. of A 63 per kilo of body weight injected into the peritoneal cavity. The injections were repeated at weekly intervals and the dose was progressively increased up to 3 gm. per kilo. The sixth and last dose of 2.5 gm. per kilo was

TABLE V.

Tolerance of Mice to Intraperitoneal Administrations of Increasing Doses of N-Phenylglycineamide-p-Arsonic Acid Given at Weekly Intervals.

No. of injection.	Dose per kilo.	No. of mice used.	No. intoxicated.	No. died.
	gm.			
1	2.00	15	5	2
2	2.25	13	0	0
3	2.50	13	2	2*
4	2.75	11	0	0
5	3.00	11	0	0
6†	2.50 (i.v.)	9	3	3

* These two mice were toxic after the first dose.

† The last injection was given intravenously.

then given intravenously. Five of the fifteen mice showed symptoms of intoxication following the administration of the first dose and two of these died. Two others died after receiving the third dose (2.5 gm.) but the remaining eleven mice survived the administration of 3 gm. per kilo with no evidence of intoxication, indicating a definite increase in their tolerance to intraperitoneal administrations of the drug. When the route of administration was changed, these animals still showed a resistance to the drug slightly greater than that of normal mice.

Experiments of a similar character were carried out with rabbits. In this case, three routes of administration were used—subcutaneous, intramuscular, and intravenous. The initial doses were placed at

what was regarded as comparable levels for the various routes of administration; *i.e.*, 0.75 gm. per kilo intravenously and 1 gm. per kilo given either subcutaneously or intramuscularly. Animals of the subcutaneous and intramuscular series received their last dose intravenously and those of the intravenous series were divided into two

TABLE VI.

Tolerance of Rabbits to Increasing Doses of N-Phenylglycineamide-p-Arsonic Acid Given at Weekly Intervals, (A) Subcutaneously, (B) Intramuscularly, and (C) Intravenously.

No. of injection.	Dose per kilo.	No. of rabbits used.	No. intoxicated.	No. died.
	gm.			
A 1	1.00	3	0	0
2	1.00	3	0	0
3	1.10	3	0	0
4	1.25	3	0	0
5	1.35	3	0	0
6*	1.10	3	0	0
B 1	1.00	3	0	0
2	1.00	3	0	0
3	1.10	3	0	0
4	1.25	3	0	0
5	1.35	3	0	0
6*	1.10	3	0	0
C 1	0.75	10	2	1
2	0.75	9	1	0
3	0.90	9	0	0
4	1.00	9	1	1
5	1.10	8	0	0
6*	1.35	8	1	1

* The last injections of Series A and B were given intravenously; in Series C, four rabbits were injected subcutaneously and four intramuscularly.

groups one of which was given the last dose subcutaneously and the other intramuscularly. The results of these experiments are given in Table VI.

Following the first dose, one rabbit of the intravenous series was extremely toxic and died within 24 hours. A second rabbit was slightly toxic but all the others remained in good condition. No

other toxic manifestations developed until the fourth dose of the series was given, when one rabbit of the nine which received the dose of 1 gm. per kilo intravenously became toxic and died after 17 days. The fourteen remaining rabbits were carried through to the conclusion of the experiment with no symptoms suggestive of a harmful effect other than slight fluctuations in weight.

The dose in the intravenous series was raised progressively from 0.75 gm. per kilo of body weight to 1.1 gm., which in our experience is almost uniformly fatal when given to normal rabbits, with the loss of only two out of the ten rabbits; the eight rabbits which received the dose of 1.1 gm. survived with no evidence whatsoever of intoxication. These eight rabbits were then given a dose of 1.35 gm. of A 63 per kilo of body weight either subcutaneously or intramuscularly with one death following an intramuscular injection. The other rabbits remained entirely normal and continued to gain weight. With the subcutaneous and intramuscular series, the dose was raised from 1 gm. per kilo of body weight to 1.35 gm. with no toxic developments, and all these animals were then given 1.1 gm. of the drug by the intravenous route. In this case, the weights declined slightly but there was no other sign of intoxication. It is to be noted that all three groups of rabbits were carried to a point where they not only survived doses which are ordinarily fatal but that when a normally lethal dose was given by a route other than that to which they had been accustomed, only one of the fourteen rabbits succumbed, while the others showed little or no ill effects.

At the conclusion of this experiment, the entire group of rabbits was killed for pathological examination. On opening these animals, there was a strong odor of garlic—a condition never noted in an animal which had died from a single dose of the drug. Pathologically, the only changes found were small subcutaneous abscesses or abscesses in the lumbar muscles of animals which had received repeated injections in the same regions, a slight fatty degeneration of the myocardium with a few patches of fibrosis, and chronic nephritis. The injuries to the heart muscle and to the kidneys were definitely more pronounced in the rabbits of the intravenous group than in the others. With reference to the kidneys of this group, we would classify them as two with slight lesions, two with moderate lesions, and four with pronounced lesions.

The total amount of drug received by each of these rabbits during a period of 36 days was 5.85 gm. per kilo in one group and 6.8 gm. per kilo in the other, or 1.437 to 1.67 gm. of arsenic administered within a period of 5 weeks. Finally, it should be recalled that the toxic dose of A 63 for rabbits is lower than that for any other animal with which we have worked except the rat, and that the mice previously referred to received more than twice these amounts of the drug.

Our limited experience with monkeys, as previously stated, merely served to indicate that under properly chosen conditions, they too would respond to repeated doses in essentially the same way as rabbits or mice. While we do not know what the lethal dose of this drug is for monkeys, it is probably not less than 1 to 1.25 gm. per kilo of body weight, or approximately the same as that for guinea pigs and considerably more than that for the rabbit. In the one instance in which gradually increasing doses of A 63 were given to a monkey, we succeeded in giving as much as 1.5 gm. per kilo as the final dose with very slight intoxication resulting. When this animal was killed 3 months later for pathological examination, the organs appeared normal.

CONCLUSION.

The essential facts to be gathered from these studies of the toxicologic action of *N*-phenylglycineamide-*p*-arsonic acid may be summarized very briefly. The substance is one which lends itself well to almost any method of administration and can be given to animals in very large doses. The tolerance of different animal species varies rather widely but with one exception the reaction of laboratory animals to toxic doses of the drug is of favorable character. That is, toxic effects are confined to doses relatively close to the minimum lethal dose and the recovery of animals from sublethal intoxications is remarkably rapid and complete. This feature of the action of the drug makes possible the repeated administration of even very large doses at comparatively short intervals of time without incurring the dangers incident to cumulative action or to superposition of toxic effects. On the contrary, by taking advantage of this peculiarity of action, it is possible to develop such a degree of tolerance on the part of animals that the dose of the drug administered can be progressively

increased to a point well above that which is fatal to the normal animal, and this stands out as the feature of the toxicologic action of *N*-phenylglycineamide-*p*-arsonic acid which is of greatest significance in the use of the drug for therapeutic purposes.