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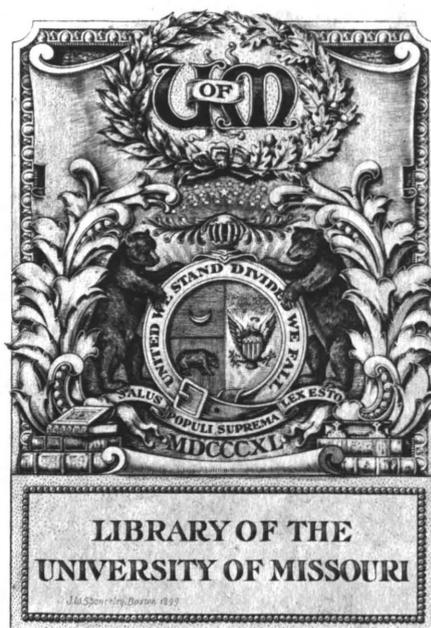
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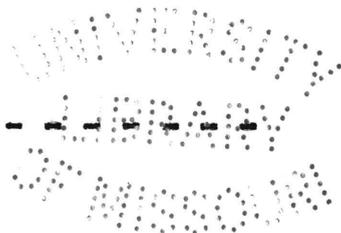
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ON THE PHARMACOLOGICAL ACTION OF CERTAIN ORGANIC DERIVATIVES OF
ARSENIC WITH SPECIAL REFERENCE TO SALVARSAN
AND SODIUM CACODYLATE.

by

James Robert McVay, A. B.



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ON THE PHARMACOLOGICAL ACTION OF CERTAIN ORGANIC DERIVATIVES OF
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SODIUM CACODYLATE.

I. INTRODUCTION.

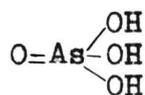
Since the introduction of Salvarsan by Ehrlich in the latter part of 1910 many facts have been brought out concerning its reactions in the body. The most important of these pharmacologically are the deaths which have been attributed to its untoward action. Many of the reports of deaths following Salvarsan injection, Ehlers (9), Gaucher (14), Paris Letter (26), Moore (25), and Fraenkel and Grouven (13), have described symptoms strikingly similar to those attributed to the action of arsenic by Cushny (7). The experiments in the present work were undertaken in order to determine whether such action was due to the drug injected or to other conditions accompanying the injection.

The steps which led up to the final production of such a drug as Salvarsan are well worth examining. For many years Ehrlich (10) had been studying the action of different chemical compounds upon the trypanosome infection in mice. It was while making these studies that he evolved his "Side-chain Theory". Later he applied this theory to the results of his researches with specifically acting chemicals, and to such treatment he applied the descriptive term "Chemotherapy". He claimed that the cells also had receptors which differed from the nutritive-receptors in that they could unite with chemical compounds. These he called "chemoreceptors". The great problem which now confronted him was to find a definite chemical compound which would unite with the "chemoreceptors" of the parasite and cause its destruction but which would not affect the cells

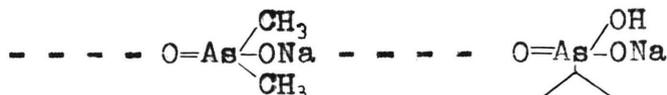
of the host. A compound possessing these qualities he called "parasitotropic" as distinguished from "organotropic" compounds, or those which acted upon the cells of the host as well as upon the invading organism. He found drugs which were highly "parasitotropic" to trypanosomes in the test tube but did not destroy them when introduced into the body of an infected animal. A chemical compound was finally prepared which when injected into the animal killed nearly all the parasites without affecting the host. When he injected this same drug into the animal to kill the remaining parasites, he was surprised to find that they had developed an immunity or "drug fastness" toward the drug. It was possible to develop parasites which were "fast" to many drugs, and Ehrlich soon recognized the necessity of preventing this. He sought for a drug which when injected into an animal would kill all the parasites at one time and thus leave no organisms to develop a "fastness". He called this treatment of completely cleansing the animal with a single injection "Therapia Sterilisans Magna".

He tested many thousands of compounds experimentally and found only three classes which were of any practical use. Of the three classes those containing arsenic were found to be the most effective. He worked successively with arsenious acid, sodium cacodylate, atoxyl, arsacetin, arsenophenylglycin, and finally with Salvarsan which was the most highly "parasitotropic" and least "organotropic" of all.

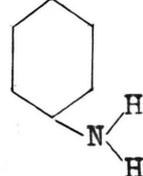
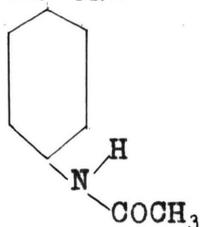
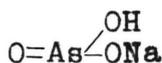
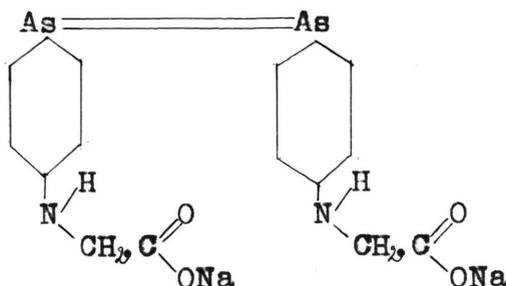
A study of the structural formulae of these compounds as given by Böös (5) will give some idea as to the reason for such success-
ion.



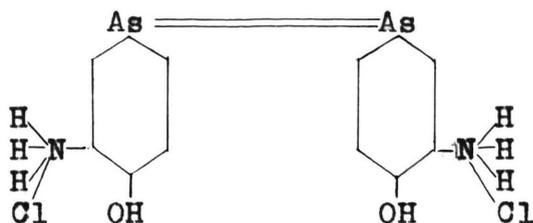
Arsenic Acid.



Sodium Cacodylate.

Atoxyl or Sodium salt of
P-amido-phenyl- arsenic
acid.Arsacetin or Sodium salt
of P-acetyl-amido-phenyl-
arsenic acid.

Arseno-phenyl-glycin.

Salvarsan, "606" or P-dioxy-M-diamido-arseno-benzol
di-hydrochloride.

From the above structural formulae it will be seen that each succeeding compound was one capable of more substitution products than its predecessor, thus increasing the possibilities of "chemo-receptors" and thereby decreasing the possibility of the development of an absolute "fastness" to the drug. Ehrlich (10) supposed

that the compound, Salvarsan, became attached to the parasite through the arsenic which is present in the relatively unstable trivalent form.

As Simon (32) has pointed out, this is the first drug possessing a specific action which was ever developed from scientific reasoning brought to its logical conclusions, and this step opens the way for a large number of such compounds. That other specifically acting drugs will be developed is shown by Ehrlich's words when he introduced Salvarsan to the world, "Die Pfosten sind in den Grund getrieben, nun gilt es zu bauen das fertige Haus".

Salvarsan met with immediate favor and has become very extensively used clinically. This use has given rise to a large number of problems concerning its action and a large amount of experimental work has been done.

Hoke and Rihl (18,19,20.) worked on the effects of the drug on the whole mammal, paying particular attention to its effect upon the organs of circulation and respiration. They conclude that the drug has an action upon the nervous centers, the peripheral vessels, and also upon the heart. This is a depressing effect and results in a fall in blood pressure similar to that seen after intravenous injections of arsenious acid. Further work on the heart by Auer (3) determined that the drug had a deleterious action on the heart in dogs. Hoke and Rihl (18,19,20.) and Auer (3) used both acid and alkaline solutions, and they found that the acid solutions were the most destructive to normal function.

The change brought about in the blood by the introduction of Salvarsan into the body has been studied by Schwaer (31) and Pawlow (27). They found a decrease in the number of red blood cells and in the percentage of hemoglobin after injection. In this it would seem to resemble the action of inorganic arsenic upon healthy

animals.

The influence of Salvarsan upon metabolism has been studied by Postojew (28) and Pawlow (27). They found a general decrease in metabolic activity in dogs which were injected with amounts ranging from .01 to .3 gm. per kilo. body weight. This influence Postojew ascribes more directly to the arsenic content while Pawlow suggests that it may also be due in part to the narcotizing action of the benzol group. Both observers found that small doses slightly stimulated the body metabolism, in this manner showing its similarity to small doses of arsenic.

Tests of Salvarsan upon the central nervous system have been made by Hoke and Rihl (18,19,20.) and by Beck (4). The former working with biological methods found a loss of tone in the nerve centers, while the latter working with mice found absolutely no changes histologically after injections of amounts which would correspond to a dose of 9 grams for a 60 kilo. man.

In order to determine the manner in which the drug acts a great deal of experimental work has been done on the duration of its action in the system, and its elimination. Fisher and Hoppe(13) found that after injection, the drug was eliminated through the urine and possibly through the mucous membrane of the stomach. Stumpke and Siegfried (33) working on rabbits concluded that the greater amount of the drug was quickly eliminated through the kidneys but some was stored in the liver cells, and they were able to demonstrate arsenic in these cells for some time after the injection. Ritter (29) confirmed the observations of Stumpke and Siegfried, finding Salvarsan stored up in the liver after repeated injections. Kolmer and Schamberg (23) after administration by mouth found arsenic in the urine and bile after 24 hours, but the

reaction disappeared after 72 hours. Aladow (2) found that arsenic was eliminated by the liver and stomach after intramuscular injections of Salvarsan. Abelin (1), by a special technic, was able to demonstrate Salvarsan in the blood for some little time after injection proving that all the drug is not changed at once to some other form of arsenic. Greven (15) found arsenic in the urine very soon after the injection of Salvarsan. Saccone (30) found that Salvarsan was eliminated as such through the kidneys and the mucous membrane of the intestinal tract. Later he was only able to demonstrate arsenic in these excretions and finally this reaction became negative. These results show that the drug must be broken down and the resulting compounds may exert some action as has been suggested by Ehrlich (10), himself.

According to Kersten (21) Ehrlich and Hata report that the "dosis tolerata", the highest dose which an animal will stand with safety, to be .1 gm. per kilo. body weight for rabbits. He found however that a dose of this size produced death in from one to two days in 50% of his rabbits. Hata, according to Marks (24), claims that the "dosis curativa", the amount sufficient to cure syphilitic rabbits in one injection, is 1/7 of the "dosis tolerata", so the compound must be markedly "parasitotropic". Herring (17) determined the lethal dose for rabbits to be .004 to .005 gm. of the acid solution per kilo body weight. Dogs were much more resistant, and withstood a dosage of .01 to .02 gm. per kilo. body weight. Herring claims that the "dosis tolerata" of Hata is not without effect upon the blood pressure. Kochmann (22) using alkaline solutions found that .100 gm. per kilo body weight injected intravenously into dogs produced death after ten hours. Rabbits he found to possess a greater tolerance, as they were able to stand a dose

of .200 gm. per kilo. body weight, which caused death only after two hours. Comparing his results with Salvarsan with those he obtained by injecting calcium arsenite, he found that rabbits could stand nearly 7 1/2 times as much arsenic when injected in the form of Salvarsan as when injected in the inorganic form.

In view of the fact that Salvarsan is after all an organic arsenic compound, it was thought advisable to compare its action with the simpler compound of this class, and sodium cacodylate was selected.

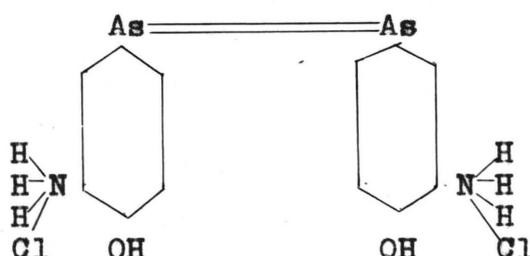
The principal work on the cacodylates in the experimental field has been that of Heffter (16). He found that after injection into the animal organism of sodium cacodylate, some was eliminated as such, a small amount however was oxidized and reduced to cacodyl oxide and arsenious acid. He attributed to the active arsenious acid any action which might follow from the injection of the cacodylates. Cloetta (6) arrived at some what similar conclusions. Dawes and Jackson (8) got practically the same results and calculated that about 6 to 10% of the sodium cacodylate was eliminated unchanged. The remainder was reduced to the more active inorganic forms, and it is to this inorganic arsenic that they ascribe the action of the drug.

It was decided after reviewing the above to attempt experiments on the frog's heart, and upon mammals, using dogs, in an effort to arrive at some conclusions as to the pharmacological action of Salvarsan on the circulatory apparatus both nervous and muscular.

II. MATERIALS AND METHODS.

Drugs Used.

The first drug used was Salvarsan or para-dioxy-meta-diamido-arseno-benzol dihydrochloride which has the following stereochemical formula,

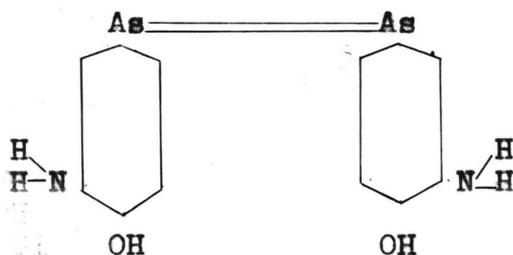


The drug is a light yellow powder and has the arsenic content of 31.6%. It comes in small ampoules containing from .1 to .6 gms. The .1 and .6 gm. sizes were the only ones used in this work. The air in the ampoules is replaced by an inert gas, nitrogen, and when opened there is an odor somewhat resembling cacodyl though not so pungent. The compound is easily soluble in water, the solution being of a bright yellow color and has a strong acid reaction. This is thought to be due to the ionization of the HCl molecules.

The ampoule containing the drug was carefully weighed, the drug taken out and the container reweighed in order to determine the exact amount. This precaution was necessary since the ampoules were generally found to contain an amount of drug varying slightly from the amount given on the label. After the weight had been determined, it was placed in a wide-mouthed 250 c.c. ground glass stoppered bottle containing a few glass beads.

When the drug was to be used for frog's heart perfusion enough

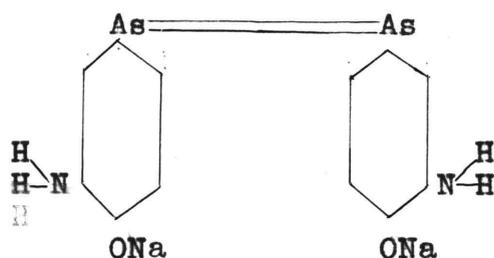
Ringer's solution was added to make the solution 1% strength. The solution had a strong acid reaction. 15% sodium hydroxide solution was carefully added drop by drop until the reaction was neutral. The drug was no longer in solution, but was in the form of a light yellow flocculent suspension, showing that the insoluble salt had been formed which has the formula,



To this suspension was added drop by drop just enough HCl so that the drug would again go into acid solution, 2 drops of HCl to 60 c.c. of the 1% Salvarsan suspension was found sufficient. 10c.c. of the 1% acid solution was then diluted with 90 c.c. of the Ringer's solution making a .1% acid solution. The drug was perfused in this strength to determine the effect of the acid solution.

To prepare the neutral suspension 10 c.c. of the 1% suspension was diluted with 90 c.c. of Ringer's solution making a .1% neutral suspension of Salvarsan and the drug was perfused in this strength.

For the alkaline Salvarsan solution 10 c.c. of the 1% neutral suspension was taken and to this 3 drops of 15% sodium hydroxide solution was added and the suspension went into solution. This solution had a strongly alkaline reaction showing that the soluble alkaline compound had been formed which has the following formula,



90 c.c. of Ringer's solution was added to this 10 c.c. of 1% alkaline solution making a .1% alkaline solution of Salvarsan in Ringer's solution and this strength was used for perfusing.

The oxidized Salvarsan solutions were prepared by exposing .1% neutral suspension to the oxidizing action of the air. The drug soon turned a muddy brown in color and after 2 to 4 days it was tested and if neutral was perfused. It generally had a slight tendency toward acidity and this was corrected with the sodium hydroxide solution. The .1% neutral oxidized solutions were then used for perfusing.

Since the acid and alkali would obviously affect the heart, it was necessary to prepare a Ringer's solution which contained the same amount of acid as the acid Salvarsan solution for the purposes of comparison. Likewise an alkaline Ringer's solution was prepared. Since 2 drops of HCl were added to 60 c.c. of the 1% neutral suspension to make it go into acid solution, 2 drops of HCl were added to 60 c.c. of Ringer's solution and used as a normal for the acid solutions. The alkaline normal was prepared by adding 3 drops of 15% sodium hydroxide solution to 10 c.c. of Ringer's solution and diluting this 10 times with Ringer's solution.

The preparation of the Salvarsan solutions was made as quickly as possible, giving due regard for accuracy. This was done in order to reduce to a minimum the probable formation of toxic oxidation

products.

The next drug used was sodium cacodylate which is the sodium salt of dimethyl arsenic acid. It is a white crystalline substance and contains 54.3% arsenic. It is easily soluble in water and the solution is slightly alkaline in reaction. The drug was prepared by adding 5 grams of the salt to 95 c.c. of Ringer's solution. The solution was then neutralized by the addition of dilute HCl. The 5% neutral solution in Ringer's solution was then used for perfusing.

In order to compare the action of the above compounds with sodium arsenate which is closely allied to sodium cacodylate the following solution was prepared. 5 grams of this drug were dissolved in 95 c.c. of Ringer's solution and the solution was neutralized by the addition of dilute HCl. The arsenate has an arsenic content of 45.7% and the 5% neutral solution in Ringer's solution was used for perfusing.

For comparing the above compounds with the more poisonous arsenious acid the following solution was made up. 1 gram of arsenic trioxide, which is highly insoluble, was added to 99 c.c. of Ringer's solution. 15% sodium hydroxide solution was now added until all the arsenic trioxide went into solution. The solution was then carefully neutralized by the addition of dilute HCl. This 1% neutral solution of arsenious acid was used as a stock solution from which various strengths were made by diluting with Ringer's solution.

In the preparation of the Salvarsan for the intravenous injection into mammals a slightly different technique was employed. After the drug had been weighed and placed in the large 250 c.c. bottle 10 c.c. of boiled freshly distilled water was added for each .1 gm.

of the drug. As there was usually .6 gm. of Salvarsan, 60 c.c. of distilled water was added making a 1% solution. This was neutralized by the addition of 9 drops of 15% sodium hydroxide solution.

To make the acid solution 2 drops of conc. HCl were added to 60 c.c. of the 1% neutral suspension and this was diluted with .8% NaCl solution to make a .1% and a .2% acid solution in .8% NaCl solution. These two strengths were used in testing the effects of the drug on the mammal.

The alkaline solutions were prepared by adding 21 drops of 15% sodium hydroxide solution to 60 c.c. of the 1% neutral suspension. The 60 c.c. of the 1% alkaline solution was then diluted with 240 c.c. of .8% NaCl solution making a .2% alkaline solution of Salvarsan which was the strength used for injecting.

Acid and alkaline normal solutions were prepared and used as in the frog's heart perfusion experiments above. The acid normal was prepared by adding 2 drops of the conc. HCl to 60 c.c. of .8% NaCl solution and then diluting this to the same amount as for the .1 and .2% acid Salvarsan solutions. The alkaline normal was prepared by adding 21 drops of 15% sodium hydroxide solution to 60 c.c. of the .8% NaCl solution and then diluting this with 240 c.c. of .8% NaCl. The normal solutions contained the same amount of acid or alkali as the drug solutions and were the same in every respect except that they did not contain the drug. A comparison of the effects produced by their injection with those produced by the injection of the drug solutions would show the effect of the drug.

Animals Used.

The frog's used in these experiments were the common leopard variety or *Rana pipiens*. In each series of experiments frogs

of similar weight and sex were used in an attempt to keep at a minimum the factor of individual variation. The technical methods employed will be described in the protocol of a typical experiment which will be given in the next chapter.

The dogs used in this work were small curs. As nearly as possible animals of the same weight and age were used. The technical methods employed will be discussed in the next chapter.

III. EXPERIMENTAL RESULTS.

The Action of Salvarsan on the Perfused Frog Heart.

It must be remembered that in Salvarsan we are dealing first of all with an arsenic compound. As such it would be expected to have some "organotropic" action upon living tissue. As a specialized type of tissue the frog's heart lends itself most readily for the pharmacological investigation into the action of Salvarsan. A comparison of the action of different solutions of Salvarsan upon the frog heart will give some idea as to the general "organotropic" effects produced by the drug. With this fact in view the following solutions of Salvarsan were tested upon the frog heart, paying particular attention to their effects as shown by changes in the rate and the amplitude of the contractions.

1. Acid Salvarsan solutions.
2. Neutral Salvarsan suspensions.
3. Alkaline Salvarsan solutions.
4. Oxidized Salvarsan solutions.

THE ACTION OF ACID SALVARSAN.

The experimental methods employed and the results obtained by perfusing .1% acid Salvarsan solutions will best be given in the protocol of the following experiment, which is typical of the series.

Series 25 Experiment No. 1.

Frog No. 1, Sex Male, Weight 26 grams. Date April 14 1913

The frog was pithed and a short time was allowed for preliminary recovery from shock. The heart was next dis-

sected out as follows. A long median incision through the skin of the abdomen served to expose the muscles of the ventral body wall. These with the attached sternum were removed exposing the heart in the pericardial sac. The pericardium was slit open and the position of the inferior Vena Cava determined. A silk ligature was then run under the vena cava and the ends brought around to the surface and loosely tied. The frog was then pinned out on the frog board, ventral side up. A V-shaped slit was made in the Vena Cava, pointing towards the heart, and the cannula inserted into the vein. The ligature was tied to firmly fix the cannula in the vein. The cannula used was the Y-shaped type having an upright pressure tube at the intersection of the Y, as first described by Gibson and Shultz (1), from this laboratory. The open pressure tube makes it possible to perfuse different drugs at a constant pressure. The drugs were perfused from Mariotte pressure bottles as described in Greeness Experimental Pharmacology. The bottles were connected to the cannula by means of a short piece of rubber tubing of small diameter in the course of which was placed a glass T-tube. This T-tube was of service in changing the drugs in the bottles without interfering with the pressure of the fluid then perfusing. A small clamp was attached to the conus arteriosus just at its bifurcation to hold the heart more firmly. A small cut was made in the conus to allow the perfused fluids to readily escape from the ventricle. A light heart lever was attached to the heart and this was adjusted to write on a smoked paper kymograph. An electric signal magnet for marking on the record the exact time the drugs were "on" and "off" and a Jacquet chronograph which marked the time in seconds served to complete the apparatus.

The heart was perfused with acid Ringer's solution and after this had been perfusing long enough to establish a normal, about four minutes, the .1% acid Salvarsan solution was perfused. The heart showed no marked immediate change. There was, however, a progressive, rather rapid but uniform decrease in both the rate and amplitude. The principal effect seemed to be a decrease in the systole as the drug did not perfuse nearly so rapidly near the end of the experiment as at the beginning. The heart also lost its elasticity early as was shown by the disappearance of the elastic rebound at the end of the diastole. The diastole itself appeared to be little affected. The Salvarsan was shut off after about four minutes of perfusion and the acid Ringer's solution was again allowed to perfuse. There was little change in the rate and amplitude when the drug was taken off. The amplitude and rate continuing to decrease. But the greatest effect is a slow gradual decrease in systole.

The effect of the acid Salvarsan on the frog heart seems to be first a slight increase in rate which is soon followed by a decrease

which persists to the end of the experiment. This preliminary stimulation of the rate is not seen with the acid Ringer's solution alone. The amplitude also shows a more marked decrease after acid Salvarsan. From these results it must be said that acid Salvarsan solutions cause a greater decrease in both rate and amplitude of the perfused frog heart than does a Ringer's solution containing the same amount of acid. A comparison of the effects as seen in Plate I, page 44, gives some idea as to the difference in the action of the drug and the action of the acid Ringer's solution. The average effect of a number of experiments is shown in Table I, page 22, The gradual decrease of rate and amplitude is shown in Graphs 1 and 2, page 48.

The Action of Neutral Salvarsan.

The neutral suspension of Salvarsan in strength of .1% has a depressing action upon the heart rate and amplitude. This depressing action on the amplitude is caused by a loss of elasticity, a decrease in diastole, and a decrease in systole. The loss of elasticity occurs first, then the decrease in diastole. The effect on the diastole begins to wear off somewhat as the systole becomes less and less complete. The greater effect, however, is upon the amplitude as was the case with acid Salvarsan. When normal Ringer's solution is again perfused the heart slowly recovers to normal rate and amplitude. The systole recovers first, then the diastole and finally the elasticity. After 29 minutes perfusion with normal Ringer's solution the rate and amplitude had practically returned to what they were before the drug was perfused. This recovery does not occur after acid Salvarsan. A comparison of the effect of neutral Salvarsan suspension in strength of .1% in Ringer's solution,

with normal Ringer's solution is shown in Plate II, page 45, in Table I, page 22, and in Graphs 1 and 2, page 48.

The Action of Alkaline Salvarsan.

The amount of alkali which is necessary to make .1% neutral Salvarsan go into alkaline solution is itself, when added to a normal physiological solution, sufficient to produce an alkaline toxicity of the living tissues of the heart. When alkaline Ringer's solution is perfused through the frog's heart there is first a rapid loss of elasticity, a marked decrease in the diastole follows but there is little effect upon the systole. The perfusion of fluid through the heart almost entirely stops. If the .1% alkaline Salvarsan is turned on at this period very little if any reaches the heart. If turned on shortly before the same effect is seen, the heart soon goes into a state of extreme systolic contraction with little dilation and no filling of the ventricle. Gradually, however the ventricle begins to relax, becoming at the same time arrhythmic and soon stops. This stoppage is generally in the nature of a heart block as the auricle keeps on contracting faintly but the rate is very much slowed. In this state the ventricle contracts when stimulated with a weak induced current, showing that it is still irritable. After varying periods of time the ventricle starts beating again, with the same rhythm as the auricle, but the amplitude diminishes rapidly and the heart soon comes to a stop. The Salvarsan was allowed to run for varying periods of time but the same effect occurred. After the alkaline Ringer's solution is again perfused there is no change. The auricle beats on for some time after the ventricle stops but grows gradually weaker. The heart stops sometimes in extreme systole but usually in extreme diastole. The same type of reaction occurred when the alkaline Ringer's solution was

perfused alone.

Several methods were devised to get the fluid to continue perfusing through the heart during the stages of marked alkaline action but they were all unsuccessful. The results shown in Table I, page 22, and Graphs 1 and 2, page 48, represent better, perhaps, the effect of an alkali in toxic doses upon the heart.

The Action of Neutral Oxidized Salvarsan.

Neutral suspensions of Salvarsan in .1% strengths which have been oxidized by exposing to the air for some time are by far the most toxic form of Salvarsan to the frog heart. There is a very marked decrease in both rate and amplitude. Very soon after the drug has started perfusing the ventricle either stops completely or arrhythmia sets in. The auricle does not stop completely but is visibly slowed. The ventricle gives a few weak contractions upon stimulation showing that the impulses from the auricle evidently do not reach it. If the heart is then perfused with normal Ringer's solution it may recover slightly but the beats are usually in groups and do not last long. The ventricle stops, then the auricle, and finally the sinus giving the effect of a progressive poisoning.

The rapid toxic action of the oxidized neutral Salvarsan suspensions is shown in Table I, page 22, The markedly depressing action upon the rate and amplitude is compared with the effect of other forms of Salvarsan in Graphs 1 and 2, page 48.

TABLE NO. I. SHOWING THE EFFECTS OF DIFFERENT SALVARSAN SOLUTIONS
UPON THE RATE AND AMPLITUDE OF THE PERFUSED
FROG HEART.

GENERAL DATA.				HEART RATE PER MINUTE.			
No. of Expts.	Av. Wt. of Frogs.	Av. Time Drug Perfused.	Drug. Strength.	Before the Drug.	Percentage Change.		
					During.		After.
					Early.	Late.	
8	28.0 gm	268 sec.	.1% "606" Acid.	41.2	01.5	-09.1	-12.6
4	26.8 "	- - - -	Acid Ringers.	39.0	-01.9	-03.2	-03.2
19	29.7 "	259 sec.	.1% "606" Neutral	37.8	-01.8	-04.3	-01.8
8	27.3 "	192 "	.1% "606" Alkalin	50.0	-07.4	-09.0	-20.0
6	38.9 "	206 "	.1% "606" Oxidiz.	59.3	-25.4	-93.1	-71.6

TABLE NO. I. (continued.)

HEART AMPLITUDE IN MM.				EFFECT ON;		
Before the Drug.	Percentage Change.			Systole	Diastole	Elasticity.
	During.		After.			
	Early.	Late.				
18.0	-06.1	-30.5	-41.6	Grad. Decreas	Little	Gradual Decrease.
18.5	-01.3	-02.7	-12.3	"	"	"
18.8	-02.2	-17.8	-12.0	"	Grad. Decre.	"
14.0	-14.2	-14.2	-35.7	Little	Rapid Dec.	Rapid Decrease.
16.0	-20.0	-98.0	-76.2	Rapid Decreas	"	"

The Action of Sodium Cacodylate.

Neutral solutions of sodium cacodylate in Ringer's solution have a very striking action on the frog's heart. When a 5% solution of the sodium cacodylate is perfused through the heart there is a rapid loss of elasticity and a very sharp decrease in systole. Thus it takes a comparatively strong solution, i. e., 5%, to produce changes acute enough to be measured by the methods here used. At the same time there is an increase in the rate at the early stage of the action of this arsenic compound. If the drug is perfused for some time the rate becomes slower and slower, the heart becomes arrhythmic and finally stops in diastole. If normal Ringer's solution is again perfused the heart recovers with remarkable rapidity, and both rate and amplitude approach very near to what they were before the drug was perfused. The rapidity with which this effect comes on and disappears suggests an ionic action similar to that seen when potassium is perfused through the frog heart. In some ways the action of the cacodylates resemble the action of the neutral Salvarsan suspensions. With the former however the depressing effect comes on much more rapidly and lasts for a much shorter time after the heart is again perfused with normal Ringer's solution. The effect of the cacodylates is shown in Plate III, page 46, figures 1 and 2, Table II, page 25, and Graphs 1 and 2, page 48, also show the depressing action of the drug and the quick recovery after it.

The Action of Sodium Arsenate.

The action of 5% neutral solutions of sodium arsenate in

Ringer's solution when perfused through the frog's heart is to cause a rather rapid decrease in the amplitude and in the heart rate. This change is not so rapid nor so marked as that which results from the perfusion of the cacodylates. The principal effect upon the amplitude is due to a decrease in systole.

When Ringer's solution is again perfused the amplitude gradually returns to normal. After some time there is a slight arrhythmia after which the rate is very much slowed. The rapid change when the drug is first perfused would suggest that the sodium arsenate had an ionic action comparable to that of the cacodylates, but the fact that the heart shows permanent damage proves that the compound is definitely toxic, in fact more toxic than the cacodylate. A comparison of the action of these two compounds is given in Table II, page 25. The general depressing effect of the arsenate is compared with the action of other arsenic compounds in Graphs 1 and 2, page 48, and Plate IV, page 47.

TABLE NO. II. SHOWING THE EFFECT OF SODIUM CACOYLATE AND
SODIUM ARSENATE UPON THE RATE AND AMPLITUDE OF
THE PERFUSED FROG HEART.

GENERAL DATA.				HEART RATE PER MINUTE.			
No. of Exp'ts.	Av. Wt. of Frogs.	Av. Time Drug Per- fused.	Drug Strength.	Before the Drug.	Percentage Change.		
					During.		After.
					Early	Late.	
25.	30.8 gm	252 sec	5% Sod. Cacodylat	51.9	-05.0	-52.4	-07.4
4.	26.2 gm	260 "	5% Sod. Arsenate	55.7	-05.7	-31.0	-29.0

TABLE NO. II. (continued.)

HEART AMPLITUDE IN MM.				EFFECT ON;		
Before the Drug.	Percentage Change.			Systole	Diastole	Elasticity.
	During.		After.			
	Early.	Late.				
18.3	-32.2	-53.0	-01.6	Rapid Decreas	Slight Increase	Rapid Decrease.
18.0	-12.0	-34.4	-01.6	"	Little.	"

The Action of Arsenious Acid.

Arsenious acid is very toxic to heart muscle. The compound evidently enters into a firm combination with the protoplasmic molecule of the muscle of the frog's heart, since the toxicity is not overcome by perfusing with normal Ringer's solution after the use of the drug. Dilute solutions of arsenious acid have a slight stimulative action upon the amplitude of the heart beat at first but this soon disappears, and a sharp progressive depression sets in which persists to the end of the experiment. The decrease in the amplitude is caused principally by a decrease in the systole which is accompanied by some increase in diastole. The influence of the arsenious acid is not so marked on the heart rate. Stronger solutions are more rapidly toxic. The effects of the drug are shown in Table III, page 27, and Graphs 1 and 2, page 48.

TABLE NO. III. SHOWING THE EFFECTS OF VARIOUS STRENGTHS OF
ARSENIOUS ACID UPON THE PERFUSED FROG HEART.

GENERAL DATA.				HEART RATE PER MINUTE.			
No. of Exp'ts.	Av. Wt. of Frogs.	Av. Time Drug Per- fused.	Drug. Strength.	Before the Drug.	Percentage Change.		
					During.		After.
					Early.	Late.	
8	21.0 gm	429 Sec.	.01%	44.0	-03.4	-09.0	-28.4
1	21.0 "	498 "	.05%	50.0	-00.0	-00.0	-00.0
12	27.6 "	296 "	.1%	52.5	-04.7	-23.8	-38.8
1	34.0 "	336 "	.2%	60.0	-08.3	-36.3	-50.0
1	31.4 "	133 "	.4%	58.0	-25.9	-100.0	-39.6
4	33.8 "	65 "	1.%	52.0	-00.7	-32.6	-49.6

TABLE NO. III. (continued.)

HEART AMPLITUDE IN MM.				EFFECTS ON;		
Before the Drug.	Percentage Change.			Systole	Diastole.	Elasticity.
	During.		After.			
	Early.	Late.				
26.0	-00.0	-11.5	-19.0	Grad. Decrease	Little.	Gradual Decrease.
23.0	04.1	-20.8	-29.0	"	"	"
17.0	03.3	-61.4	-93.5	"	"	"
17.0	-05.8	-88.8	-99.0	Rapid Decrease	"	Rapid Decrease.
20.0	00.5	-55.0	-90.0	"	"	"
13.5	-07.4	-60.7	-94.8	"	"	"

TABLE NO. IV. COMPARING THE AVERAGE ACTION OF VARIOUS ARSENIC COMPOUNDS UPON THE PERFUSED FROG HEART. THE DRUGS COMPARED ARE ACID SALVARSAN, ACID RINGER, NEUTRAL SALVARSAN, ALKALINE SALVARSAN, OXIDIZED SALVARSAN, SODIUM CACODYLATE, SODIUM ARSENATE, AND ARSENIOS ACID.

GENERAL DATA.				HEART RATE PER MINUTE.			HEART AMPLITUDE IN MM.			EFFECT ON;			REMARKS.		
No. of Experiments performed.	Average Wt. of frogs in the set.	Average Time Drug Perfused in Seconds.	Strength of Drug.	Before the Drug.	Percentage Change.		Before the Drug.	Percentage Change.		Systole.	Diastole.	Elasticity.			
					During.	After.		During.	After.						
					Early.	Late.		Early.	Late.						
8	28.0 gm.	268	0.1% Acid Salvarsan.	41.2	-01.5	-99.1	-12.6	18.0	-06.1	-30.5	-41.6	Grad. Decrea.	Little.	Gradual Decrease.	Effect slow, scarcely perceptible on chart.
4	26.8 "	- -	Acid Ringer.	39.0	-01.9	-03.2	-03.2	18.5	-01.3	-02.7	-12.3	"	"	"	Very slow gradual decrease.
19	29.7 "	259	0.1% Neut. Salvarsan.	37.8	-01.8	-04.3	-01.8	18.8	-02.2	-17.8	-11.0	"	"	"	Principal effect on systole and elasticity.
8	27.3 "	192	0.1% Alk. Salvarsan	50.0	-07.4	-09.0	-20.0	14.0	-14.2	-14.2	-35.7	Little	Rapid Decrease	Rapid Decrease.	Principal effect on diastole and elasticity.
8	38.9 "	269	0.1% Oxidized Salvarsan.	59.3	-25.4	-93.1	-71.6	16.0	-20.0	-98.0	-76.2	Rapid Dec.	Little	Rapid Decrease.	After this drug, heart arrhythmic. Little Ringer perfuses.
25	30.8 "	252	5% Sodium Cacodylate.	51.9	-00.5	-52.4	-07.4	18.3	-32.2	-53.0	-01.6	"	"	"	Effect comes on rapidly and goes off rapidly.
4	26.2 "	260	5% Sodium Arsenate.	55.7	-05.7	-31.0	-29.0	18.0	-12.0	-34.4	-01.6	"	"	"	Decrease rapid, recovery slow.
12	27.6 "	296	0.1% Arsenious Acid.	52.5	-04.7	-23.8	-38.8	17.0	-03.3	-61.4	-93.5	Grad. Dec.	Little	Grad. Decrease.	Heart Gradually weakened and stopped in extreme diastole.

The Action of Salvarsan on the Mammal.

The experiments on the frog heart show that Salvarsan as such is only slightly "organotropic". But in the frog heart oxidation processes go on very slowly and toxic oxidation products, if any are formed, would be produced very slowly. In the mammalian body, however, the oxidation processes by which substances are oxidized and reduced take place relatively fast. For this reason the body of the mammal is of excellent service in testing the effect of such compounds as Salvarsan where there is a possibility that the principal "organotropic" or toxic action, if one occurs, is due to the oxidation and reduction products formed.

Young dogs were selected as a type of mammal upon which to make this study as they react more easily, and thus minute changes are seen and recorded which would be lost if adult animals were used.

The Action Of Acid Salvarsan.

An idea of the technical methods employed and the results obtained by intravenous injection of .1% and .2% acid Salvarsan solution can best be seen in the following protocol.

Experiment No. VIII. Date, January, 18 1913.

10:30 A.M. The dog was weighed and the following weight recorded.

Weight of dog and box	7330 grams.
" "	1878 "
Weight of dog	3452 "

The dog was a female pup about 3 1/2 months old.

10.55 A.M. The dog was anesthetized with ether and ~~securely~~ fastened to the animal board.

11:05 A.M. Cannula put in the right carotid for taking blood pressure. Vagus nerves were dissected out and ligatures passed around them, but not tied, and the nerves then dropped back in their beds until time for stimulation. A tracheal cannula was inserted and the anesthetic given from an automatic anesthetic bottle which secured a greater constancy of anesthesia and warmed the ether vapor used to body temperature, thus reducing to a minimum the irritating action of the vapor on the mucous membranes of the respiratory tract

A side tube from the tracheal cannula recorded the changes in respiratory rate and amplitude. The abdomen^{was opened} by a midline incision extending from the lower part of the xiphoid cartilage to about an inch below the umbilicus. The left kidney was secured, and the perirenal fat stripped off, and the organ placed in a renal oncometer to record changes in the peripheral circulation as shown by the kidney. The abdominal wound was loosely closed by a continuous suture. The femoral vein was secured by making a short incision on the inner side of the thigh. The vein was dissected out for a distance of about 2 cm. and two ligatures were passed around it. The distal ligature was tied, a V-shaped cut, pointing towards the heart, made in the vein and the injecting cannula inserted, and securely fixed in the vein with the proximal suture. The injecting cannula was a 4-way glass cannula, one limb was put in the vein, to another was attached a tube containing the drug, to a third limb was attached a tube leading from the burette containing the acid saline solution and to the fourth limb a small tube was attached which served as an air trap, and as a means of washing out the tubes and cannula. The injecting burettes were jacketed and kept surrounded with warm water at 40 degrees C., so that the solutions were about body temperature when injected. The injecting apparatus is one which has been developed and is used in this laboratory.

12:00 M. The operation was completed and all records started. In Table No. V, page 31, are shown the results of the various tests made during this experiment.

TABLE NO. V. SHOWING THE RESULTS OF THE VARIOUS TESTS MADE
UPON THE CIRCULATION, RESPIRATION, AND GENERAL NERVOUS
SYSTEM IN THE MAMMAL IN A TYPICAL ACID SALVARSAN

EXPERIMENT.										DOSAGE.				HEART RATE PER MINUTE.									BLOOD PRESSURE IN MM. MERCURY.										
No.	Time. P.M.	Temperatures.				Drug or Stim. Amount strength, etc.	Strength of Coil.		Time of In- jection in seconds.	Rate of In- jection per second.	Dose in Mg. of "606"	Dose "606" in mg. per kilo bodyweight.	Before.			During.			After.			Before.			During.			After.					
		R e c t a l	A n t e r o r i a l	R e c t a l	D i r e c t a l		Sec. from Prim.in cm.	Length of Stim. in Seconds.					120 Sec.	60 Sec.	10 Sec.	10 Sec.	20 Sec.	60 Sec.	120 Sec.	20 Sec.	60 Sec.	120 Sec.	120 Sec.	60 Sec.	120 Sec.	20 Sec.	20 Sec.	60 Sec.	120 Sec.	20 Sec.	20 Sec.	60 Sec.	120 Sec.
1.	12.41					Stim. Rt. Vag	6	5	-	-	-	-	156	174	162	000	---	---	---	162	168	---	76	78	77	48	---	---	---	72	79	---	
2.	12.44					" " "	6	4	-	-	-	-	---	150	168	000	---	---	---	150	165	---	---	77	77	46	---	---	---	68	75	---	
3.	12.50	34	40	24	40	34.5cc. .8% Acid NaCl.	-	-	126	-	-	-	---	162	168	159	165	162	---	150	158	---	---	68	72	72	69	65	---	66	66	---	
4.	12.55					Stim. Rt. Vag.	6	5	-	-	-	-	---	156	156	000	---	---	---	126	156	---	---	64	64	41	---	---	---	53	63	---	
5.	12.59					" " "	6	6	-	-	-	-	---	156	162	144	000	---	---	---	126	159	---	63	63	63	41	---	---	---	51	61	---
6.	1.00	34	40	25	40	34.5cc. .1% Acid "606"	-	-	140	.24 cc.	32.14	9.31	---	156	159	156	156	162	159	147	144	138	---	60	61	61	62	64	60	49	36	32	
7.	1.05					Stim. Rt. Vag.	6	5	-	-	-	-	---	147	144	138	000	---	---	---	122	144	141	48	36	32	26	---	---	---	31	30	28
8.	1.07					" " "	6	5	-	-	-	-	---	122	144	141	000	---	---	---	132	144	141	31	30	28	24	---	---	---	29	32	34
9.	1.17	34	40	25	40	34.5cc. .1% Acid "606"	-	-	182	.18 cc.	34.5	10.00	---	138	138	138	138	138	144	144	144	42	44	46	47	48	51	54	56	56	54		
10.	1.41					Stim. Rt. Vag.	6	5	-	-	-	-	---	174	174	174	000	---	---	---	162	174	174	65	63	62	35	---	---	---	59	60	56
11.	1.45	34	40	26	40	100 cc. .1% Acid "606"	-	-	1400	.071cc.	100.0	28.81	---	174	174	174	174	177	180	174	180	174	174	58	58	60	59	59	63	64	64	64	
12.	2.30					Stim. Rt. Vag.	6.	6	-	-	-	-	---	174	174	180	006	---	---	---	174	162	168	60	59	58	38	---	---	---	56	56	56
13.	2.35	34	40	27	40	97 cc. .1% Acid "606"	-	-	734	.130cc.	97.0	28.18	---	174	174	174	174	174	174	180	174	174	56	56	54	56	56	60	63	68	66	63	
14.	2.47					Stim. Rt. Vag	6	5	-	-	-	-	---	174	174	168	006	---	---	---	174	162	168	66	63	61	38	---	---	---	57	59	59
15.	2.49					" " "	6	5	-	-	-	-	---	174	162	168	024	---	---	---	168	156	168	57	59	59	36	---	---	---	56	58	57
16.	2.51					" " "	0	112	-	-	-	-	---	---	156	168	000	---	---	---	156	174	168	---	58	57	34	---	---	---	58	55	54
17.	3.00	34	40	27	40	75 cc. .8% Acid "NaCl."	-	-	965	.077	-	-	---	174	174	168	168	---	162	162	165	165	---	55	53	54	56	---	54	57	60	62	---
18.	3.17					Stim. Rt. Vag.	6	5	-	-	-	-	---	---	165	165	012	---	---	---	158	168	174	---	60	62	43	---	---	---	55	56	58
19.	3.51					" " "	6	5	-	-	-	-	---	168	168	168	018	---	---	---	162	174	174	45	45	45	43	---	---	---	43	44	46
20.	3.54	34	40	27	40	143 cc. .2% Acid "606"	-	-	1397	.102	286.0	86.03	---	162	174	174	168	168	168	168	126	132	---	45	44	46	46	48	49	51	39	36	---
21.	4.20					Stim. Rt. Vag.	6	6	-	-	-	-	---	---	132	132	015	---	---	---	132	132	132	---	36	41	28	---	---	---	37	37	37
22.	4.33	34	40	27	40	100 cc. .2% Acid "606"	-	-	600	.160	200.0	57.30	---	128	132	135	138	138	138	042	x	x	x	35	39	32	34	35	39	35	x	x	x

VOLUME CHANGE IN KIDNEY IN MM.									RESPIRATION RATE PER MINUTE.									RESPIRATION AMPLITUDE IN MM.									REMARKS.			
Before.			During.			After.			Before.			During.			After.			Before.			During.			After.						
120 Sec.	60 Sec.	10 Sec.	10 Sec.	20 Sec.	60 Sec.	120 Sec.	20 Sec.	60 Sec.	120 Sec.	120 Sec.	60 Sec.	120 Sec.	10 Sec.	20 Sec.	60 Sec.	120 Sec.	20 Sec.	60 Sec.	120 Sec.	120 Sec.	60 Sec.	10 Sec.	10 Sec.	20 Sec.	60 Sec.	120 Sec.		20 Sec.	60 Sec.	120 Sec.
113	113	113	101	---	---	---	114	114	---	96	102	99	000	---	---	---	84	880	---	12	12	13	00	---	---	---	14	13	---	
---	113	112	99	---	---	---	113	112	---	---	84	84	000	---	---	---	96	102	---	---	13	13	00	---	---	---	14	13	---	
---	112	112	112	112	112	---	113	112	---	---	99	99	87	96	102	---	93	93	---	---	13	13	13	14	15	---	14	15	---	
---	113	112	101	---	---	---	113	113	---	---	93	96	000	---	---	---	102	90	---	---	14	14	00	---	---	---	14	14	---	
113	112	113	102	---	---	---	113	112	---	90	87	84	000	---	---	---	93	84	---	14	15	14	00	---	---	---	13	14	---	
---	113	112	113	113	114	112	111	111	110	---	81	84	84	87	90	86	84	90	90	---	14	14	14	14	13	13	15	15	14	
111	111	110	107	---	---	---	111	110	111	84	90	90	000	---	---	---	90	93	102	15	15	14	00	---	---	---	13	13	12	
111	110	111	107	---	---	---	111	111	111	90	93	102	000	---	---	---	90	90	96	13	13	12	00	---	---	---	13	14	14	
111	111	111	112	112	112	112	112	111	111	108	102	90	90	90	99	111	99	99	99	12	13	14	15	14	14	13	13	14	14	
109	109	109	98	---	---	---	110	110	109	108	120	120	72	---	---	---	144	120	87	16	16	16	10	---	---	---	14	14	15	
109	109	109	110	110	110	110	110	110	110	84	96	93	99	96	90	96	132	138	144	15	15	16	16	15	15	14	14	14	15	
109	109	109	98	---	---	---	110	110	109	108	114	108	60	---	---	---	108	102	99	16	16	15	6	---	---	---	14	14	15	
110	109	109	109	109	110	109	110	110	110	102	99	102	108	108	114	111	108	96	114	14	15	15	15	15	15	15	16	17	17	
110	110	110	100	---	---	---	111	111	111	96	114	108	30	---	---	---	111	100	108	17	17	16	6	---	---	---	15	17	16	
111	111	111	101	---	---	---	111	111	111	111	100	108	18	---	---	---	108	90	90	15	17	16	5	---	---	---	16	17	16	
---	111	111	101	---	---	---	112	111	111	---	90	90	12	---	---	---	105	84	90	---	17	16	6	---	---	---	15	16	17	
111	111	111	111	---	112	111	109	109	---	84	84	90	96	---	108	108	105	96	---	16	17	17	15	---	19	18	17	16	---	
---	109	109	99	---	---	---	109	109	109	---	105	96	12	---	---	---	96	84	105	---	17	16	6	---	---	---	16	17	16	
110	110	110	104	---	---	---	110	110	110	84	90	84	18	---	---	---	84	78	84	15	15	15	5	---	---	---	14	14	15	
110	110	110	110	110	110	109	109	110	---	84	78	84	84	81	84	84	45	45	---	14	14	15	16	15	16	16	15	14	---	
---	109	109	105	---	---	---	109	110	110	---	45	45	48	---	---	---	44	42	42	---	14	14	14	8	---	---	---	15	14	---
110	109	110	110	110	110	108	109	110	110	30	24	24	30	26	24	12	x	x	x	15	16	15	15	15	16	19	x	13	14	

Traube Herring waves in B.P. and Onc. Tracing. 17 min. after drug started Resp. labored.

12 min. after injection started Traube-Herring waves appeared.

* Heart and Resp. very irregular. 12 min. after inject. started Resp. stopped. 12 min. 80 sec. heart stopped. 17 min. Bl. Pr. 2 mm. Onc. 109.

The three principal reactions studied were;

The Effect of Salvarsan upon the Organs of Circulation.

The Heart Rate.

The Peripheral Changes in Kidney.

The Blood Pressure.

The Effect of Salvarsan upon the Respiration.

The Rate.

The Amplitude of the Respiratory Movement.

The Effect Upon the Central Nervous System as Shown
by the Variations in Sensitiveness of the Vagus
Nerve.

The Organs of Circulation.

The Respiration.

The Central Nervous System.

The Action of Acid Salvarsan.

The Organs of Circulation.

In Table VI, page 35, is shown the percentage change caused by acid Salvarsan on the circulatory system. Small amounts injected intravenously cause at first a slight rise in blood pressure. This is followed by a rather marked fall which continues for some time after the drug has been injected. The heart rate is at first increased and then slowed, but there is little effect upon the kidney. Injections of equal amounts of acid saline solution also cause a fall in blood pressure and a decrease in heart rate, although not quite so marked as with the Acid Salvarsan. After repeated injections of small doses there is a slight rise in blood pressure which may possibly have been due to the anesthetic.

Large doses of 0.2% acid Salvarsan, amounts equalling 53% of the total quantity of blood, injected slowly cause at first a slight rise and then a marked fall of blood pressure. The heart rate is slowed but the kidney volume is little changed. If a dose of this volume be repeated in a short time the blood pressure falls rapidly, and the heart becomes irregular and soon stops in extreme diastole. So great a change in the mammalian heart can not be ascribed to the solvent, hence we must conclude that Salvarsan in acid solution is directly toxic to the heart.

The kidney seems little affected in so far as revealed by our experiments.

The Respiratory Mechanism.

Small amounts of .1% acid Salvarsan, doses equalling 13% of the total quantity of the blood, cause an increase in the respiratory rate which lasts for some little time after the drug is injected. The respiratory amplitude is at the same time slightly decreased. This stimulation is no doubt due to the direct action of the Salvarsan on the respiratory center in the medulla.

Acid saline solutions also cause an increased rate and a decreased amplitude so this effect must be partially attributed to the action of the solvent.

Large doses, 0.2% acid Salvarsan, cause a slight increase in the respiratory rate which is followed by a marked fall. The effect on the amplitude varies, usually, however, there is an increased amplitude associated with a decreased rate. If the larger dose is repeated the respiration becomes slower and slower and stops sometime before the heart ceases to beat.

On the Central Nervous System.

Stimulation of the intact Vagus nerve calls forth about the same changes after the acid Salvarsan as before. The blood pressure falls about the same amount, the slowing of the heart is the same and the kidney volume remains unchanged. The respiratory rate and amplitude are affected in the same way. It may be concluded, then, that acid Salvarsan has very little effect upon the Central Nervous System other than the stimulative action upon the respiratory center referred to above.

TABLE NO. VI. SHOWING THE EFFECT OF ACID SALVARSAN ON THE
CIRCULATION, RESPIRATION, AND CENTRAL NERVOUS
SYSTEM IN THE MAMMAL.

Experiment and Injection Number.	Time of Injection.	Amount and Strength of Drug.	DOSAGE.		HEART RATE PER MINUTE.						BLOOD PRESSURE IN MM. OF MERCURY.				
			Time of Inject. in Sec.	Rate of Inject. in cc. per Sec.	Dose "606" in Mgr. per kilo. body weight.	Before the Drug. Av. 60-10 Seconds.	Percent of Change.				Before the Drug. Av. 60-10 Seconds.	Percent of Change.			
							During Drug.		After the Drug.			During the Drug.		After the Drug.	
							20 Sec.	120 Sec.	20 Sec.	120 Sec.		20 Sec.	120 Sec.	20 Sec.	120 Sec.
VIII #1	12:50 P.M.	34.5 cc. Acid NaCl	126	.261	- - - -	165	±00.0	-01.8	-09.0	-03.6	70	-01.4	-07.1	-05.7	-05.7
" #2	3:00 P.M.	75.0 cc. Acid NaCl	965	.070	- - - -	171	-05.3	-05.3	-03.5	-03.5	55	+05.6	+07.6	+13.2	+18.8
" #3	1:00 "	34.5 cc. .1% "606"	140	.240	9.31	157	-00.6	+01.3	-06.3	-12.1	60	+03.3	-00.0	-21.6	-46.4
" #4	1:17 "	34.5 cc. .1% "606"	182	.180	10.00	138	-00.0	+06.5	+01.5	+04.3	45	+06.6	+20.0	+24.4	+20.0
" #5	1:45 "	100 cc. .1% "606"	1400	.070	28.81	174	+01.7	-00.0	+03.4	-00.0	59	-00.0	+08.4	+08.4	+08.4
" #6	2:35 "	97 cc. .1% "606"	734	.130	28.18	174	-00.0	-00.0	+03.4	-00.0	55	+01.8	+14.5	+23.6	+14.5
" #7	3:54 "	143 cc. .2% "606"	1397	.100	86.03	174	-03.4	-03.4	-25.4	-21.2	45	+06.6	+13.3	-13.3	-20.0
" #8	4:33 "	100 cc. .2% "606"	600	.160	57.30	133	+03.7	-68.4	x	x	35	-00.0	-00.0	x	x

x. Heart and Respiration grew very irregular. Resp. rate slowed. 12 min. after injection started Resp. stopped. 12 min. 30 sec. heart stopped. 17 min. blood pressure 2 mm. Oncometer 109 mm.

PERIPHERAL EFFECT. (Oncometer Change.)					RESPIRATORY RATE PER MINUTE.					RESPIRATORY AMPLITUDE IN MM.				
Before the Drug. Av. 60-10 Seconds.	Percent of Change.				Before the Drug. Av. 60-10 Seconds.	Percent of Change.				Before the Drug. Av. 60-10 Seconds.	Percent of Change.			
	During the Drug.		After the Drug.			During the Drug.		After the Drug.			During Drug.		After the Drug.	
	20 Sec.	120 Sec.	20 Sec.	120 Sec.		20 Sec.	120 Sec.	20 Sec.	120 Sec.		20 Sec.	120 Sec.	20 Sec.	120 Sec.
112.	-00.0	-00.0	+00.8	-00.0	99	+03.0	+03.0	-06.0	-06.0	13	+07.6	+15.3	+07.6	+15.3
111.	-00.0	-00.0	-01.8	-01.8	87	+10.3	+24.0	+20.6	+10.3	17	-11.7	+05.8	-00.0	-05.8
112	+00.8	-00.0	-00.8	-01.7	82	+06.0	+04.8	+02.4	+09.7	14	-00.0	-07.1	+07.1	-00.0
111	+00.9	+00.9	+00.9	-00.0	96	+06.8	+05.2	+03.1	+03.1	13	+07.6	-00.0	-00.0	+07.6
109	+00.9	+00.9	+00.9	+00.9	94	+02.1	+02.1	+37.2	+53.1	15	-00.0	-06.6	-06.6	-00.0
109	-00.0	-00.0	+00.9	+00.9	100	+08.0	+11.0	+08.0	+14.0	15	-00.0	-00.0	+06.6	+13.3
110	-00.0	-00.9	-00.9	-00.0	81	-00.0	+03.6	-44.4	+44.4	14	+07.1	+14.2	+07.1	-00.0
109	+00.9	-00.9	x	x	24	+08.3	+50.0	x	x	15	-00.0	+20.0	x	x

The Effect Of Alkaline Salvarsan.

The Organs of Circulation.

Small doses of 0.2% alkaline Salvarsan solution, amounts ranging from 12% to 19% of the total volume of the blood, cause very little change which cannot be accounted for by changes in experimental conditions. A total dose of 89.7 mg. per kilo body weight was injected into one animal, yet no appreciable change followed except that of a slight increase in blood pressure. This change was most likely due to the increase in the volume of fluids in the blood vessels of the body. This amount per kilogram is far above the maximal therapeutic dose given intravenously to man, i. e., 89.7 mg. for the dog, as against 10 mg. for the man.

Large doses of 0.2% Alkaline Salvarsan solution, amounts ranging from 33% to 57% of the total volume of the blood, cause a marked increase in blood pressure. There is also a marked increase in the heart rate and a slight increase in the volume of the kidney. The total amount of the drug injected was equal to 140 mg. per kilo. body weight. As this dose is 14 times the therapeutic dose Salvarsan certainly could not be called very "organotropic".

The Respiration.

Small doses have very little effect upon the respiratory rate or amplitude. Large doses cause an increase in both rate and amplitude.

The Central Nervous System.

After intravenous injections of small doses of the alkaline

Salvarsan solution there is little effect upon the Central Nervous System. Large doses cause a stimulation of the respiratory center, and probably of the cardiac acceleratory center. The general effects produced by stimulation of the intact Vagus remain the same after the drug as before.

In Table VII, page 38, is shown the effects of Alkaline Salvarsan solutions upon the mammal.

TABLE NO. VII. SHOWING THE EFFECT OF ALKALINE SALVARSAN ON THE CIRCULATION, RESPIRATION, AND CENTRAL NERVOUS SYSTEM IN THE MAMMAL.

Experiment and Injection Number.	Time of Injection.	Amount and Strength of Drug.	DOSAGE.			Before Drug Av. 60 Seconds	HEART RATE PER MINUTE.				Before the Drug. Av. 60-10 Seconds.	BLOOD PRESSUER IN MM. OF MERCURY.			
			Time of Inject. in Sec.	Rate of Inject in cc. per Sec.	Dose "606" in Mgr. per kilo. body weight.		Percent of Change.					Percent of Change.			
							During the Drug.		After the Drug.			During the Drug.		After the Drug.	
							20 Sec.	120 Sec.	20 Sec.	120 Sec.		20 Sec.	120 Sec.	20 Sec.	120 Sec.
VI #1	4:18 P.M.	100 cc.	295	.340	- - - -	210	-07.1	-04.1	-00.0	-00.0	156	-00.6	-09.6	-19.6	-25.6
" #2	4:40 "	.8% NaCl 137 cc.	652	.211	20.	205	+01.6	-01.9	-01.9	-07.8	129	+01.5	+00.7	-05.3	-06.1
" #3	4:55 "	.2% "606" 137 cc.	793	.173	20.	189	-01.6	-00.0	-06.3	-07.8	121	-03.3	-02.4	-22.3	-23.9
" #4	5:58 "	.2% "606" 137 cc.	415	.330	20.	198	-03.0	-06.0	-00.0	-03.0	102	-00.9	-00.0	+15.8	-07.8
" #5	6:29 "	.2% "606" 205 cc.	892	.228	<u>29.7</u>	195	-01.5	+01.5	-06.1	-04.6	93	-02.1	+07.5	-12.9	-17.2
					89.7										
VII.#1	3:20 "	138 cc. .2% "606"	550	.250	51.63	178	+01.1	-05.6	+05.7	+05.7	108	+02.7	+05.5	+09.9	+12.0
VII.#2	3:40 "	236 cc. .2% "606"	1014	.232	<u>88.49</u>	171	+01.7	+10.3	+44.8	+44.8	92	+04.3	+13.0	+22.8	+21.7
					140.12										

Experiment No. VI. Weight of Dog. 13,795. grams.

Experiment No. VII. Weight of Dog. 5.345. grams.

PERIPHERAL EFFECT. (Oncometer Change.)					RESPIRATORY RATE PER MINUTE.					RESPIRATORY AMPLITUDE IN MM.				
Before the Drug. Av. 60-10 Seconds.	Percent of Change.				Before the Drug. Av. 60-10 Seconds.	Percent of Change.				Before the Drug. Av. 60-10 Seconds.	Percent of Change.			
	During the Drug.		After the Drug.			During the Drug.		After the Drug.			During the Drug.		After the Drug.	
	20 Sec.	120 Sec.	20 Sec.	120 Sec.		20 Sec.	120 Sec.	20 Sec.	120 Sec.		20 Sec.	120 Sec.	20 Sec.	120 Sec.
110	-00.0	-00.0	-00.0	-00.0	30	-20.0	-10.0	-00.0	-00.0	8	-12.5	+12.5	+12.5	+12.5
112	+00.8	+00.8	+00.8	-00.0	42	-00.0	-07.0	+57.0	+50.0	8	-00.0	+12.0	+25.0	+25.0
112	-00.0	-00.0	-00.0	+00.8	70	-05.1	-05.1	-25.9	-29.8	8.5	+05.8	+17.0	+29.4	+29.4
112	-00.0	-00.0	-00.0	-00.0	55	-07.2	-00.0	-12.3	-23.6	12.	+16.6	+25.0	+41.6	+41.6
112	-00.0	-00.0	-00.0	-00.0	51	+05.8	+05.8	-05.8	-05.8	15.	-00.0	-00.0	-13.3	-13.3
110	-00.0	+00.8	+01.8	+01.8	67	+07.4	+01.1	+29.8	+16.4	11.	-09.0	-09.0	+09.0	+09.0
106	-00.0	-00.0	+08.6	+04.8	67	+07.4	+16.4	+18.0	+18.0	10	+10.0	+10.0	+30.0	+40.0

IV. GENERAL DISCUSSION OF RESULTS.

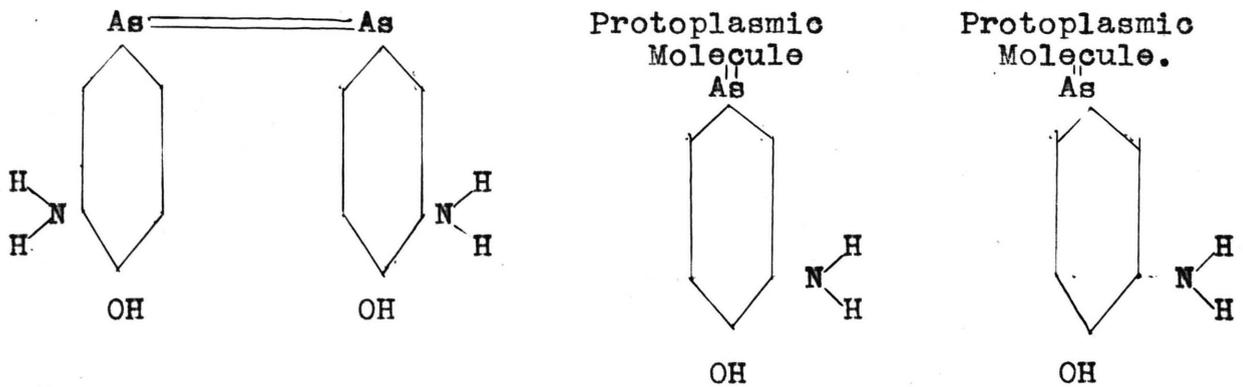
A Comparison of the Action of Different Arsenic-Containing Drugs Upon the Perfused Frog Heart.

All the compounds used contained arsenic hence may legitimately be expected to show some toxic action to all animal tissues. From Salvarsan which contains 31.6% to Sodium Cacodylate which contains 54.3% arsenic in the molecule, there is a wide variation in the type of action on the frog heart. This cannot be explained by a difference in the amount of arsenic present.

That Salvarsan is "organotropic", i. e., toxic to tissue cells to a certain extent is not denied even by its most ardent advocates. It is a highly reduced product and would naturally be easily oxidized in the tissues. This is shown to be the case by the effects of the neutral suspension of Salvarsan on the frog heart. The oxidation products are easily removed from the heart, however, as shown by the partial recovery when Ringer's solution is perfused after the drug.

The oxidized Salvarsan presents quite different features. It is highly "organotropic". It must be added, however, that there is a slight tendency for the heart to recover after the oxidized drug is perfused, resembling in a way the neutral suspension. This would indicate that the oxidation products formed outside the body were of a somewhat similar nature to those formed in the tissue, but outside the body they are formed in a much greater quantity. A probable reason for this is that in the tissue more Salvarsan molecules attach themselves to the protoplasm and in that form are not readily oxidized by the tissues, while outside the body all the molecules of the drug lend themselves to ready oxidation.

The fact that Salvarsan has been found in large quantities in the liver cells after intravenous injections would tend to show that certain cells do fix this substance unchanged, thus effectively removing it from the blood stream. The fact that after intravenous injection Salvarsan as such can be demonstrated in the urine for only a comparatively short time would also tend to show that the majority of the molecules attach themselves to the chemoreceptors of the tissue cells, whence it is slowly oxidized and absorbed. It has been suggested that this attachment is through the arsenic bond, if so it might be represented as follows,



Since the Salvarsan compound is only slightly "organotropic" only a very few such combinations could take place with the cells the majority having no "chemoreceptors" for the arsenic in this form. The cells of cardiac tissue evidently have some such receptors and when the union of the cell and the compound takes place there is a toxic action. However, after Salvarsan is oxidized it acquires a greater toxicity, due to the fact, that the oxidation products find a more ready attachment to the cardiac cells!

Sodium Cacodylate in the body of mammals is partially reduced and oxidized to cacodyl oxide, arsenates, and arsenites. It takes some time for this reaction to take place, however, and it is very doubtful whether such a reaction can be assumed to be complete when the compound is perfused for only a short time through the heart. Since the depression is quickly removed by perfusing normal Ringer's solution it certainly suggests an ionic action. A large number of the cacodylate ions are present and become loosely attached to the cells. When the Ringer's solution alone is again perfused the cacodylate is quickly displaced and the heart resumes its normal function. The same type of recovery is seen also after the cacodylate.

Sodium Arsenate is another compound which shows this general type of reaction. It differs chemically from Sodium Cacodylate only by having two OH ions in the molecule where the latter has two CH ions. The arsenate has a much more permanent action than the cacodylate due to the fact that it enters into a firmer combination with the cardiac protoplasm.

Arsenious acid is one of the most toxic compounds used. Arsenic in this simple form evidently unites into a stable compound with the protoplasmic molecule, and quickly leads to protoplasmic destruction. It is much more toxic than the arsenates from which it differs only by the absence of an oxygen atom.

Considering all the above compounds it appears that all the compounds which contain arsenic, whether organic or inorganic, are poisonous to the tissue of the frog's heart. The more complex the compound in which the arsenic is contained the less will be its toxic action.

A Comparison of the Action of Acid and Alkaline
Salvarsan on the Mammal.

It has long been known that when acids were introduced into the circulation there was a fall in blood pressure. This fall is due to the direct action of the acid upon the muscular coats of the vessels and upon the heart, causing a dilation. This fall in blood pressure is seen when acid saline is injected. That repeated injections of small amounts do not continue to cause a marked fall in blood pressure may be explained by the fact that the increased amount of fluid in the body, and the stimulating action upon the vasoconstrictor mechanism compensates for the dilating action which small amounts of acid have. When a larger amounts of the acid are introduced there is a toxic action upon the tissue in general. The blood pressure rapidly falls and the respiration becomes slower and slower until the animal dies. Whether death in this experiment was due to the action of the acid or to the Salvarsan cannot be definitely stated. Judging from the experiments upon the frog heart it would be supposed that the drug has a toxic action on the heart muscle and that death was due to the combined action of the Salvarsan and of the acid.

With alkaline solutions of Salvarsan the effect is very much like that seen with alkaline Saline. There is a general rise in blood pressure due to the constricting action of the alkali upon the heart and vessels and to an increase in the body fluid. From the experiments tried it could not be claimed that .2% solutions of alkaline Salvarsan injected intravenously into the mammal produced any more action than would follow the injection of an equal amount of the same strength alkaline solution.

I desire to thank Dr. Greene for valuable direction and assistance in the preparation of this work.

IV. SUMMARY OF THE RESULTS.

The general results presented in this paper are summarized as follows;

1. All compounds tested which contain arsenic in the molecule are more or less toxic to the heart tissue of frogs.

2. Acid Salvarsan solutions are more toxic to the tissues of the frog heart than a solution containing a similar amount of acid without the Salvarsan.

3. Neutral Salvarsan suspensions in strengths of .1% are depressing to the frog heart, but there is a tendency toward recovery after the drug is perfused.

4. The amount of alkali necessary to make neutral Salvarsan suspension go into alkaline solution is in itself toxic to the tissue of the frog heart.

5. Oxidized neutral suspensions of Salvarsan are very toxic to the frog heart tissue.

6. Sodium Cacodylate is rapidly depressant to the heart tissue of the frog, but the heart recovers rapidly after the drug.

7. Arsenious acid is rapidly and permanently toxic to the frog heart.

8. Accumulated doses of 140 mg. per kilo. body weight, fourteen times the therapeutic dose for man, of the .2% alkaline Salvarsan solution when injected into the veins of a dog do not cause death in three hours.

9. Accumulated doses amounting to 219 mg. per kilo. body weight of the acid Salvarsan solution, injected intravenously, cause death of the dog within thirty minutes.

PLATE I.

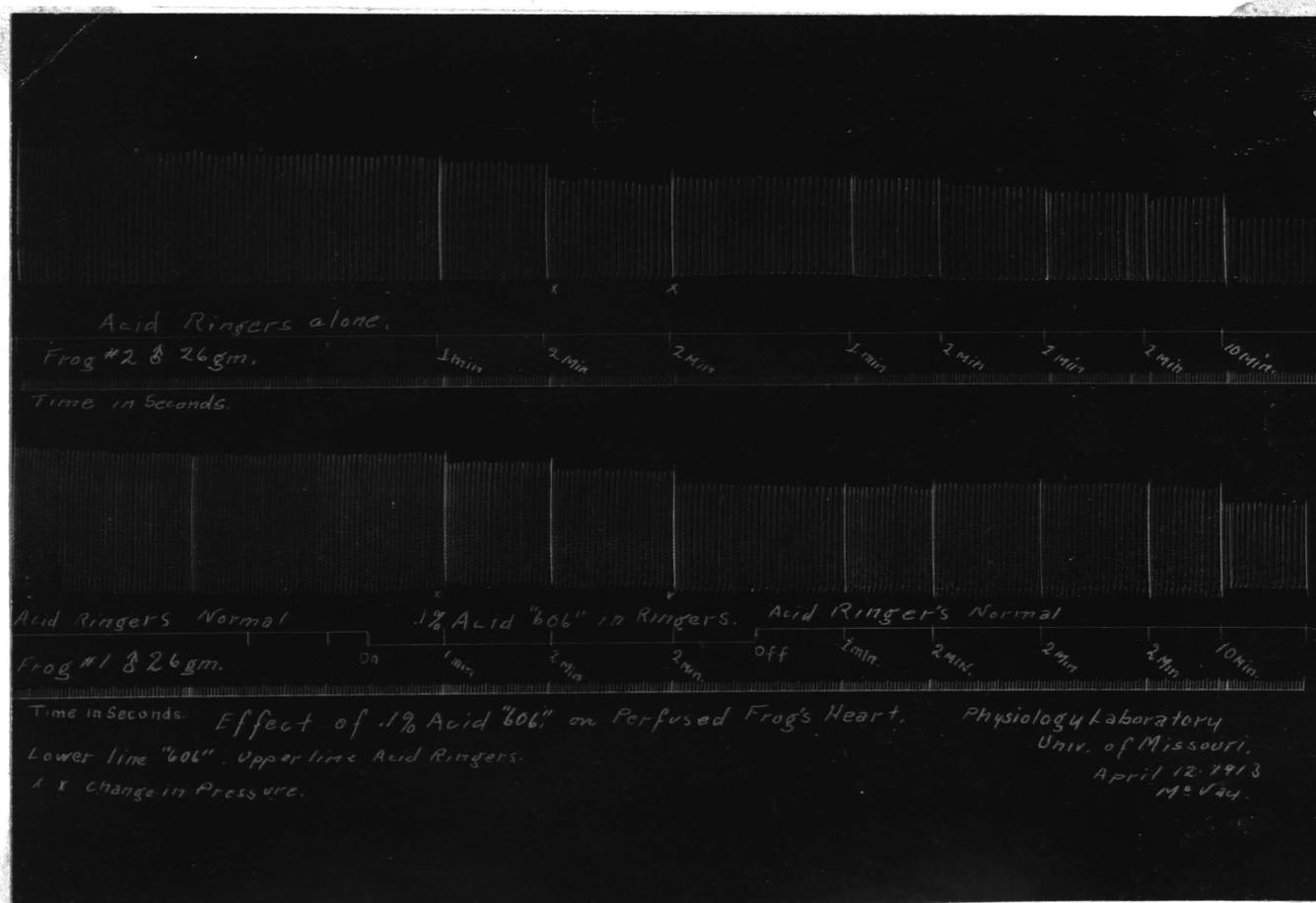


Figure No. 1. (Lower Line.) Shows Effect of .1% Acid Salvarsan upon the perfused frog heart. Shows ^{comparatively} little effect, except a gradual decrease in systole, and a loss of elasticity.

Figure No. 2. (Upper Line.) Shows Effect of Acid Ringer's solution containing same amount of acid as in Acid Salvarsan. It is very similar to the Acid Salvarsan tracing.

PLATE II.

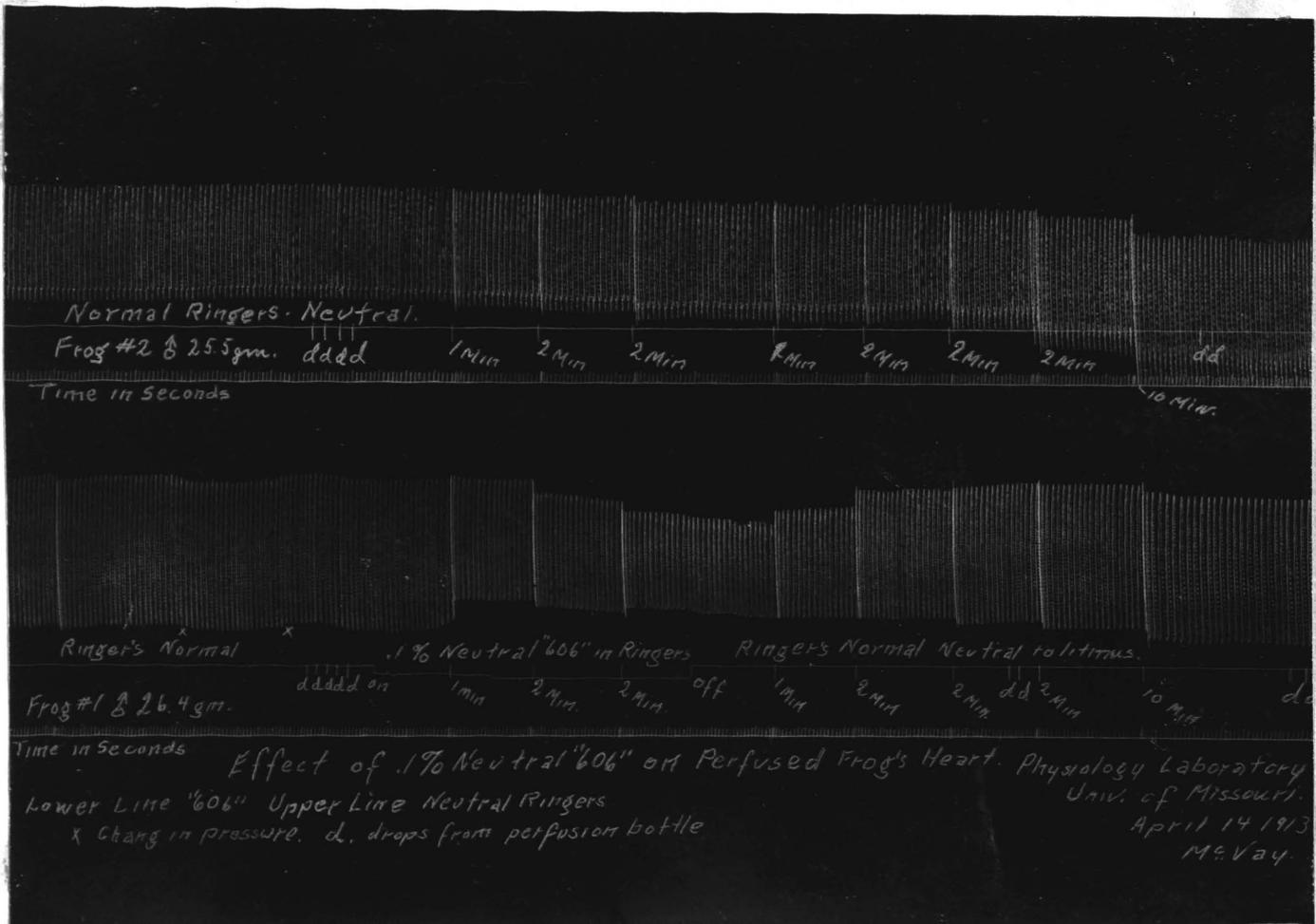


Figure No. 1. (Lower) Shows effect of .1% Neutral Suspensions of Salvarsan upon the Perfused Frog Heart. There is a rapid loss of elasticity, decrease in diastole, and later a decrease in systole. After the drug is "off" and normal Ringer's solution again perfusing there is almost complete recovery.

Figure No. 2. (Upper.) Shows effect of Normal Ringer's solution on the Perfused Frog Heart. Very little change except a relaxation of the whole heart.

PLATE III.

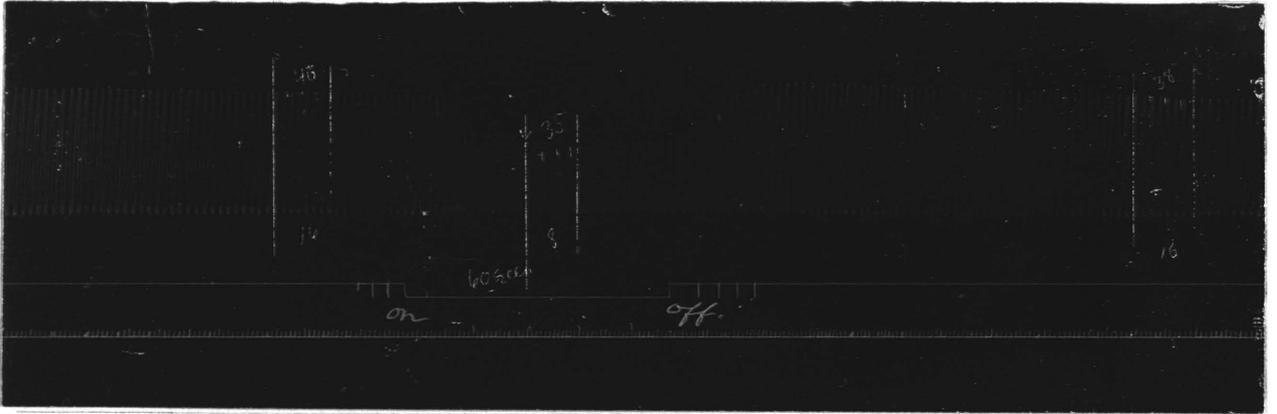


Figure No. 1. Showing Effect of Sodium Cacodylate on perfused Frog heart. Drug perfused for 60 sec. Amplitude decreased 50% Not much decrease in rate.

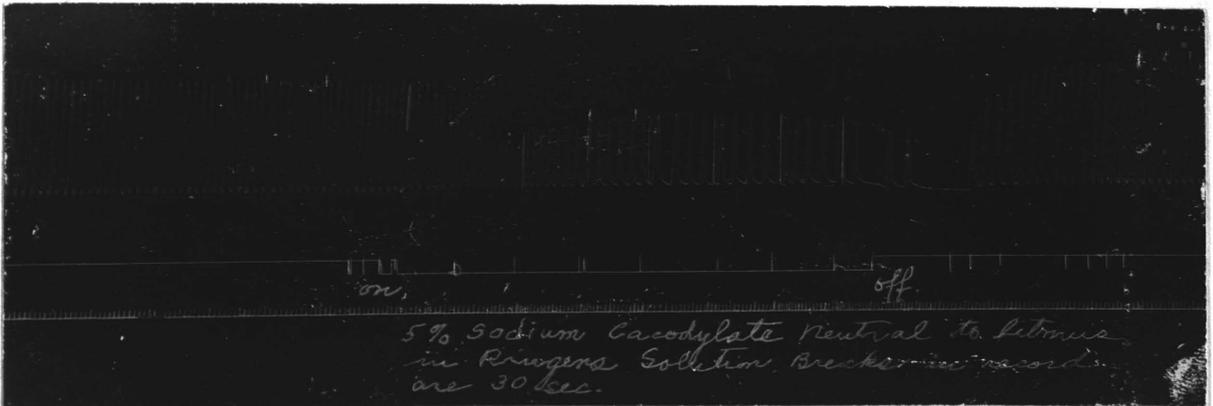


Figure No. 2. Showing Effect of Sodium Cacodylate on perfused frog heart. Drug perfused 308 sec. 50% decrease in Amplitude and Rate. 60 Sec. after drug "off" amplitude and rate have practically recovered.

PLATE IV.



Figure No. 1. Showing Effect of 5% Neutral Sodium Arsenate on the perfused frog heart. Drug perfused for 124 sec. Decreased not so marked as with Cacodylate.

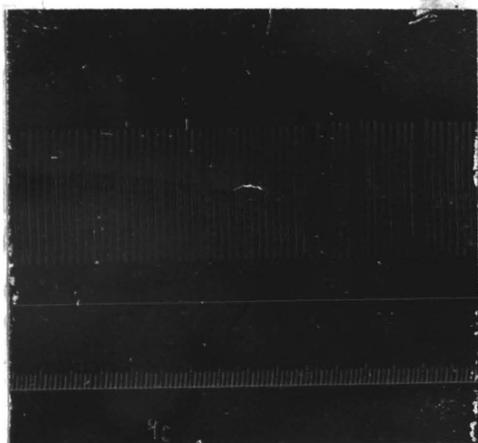


Figure No. 1 (b) 120 Sec. interval between this and Figure 1., above.

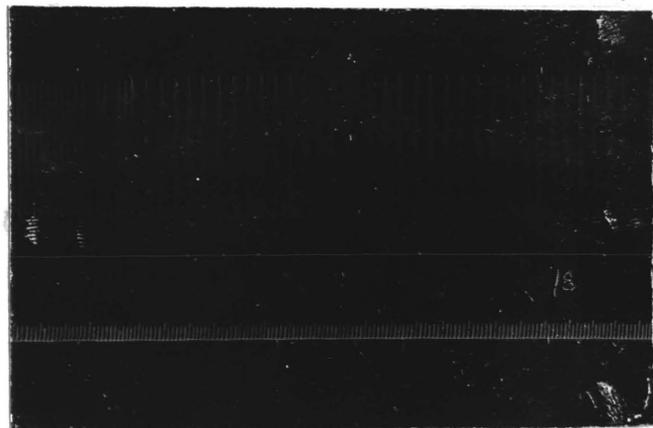
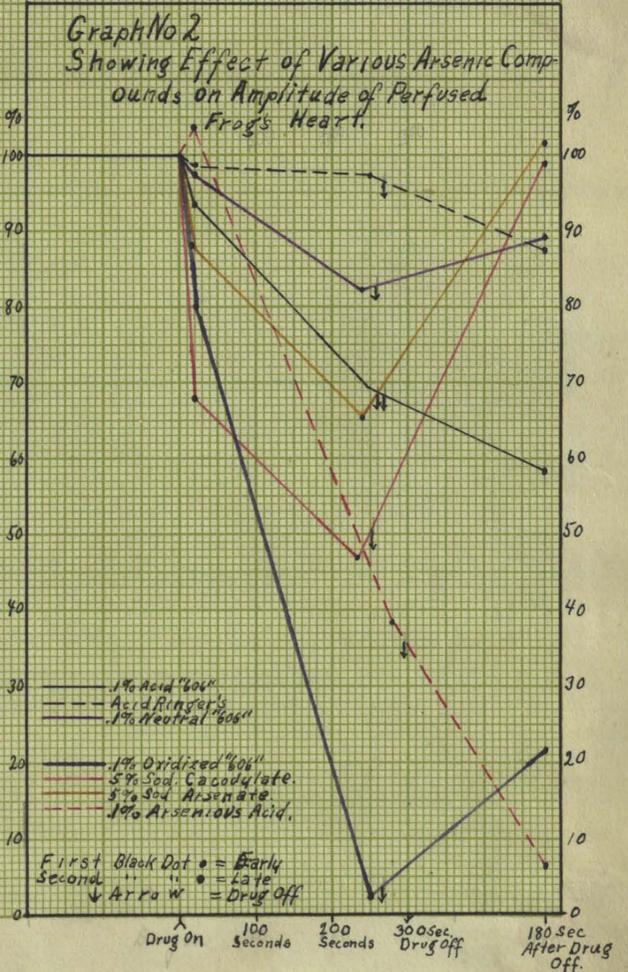
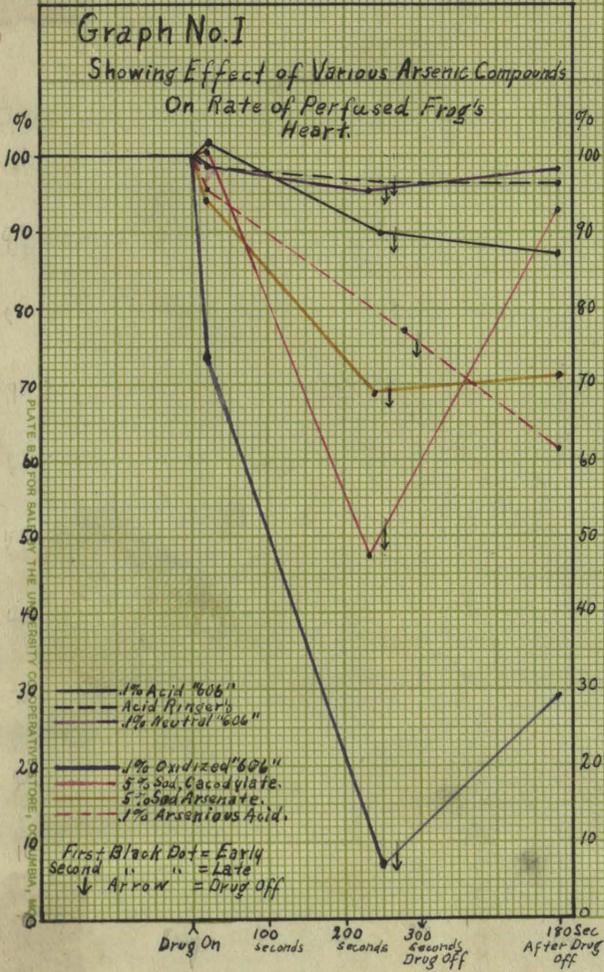


Figure No. 1 (c) 300 Sec. interval between this and Fig 1 (b). Rate very much slowed.

GRAPHS NUMBERS 1 AND 2.



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