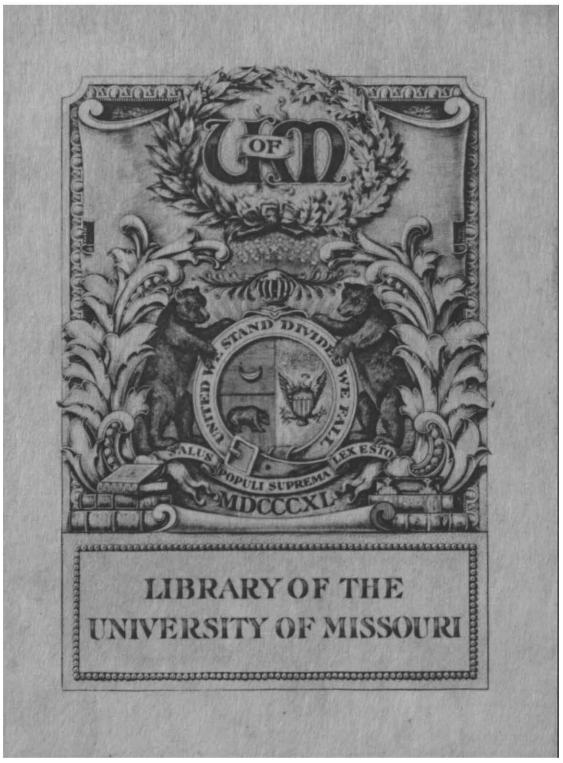

THE PHARMACOLOGICAL ACTION OF CERTAIN BROMIDE SALTS

by

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THE PHARMACOLOGICAL ACTION OF CERTAIN BROMIDE SALTS.

I Introduction.

The physiological interest in the bromides dates from 1828 when Ballard[6] first prepared potassium bromide. Altho the bromides have been subject to a great deal of investigation, little is known of the manner of their pharmacological action, i.e., whether the action is a direct effect or is produced indirectly through changes in metabolism. The early work, from 1828-1875, was characterized by investigations upon the general therapeutic actions and the action upon the reflex mechanism. Eulenburg and Guttmann[4] were the first to make a careful and systematic study. Krosz[6] in 1877 gave a careful review of the older literature. The study of the bromides took a new impetus in the latter part of the sixties which has continued to the present day. Rabuteau[13], in 1868, was the first to recognize a bromide retention in the animal organism after continued bromide feeding. This was of great interest, and contrary to all expectations, because other members of the halogen family, particularly the iodides, were eliminated with great ease.

Comparatively little work has been done in recent years upon the effect of the bromides upon the heart. Ringer[14] was among the first to prepare an artificial perfusing medium which was able to maintain the rhythmical contractions in a

frog heart for several hours. He found that the salts necessary to maintain the rhythm in the frog heart were the chlorides of sodium, potassium, and calcium. It was pointed out by Ringer(14) and later by others that the cation was responsible for this reaction. This conception led many to study the action of these cations combined with various anions upon the heart. Under the stimulus of this physiological view, the bromides have been studied quite indirectly. Loeb and his pupils have made the principal contributions in this line. Skeletal and smooth muscle was later studied in the same way. Loeb(8) pointed out that the bromide ion excites the muscular tissue more than the chloride ion, and also lowers its irritability in a shorter time. Stiles(15) showed that the bromide ion among others is able to produce rhythmical contractions in smooth muscle tissue. He successfully substituted an isotonic sodium bromide solution for the sodium chloride in a Ringer's solution. Lingle(12) found that a sodium bromide solution equimolecular with .7% sodium chloride, produced rhythmic contractions in an isolated strip of a turtle's heart, in a shorter time than did the chloride. And he also observed that the contractions were stronger, and of a longer duration than in a sodium chloride solution. Benedict(1) was able to substantiate Lingle's findings, and went a step further by substituting the calcium chloride by calcium bromide. The bromide solution was equimolecular to the chloride generally used. He found that the heart strips commenced after a shorter latent period, are more regular, and are of a longer duration. Benedict(1) accordingly concludes, that the bromide

ions are more stimulating than the chloride ions. La France[7] showed that sodium bromide in certain concentrations, one part of seventeen hundredths Normal solution in three parts of a Ringer's solution, prolongs the life of the heart, and increases the rhythm and the force of the contractions. Lussanna[11], on the other hand, found that sodium bromide decreased the irritability of the frog's heart. He perfused the frog's heart with a Ringer's solution and compared the irritability of the heart, by means of electrical stimulations, with a Ringer's and a Ringer's plus .18% sodium bromide perfusion.

Busquet and Pachon[2] studied a number of potassium salts on the heart of a rabbit and found potassium bromide more toxic than many other salts, such as the chlorate and organic preparations. Potassium chloride falls in the same group with the bromide, but is perhaps less toxic. They found no depression with sodium bromide. They conclude that the intensity of toxicity depends upon the dissociation coefficient of the salts. Loeb[9] studied the effect of various salts upon certain marine animals. He determined that the center of a Gonionemus bell can contract in a sodium bromide solution equally as well as in a sodium chloride solution.

The purpose of the work on which this paper is based has been to study the effects of some of the bromide salts upon the isolated heart of a frog as revealed by substituting the bromides of potassium, sodium, and calcium in a Ringer's solution. These experiments on the heart have been supplemented by a study of the histological changes which occur in the Purkinje cells of the cerebellum of the cat after prolonged bromide feeding.

II. Materials and Methods.

1. Animals Used.

The animals used in these experiments were the frog for the perfusion experiments and the cat for the nerve experiments. The frogs were of the ordinary leopard variety, Rana Pipiens. Young and active cats were chosen for the nerve experiments to insure normal Purkinje cells.

2. Salts Used.

The salts used were, potassium chloride, sodium chloride, calcium chloride, sodium bromide, potassium bromide and calcium bromide. The chemically pure salts were used but none were re-tested as to their purity. The crystalline calcium chloride was used instead of the fused salt. A stock solution of this salt was prepared and its calcium content determined gravimetrically. The Ringer's solution which was always used for the normal perfusing medium, was made up in the following composition,

sodium chloride	.73%
potassium chloride	.03%
calcium chloride	.026%

A stock solution was prepared for each salt and of such concentration that 100 c.c. of the stock sodium chloride, and 10 c.c . of the stock potassium and calcium chlorides, respectively, contained the required amounts of these salts in one liter of Ringer's solution. In making up the solutions the sodium chloride was measured in an ordinary graduated cylinder, the potassium and calcium chlorides were measured in a burette graduated in tenths of a cubic centimeter, and the required amount of distilled

water was added to make the desired concentration of one liter. This constituted a normal Ringer's solution. The bromide solutions were prepared in the same manner. All of the bromide solutions were equimolecular with the corresponding chloride solutions. The following concentrations were used,

sodium bromide	1.29%
potassium bromide	.048% and 1.49%
calcium bromide	.042%

In the nerve experiments, sodium bromide was used in a concentration of 12.9%, and potassium bromide as the pure salt.

3. Perfusion Method.

The method of perfusion of the frog heart used in these experiments was the laboratory method which is described in detail in Greene's Experimental Pharmacology, pages 68 and 69. The essential steps,however, will be described here.

A frog was pithed, its thorax opened,and a cannula introduced into the ascending vena cava. This was a four way cannula, which was first described from this laboratory by Gibson and Schultz (5). It was provided with an open pressure tube, placed at the junction of the two arms. This tube served both as a pressure gauge and as an overflow. The arms of the Y-shaped cannula were connected by a 4 mm black rubber tubing with two Mariotte's pressure bottles. A T-tube was placed on each side between the bottles and the cannula so that the drug could be removed more readily from the pressure bottle without changing the pressure. The normal Ringer's solution was always placed in one bottle, the right, and the drug was placed in the other, the left. The heart was anchored by a tiny supported clamp which was fastened on the conus arteriosus at the bifurcation of the

vessel. This support firmly anchored the ventricle. A small V-shaped slit was cut into the conus arteriosus above the anchor clamp, to produce a ready outlet for perfusion fluid. A small S-shaped platinum wire is then hooked into the apex of the ventricle and was connected by a thread to a light straw lever, which recorded the heart contractions upon a smoked cylinder. The magnification of this lever was 1 : 9. A second's magnet or a Jaquet's chronograph recorded the speed of the drum. After the cannula was tied into place, the normal Ringer's perfusion was commenced. As soon as the heart rate and the amplitude of the contractions became regular, the Ringer's solution was cut off by means of a pinch cock and at the same instant the drug was allowed to perfuse into the heart. The time of the perfusion was recorded upon the record. The drug was tested at successive intervals. A toxic drug was tested for but a short time, whereas a drug having a slight action was allowed to perfuse for longer periods of time. In some of these tests the drug perfused for one and a half hours. There was no set rule as to how long a drug should perfuse. Usually, it was left to perfuse until a change was noticeable. When a change occured the normal Ringer's solution was again passed thru the heart to determine whether the heart would recover from the drug effect. It is therefore evident that an experiment upon a single frog could cover a large series of tests, especially when the drug action was slight. The total duration of an experiment was from two to ten hours. At the end of such a long experiment the rate and amplitudes were usually very much decreased. Such a heart, however, could still be used for further tests, as the rates and amplitudes just before the drug was injected were used as a new normal. After the record was completed,

changes in rate and amplitude were studied. The heart rate was counted for a small interval of time, ten seconds, and was then converted into the number of contractions per minute. The amplitude of the contractions was measured in millimeters. These measurements were recorded and tabulated.

4. The Method Used in the Bromide Feeding Experiments.

The cat was used to study the action of the bromides upon the central nervous system. The plan followed was to intoxicate the cat and retain it in profound depression for a long period of time. The drug was administered either in gelatin capsules or by means of subcutaneous injections. Observations were made each day as to physiological symptoms of depression. After the animals were so intoxicated that their death was expected within a day, they were killed with ether. The cerebellum was removed and sections were taken from the vermis and the lateral lobes. These were placed in a fixing solution, which consisted of 5% of formalin in a saturated solution of mercuric chloride. The sections were then passed thru the graded and iodized alcohols and were imbedded in paraffin. The thickness of the sections was 5 μ. These sections were stained in erythrosin and a saturated solution of toluidin blue and mounted in balsam.

III. Experimental Results.

A. On the Heart.

1. Pure Sodium Bromide Solution in Comparison with Sodium Chloride Solution interpreted thru the Effect on the Rate and Amplitude of the Frog Heart.

In this series of experiments, it was attempted to determine the action of the bromide ion by making a direct comparison between the physiological saline solution with equimolecular sodium bromide solution which contains the same base. In such a solution, the dissociation coefficient constants of the two salts are so nearly the same that similarity of pure physical action may be assumed. The one crucial difference is the replacement of the chloride ion by the bromide ion. Any difference in action must, accordingly, be attributed to either of two factors, (1), to the absence of the chloride ion, or (2), to the presence of the bromide ion.

It was found that the effect of the perfusion of sodium bromide on the rate and the amplitude of the frog heart is indeed closely comparable to that of a pure solution of sodium chloride. However, the sodium chloride action is characterized at first by a slight rise in irritability of the heart as shown by the increase in the amplitude of the contractions. The later action is followed by a gradual decrease in tone. This effect of sodium chloride has been described in detail in the literature. The chloride ions were entirely replaced by the bromide ions in this series of experiments, yet there was no marked and fundamental difference in the reaction of the heart except for a slightly

greater depression in the later stages of the perfusion. This can be illustrated in one of the typical experiments. Table I.

The changes in the heart rate and in the amplitude of the contractions are indeed slight, as in the majority of the experiments of this series, but they are constant and are to be relied on as determinative for the point at issue. In test No.1 the rate was increased under the influence of the sodium chloride solution,and the amplitude was decreased. In test No.2 the rate was only slightly increased at first and then became normal. Immediately afterward, when sodium bromide was substituted the rate was at first increased slightly more than in the second test with sodium chloride, but later it became less than the normal. The amplitude of the contractions was decreased in all cases, but it recovered quickly after the heart was washed out with the Ringer's solution. Plate I, shows the gradual decrease in the amplitude of the contractions, from which the heart recovered five minutes after the drug was removed.

Table VI shows the total averages of all the tests that were tried in this series. The facts presented in the column which gives the average percentage changes during the sodium bromide perfusion, also show that the bromide ion is more stimulating on the heart rate than is the chloride ion. And in the following column of the table, which gives the percentage changes between the rate before the drug was used and the rate after the drug was removed, it is shown that the bromide rates are decreased more than are the chloride rates. The changes in the amplitude of the contractions are for the most part the same in the two solutions. Table VI however shows a marked average decrease with the chloride

perfusion. This is not quite typical and was produced by several readings falling unusually low, which probably should not have been computed in the averages.

Apparently, there is little difference between the chloride and the bromide ion, in their ability to sustain the heart contractions. The general effect is the same for both solutions. But that the action is not identical is shown in the minor differences, the slightly greater increase in rate followed by the slightly greater depression with the bromide perfusion than with the chloride perfusion.

Benedict[1] and also Lingle[12] reported that the sodium bromide stimulates the amplitude of the contractions of turtle heart strips. This does not seem to be the case with the contractions of the frog heart during the perfusions. Lussanna[11], who tested the irritability of the heart electrically, found that the sodium bromide solution of .18%, equivalent to .1% sodium chloride, diminished the irritability of the heart to stimulations more than did a Ringer's solution, and also more than occured in a Ringer's solution to which .7% sodium chloride had been added. He accordingly concluded that the bromide ion diminishes the irritability of the frog heart to electrical stimulation slightly more than does the chloride ion. He did not, however, make the crucial test as between the irritability in pure sodium chloride and sodium bromide solutions respectively. It is now well established that living organs perfused with a Ringer's solution maintain a more normal irritability than when sodium chloride alone is used.

My tests show that the bromide ion is, at first, more stimulating to the heart rate than the chloride ion and that it lowers the cardiac irritability in a shorter time. This agrees with Loeb's (8) findings of the action of the bromides on skeletal muscle. Stiles (15) working on smooth muscle, showed that the sodium bromide was stimulating, but he did not think that the tissue became exhausted any sooner than it did with the chloride solution. It is therefore evident that all these observations on the different types of muscular tissue are in substantial agreement, and that my observations upon the frog heart are also in accord.

2. <u>The Effect of Potassium Bromide Solution on the Rate and Amplitude of the Frog Heart</u>.

The tests in this series attempted to determine whether potassium bromide and potassium chloride stood in the same relation to each other as did sodium bromide to sodium chloride. A comparison between the potassium salts is rendered difficult on account of the toxic effect of the potassium base which is apt to overshadow the small differences shown to exist between the anions in the last series. However, to make a comparison between the potassium bromide and the chloride, it is essential that the physical constants remain the same as in the sodium bromide and the chloride. Accordingly, both potassium salts were made equimolecular with sodium chloride .73%. This concentration is exceedingly toxic to the ventricle of the heart, as has been established for a long time. As small doses as .06% of potassium chloride are able to produce great irregularities in the frog heart. The isotonic concentrations used were .93% potassium

chloride and 1.48% potassium bromide solutions. It need not be emphasized that any marked action obtained with these concentrations will be due largely to the potassium ion, provided that this concentration reaches the heart. In order to reduce the potassium effect the perfusion was allowed to flow very slowly so that its action would be postponed.

Contrary to expectations, only a slight difference was observed in some experiments. The average result obtained, recorded in Table VI show that the rate at first was slightly increased with the potassium bromide solution and slightly decreased with the potassium chloride solution. In both cases, however, the end result was ventricular inhibition.

The amplitude of the contractions were in both cases decreased showing no appreciable difference between the two salts.

3. <u>The Effect of Substituting Sodium Bromide in Ringer's Solution on the Rate and the Amplitude of a Frog Heart.</u>

It has already been seen in section 1, that the sodium bromide can be substituted for the sodium chloride without producing any marked ill effects. It was hoped that in substituting sodium bromide for the chloride in a Ringer's solution, one would be able to determine the action of the bromide ion in an otherwise balanced solution. If the chloride ion is an indifferent ion and the bromide ion so depressant as is currently assumed, then the substitution of the bromide salts in the Ringer's solution ought to change it from a normal sustaining fluid to one that is characteristically depressant.

In the series of the experiments presented, it is hoped to analyze this problem.

The first substitution tested was the replacement of sodium bromide for the chloride in a Ringer's solution in equimolecular concentration. Such a solution according, to Stiles (15), retains about one fifteenth or 6% of the chloride ions present in the standard Ringer's solution. The remainder accordingly consists of bromide ions. In such a solution the physical constants are the same as are found in a Ringer's solution. Any difference in action between a substituted sodium bromide Ringer's solution and the standard Ringer's solution will have to be attributed to either of two causes, (1), to the absence of the chloride ion or (2), to the presence of the bromide ion.

To our surprise as in section 1, the frog heart showed no marked difference under the influence of sodium bromide in a Ringer's solution, which is clearly shown in a typical experiment recorded in Table II.

The action produced by the bromide ion is slight on the heart rate and in the amplitude of the contractions, but it seems that both the heart rate and the amlitude of the contractions are stimulated at first and later depressed. However slight this may be, it seems to be a constant property of the bromide ion on cardiac tissue. Table VI, which is based on experiments on eight frogs including some thirty tests, show this same general tendency.

Stiles (15) who worked with smooth muscle reported that a strip of the oesophagus " gave excellent contractions lasting twenty four hours " in a Ringer's solution in which the sodium chloride was replaced by the sodium bromide solution. At another

place Stiles said that the bromide ion increases the irritability of smooth muscle but he did not think that it was exhausted in a shorter time than when the chloride solution was used. As has already been mentioned above Loeb found that the bromide ion exites skeletal muscle at first and lowers its irritability in a shorter time than the chloride ion. This seems to be in perfect accord with my results and one certainly cannot say that the bromide ion is very unfavorable to cardiac tissue.

4. The Effect of Sodium Bromide Substituted in a Ringer's Solution without the Calcium Constituent of the Ringer's Solution, on the Heart of the Frog.

After it was found that sodium bromide substituted in a Ringer's solution had little effect upon the heart rate and the amplitude of the contractions of the frog heart, it seemed essential to determine whether the calcium cation in the Ringer's solution had any antagonistic influence on the bromide ion. A solution containing sodium bromide 1.29% and potassium chloride .03% was compared with a solution containing sodium chloride .73% and potassium chloride .03%. Before and after these two solutions were used, the heart was washed out with the Ringer's solution to bring the heart back to normal as it is an established fact that the calcium cation is neccessary for the force of the heart contraction. These solutions were not left on for a long time as the absence of the calcium cation of a Ringer's solution rapidly decreases the amplitude of the contractions to zero.

A difference between the two solutions could not be observed, except that the solution containing the sodium bromide showed a slight increase in rate followed by depression, whereas

the other solution containing the chloride showed only a slight depression in rate. The amplitudes of the contractions were for the most part the same in both solutions. They gradually decreased owing to the absence of the calcium ion. Table VI.

This therefore shows that the calcium ion has no antagonistic influence on the bromide ion on the heart rate of the frog.

5. The Effect on the Rate and Amplitude of the Frog Heart of the Sodium Bromide and the Potassium Bromide Substitution for their Corresponding Chloride Constituents in a Ringer's Solution.

After having substituted the sodium bromide in a Ringer's solution, without obtaining any unfavorable results on the heart of the frog, it was logical to carry this substitution a little further by also replacing the potassium chloride by the bromide in equimolecular amounts. This solution then contained the following constituents, sodium bromide 1.29%, potassium bromide .048% and calcium chloride .026%. The only difference between this solution and the solution used in section 2, is that the chloride ions have been slightly decreased and the bromide ions have been slightly increased. Accordingly, I did not expect to see a change remarkably different from that which was observed in section 2. Unfortunately there is only one experiment available in this series. In this experiment however twelve tests were made, most of which indicate one and the same general tendency. This experiment was of an eight hour duration. Since it shows typical results it is given in full. Table III.

All the tests taken were averaged and the percentage changes calculated for both the rates and the amplitudes. The

general tendency, previously described for the bromides is again shown, slight primary stimulation followed by extremely slight depression. After the drug was taken off the depression persisted, i.e. itwas not removed immediately. But it is clear that such a heart is recoverable as is seen in the amplitude changes of the twelveth test of Table III. Altho this experiment lasted eight hours, the heart still showed a fine series of contractions at the end of the experiment. It is also noteworthy to observe the absence of ahy marked or fundamental action on the heart, during the experiment, altho the perfusion periods had been comparatively long. This shows then as in the previous experiments that the action of the bromide ion is not remarkably different from the action of the chloride ion.

6. The Effect on the Rate and Amplitude of the Frog Heart of a Ringer's Solution Composed Wholly of Bromide Salts.

As a final and crucial test of this series all of the chloride salts in the Ringer's solution were replaced by the corresponding bromide salts. The results of this series were based on experiments on five frogs, representing a series of fifty eight individual tests, Table V. The perfusion in this series represented alternate tests of a bromide and a chloride Ringer's solution. It is quite apparent in Table V, that the action of the bromide ion is extremely slight. Several types of results were obtained. In experiment # 1 there was no stimulation in rate but only a slight depression. Experiments # 2 and # 3 show a slight stimulation in rate followed by depression as has been observed in previous experiments. And the last two experiments show a slight increase in rate without

the depression. An increase in the amplitude of the contractions has been observed only in few instances and it is not so pronounced as has been shown in the previous experiments. Experiment # 4 is given in detail in Table IV, as it is remarkable that after eight hours of alternate drug perfusion the rate is actually higher than at the beginning of the experiment. In this experiment the rate was increased 30% whereas when a chloride Ringer's perfusion is continued for the same length of perfusion time the rates are decreased ~~for~~ about 30%. This in itself shows the slight toxicity of the bromide ion.

In one experiment a bromide-chloride Ringer's mixture was prepared. This solution contained a one fourth bromide and three fourths chloride Ringer's solution. The rates and amplitudes were increased without secondary depression. This would seem to show that the results obtained in these experiments were largely due to ionic action.

Loeb (10) has introduced a theory that the ions of the blood salts form ion-proteins in the heart tissue. Nearly all of the above described results can be explained by this theory. First of all, one must conclude that the bromide ion is not very much unlike the chloride ion on account of the slight variation of the actions. In the case where sodium bromide was used in a pure solution the sodium ion, by the theory, tends to replace the calcium and the potassium ions of the heart proteins. Therefore the heart substance contains an increase of sodium proteins, which continues to contract rhythmically but with decreasing amplitude. Such a changed protein naturally will show different properties, just as much as when sodium combines with bromine to form the inorganic sodium bromide. Loeb showed

this very beautifully when he produced rhythmical contractions in skeletal muscle by placing the latter in a solution of sodium bromide or chloride, whereas the addition of potassium and of calcium salts inhibits the rhythmical contractions. The explanation is that there is a different ion-protein in skeletal muscle, when the sodium ion alone is present the irritability is increased, but when the potassium and the calcium ions replace the sodium ion a different protein is formed which does not contract rhythmically in skeletal muscle. The rhythmic property of cardiac muscular tissue is principally regulated by the cations, according to the current view, but there is no reason why the property of the cardiac tissue should be assumed to remain exactly the same when the anions of the heart have largely been replaced by the bromide ion. Since the reaction of the chloride and the bromide ions are essentially similar, the substitution of the chloride ion by the bromide ion would have little effect upon the ion-protein of the heart. It had been noted in the above experiments that there existed a primary increase followed by secondary depression in rate and amplitude. It is quite probable that during the anion displacement, when the chloride ions are in excess of the bromide ions the irritability of the tissue is increased, whereas when the chloride ions ions have been almost entirely replaced by the bromide ions the tissue becomes less irritable. If this is true one should expect that after the heart became largely a bromide protein and the bromide ions were gradually replaced by the chloride ions a point should be reached when the tissue became more irritable

on account of the excess of the chloride ions. But after the bromide ions have become entirely replaced the tissue should be less irritable than when both ions were present. This has really been observed in a number of cases. That is, one frequently sees an increased rate after the Ringer's solution is turned on which later becomes decreased. To test this relation more closely the bromide-chloride mixture was prepared with the result that no depression occured, showing that it takes a longer period to replace a sufficiently large number of the chloride ion to produce depression. All these facts seem to indicate that the bromide action is essentially ionic.

In the beginning of Chapter III it was hinted that the action of the bromide ions, on cardiac tissue, determined by substitution methods probably could be attributed to either the absence of the chloride ion or to the presence of the bromide ion. It has been seen above that the preponderance of bromide ions leads to slight depression and the preponderance of the chloride ions in the mixture leads to slight stimulation. One is led to believe that the bromide action on cardiac tissue cannot be attributed to one causation alone but must neccessarily be attributed to both the absence of the chloride ions, and the presence of the bromide ions. These are factors which are inseparable.

In conclusion, it is apparent that the bromide ion as such is far less toxic to the heart than has generally been supposed.

B. Experiments on the Central Nervous System.

Experiment have been performed also on the central nervous system. There are many methods in use to determine the action of drugs upon the nervous system. In this work two methods were used, namely, (1), the effect of the bromides on the external physiological reactions determined by direct observations on the intoxicated animal, (2), the effect of the bromides on the histological changes of the Purkinje cells of the cerebellum after a prolonged intoxication. Obviously, one and the same animal can be used for both methods. The cat was used for this purpose. The Purkinje cell was chosen for the histological study as Professor Dolley had already determined the normal physiological changes produced by functional activity i.e. stimulation. Recently, in contrast to this former work he studied depression, i.e. cessation of function. The experiments on the Purkinje cells in this series were performed under his direction.

It was the plan thruout the series of the experiments to prolong the depression as long as possible. The details of the experiment with potassium bromide are presented in the following protocol.

The Effect of Potassium Bromide Feeding on the General Physiological Reactions of the Cat.

Date	Weight of Cat in Grams.	Dose of KBr in Grams.	Notes.
Jan. 20.	3240	2	Bromide fed in 100 c.c. of milk per stomach tube, vomited.
Jan. 21.	3236	1.5	Bromide in gelatin capsules in three doses. No apparent symptoms.
Jan. 22.	3195	1.5	Vomiting, loss of appetite, general depression and muscular weakness.
Jan. 23.	2497	1.5	100 c.c. of milk per stomach tube. General depression more pronounced. Sharp loss of weight, pronounced weakness of hind legs.
Jan. 24.	2234	1.5	Vomiting, diarrhoea, drowsiness. Hardly able to retain equilibrium in the sitting position.
Jan. 25.	1946	1	Vomiting two hours after the dose was given. Fed 100 c.c. of milk per stomach tube. No change in symptoms.
Jan. 26.	1720	1	Fed 100 c.c. of milk per stomach tube. No vomiting, lies in stupor. Showed no nausea when drug was given. Less irritable to sensory stimuli,i.e. pinching, or touching cornea. Difficulty in retaining equilibrium when sitting. Staggers when walking.

Date	Weight of Cat in Grams.	Dose of KBr in Grams.	Notes.
Jan. 27.	1583	1	Vomiting, diarrhoea. Symptoms the same as on previous day, but more pronounced.
Jan. 28.	1520	.5	50 c.c. of milk per stomach tube. Depression more pronounced.
Jan. 29.	1203	-	100 c.c. of milk. Comatose, cannot get on legs.
Jan. 30.	1145	-	Coma. Body temperature low. When sufficiently stimulated it would attempt to walk. Clapping the hands would upset its balance while in a sitting position. Respiration and heart action slow and weak. The cat was killed with ether and samples of the cerebellum removed.

This experiment showed that potassium bromide is very toxic to the nervous system of the cat. The general symptoms are at first a general depression manifested by quietness on the part of the animal which later passes into poor coordination of the hind limbs, assumed to be a nervous disturbance. This loss of irritability gradually progresses forward, but the front legs never became as much affected as the hind limbs. The vomiting was probably produced reflexly by the local irritation of the concentrated salt in the stomach. The diarrhoea produced is probably a salt action. The respiration and the heart action is depressed. The loss of temperature is probably due to the slow heart. Krosz (6) has pointed out that the decrease of the heart rate, temperature, respiration and paresis of muscles and nerves is due to the potassium constituent of the salt, whereas the bromide constituent produces slight slowing of the heart rate and paresis at the nerve synapse. He was also of the opinion that the decrease of the temperature and respiration was contributed to by the slowing of the heart.

There was a marked loss of weight quite apparent in this experiment. It was therefore neccessary to determine whether this loss of weight was produced by a decreased nourishment of the animal or whether it was a manifestation of a potassium bromide interference with metabolism. Accordingly, a control experiment was performed in which a cat was fasted. But plenty of water was given. The weight of the animal was taken each day. This experiment lasted almost three times as long as the potassium bromide experiment. The fasting cat did not manifest any great weakness until the last day. But even then it had

no difficulty in locomotion: Neither was the loss in weight so marked as in the potassium bromide fed animal. See Table VII. This tends to show that potassium bromide produces changes in the metabolism so that more tissue substance is used up than in the fasting animal.

It was then neccessary to determine whether the changes observed in the potassium bromide fed animal were produced by the potassium ion or the bromide ion. Accordingly a cat was given subcutaneous injections of sodium bromide. The details are recorded in the following protocol.

The Effect of Sodium Bromide Injections on the General Physiological Reactions of the Cat.

Date.	Weight of Cat in Grams.	Dose of NaBr in c.c. of 12.9%.	Notes.
Mar. 25.	1295	1.3	Irritation at point of injection which passed away in a few minutes. Lively and playful.
Mar. 26.	-	5.2	No change in reaction.
Mar. 27.	-	2.6	Slightly less playful.
Mar. 28.	1352	5.2	Slightly more less playful.
Mar. 29.	1382	5.2	Refuses to play, loss of appetite, merely nibbles at food. Loud sounds do not startle it.

Date.	Weight of Cat in Grams.	Dose of NaBr in c.c. of 12.9%.	Notes.
Mar. 30.	1250	2.6	Vomiting one hour after dose; some paralysis of hind limbs which was most marked in the left leg. Twelve hours after dose the front legs show some paralysis.
Mar. 31.	1190	2.6	Vomiting two hours after dose; left eye partially closed. Poor coordination of leg movements. Can walk slowly but falls over when it attempts to turn.
Apr. 1.	1170	-	Animal found dead but was still warm. Samples of cerebellum taken.

It is quite apparent that the symptoms from sodium bromide to those produced by potassium bromide are quite similar. Vomiting was obtained altho the salt was not given per mouth. The potassium bromide fed animal showed a more marked depression than the sodium bromide animal, but the symptoms were essentially the same. From these physiological reactions it seems that potassium bromide is a more powerful depressant than sodium bromide. This was to be expected as it is known that potassium as such has a strongly depressant action. Potassium bromide therefore is doubly depressant.

Depression Changes in the Purkinje Cells of the Cerebellum.

Normal Purkinje cells were studied in order to compare the action of the bromides on this structure. In a section of the cerebellum of a normal cat, one generally finds that the majority of the Purkinje cells are in a resting stage. Such cells are characterized by having a nucleus plasma relation of 1 to 11. The cell wall of both the nucleus and the cytoplasm is regular. The cytoplasm shows distinct blue staining granules called chromatin. The nucleus contains red staining granules and a large blue karyosome when stained with toluidin blue.

The Purkinje cells of the cerebellum taken from the potassium-fed animal showed certain marked changes in the cell structure. The cytoplasm was cloudy and indistinct, having a slate colored appearance. Some cells show almost the entire absence of chromtin. Within the cytoplasm there are found pinkish staining bodies varying in size without a definite outline.

These were yolk bodies according to Professor Dolley's interpretation. The nucleus has become larger, thereby decreasing the nucleus plasma relation. Frequently one sees the chromatin accumulated along the nuclear wall. In most cells the karyosome was in the process of breaking up or was broken up in the more severe types. These are the main histological symptoms of depression observed in the Purkinje cells of the cerebellum.

The cells in the fasting and the sodium bromide-fed animals showed the same changes as in the potassium bromide-fed animal, except in a lesser degree. Potassium bromide seemed to produce the most vigorous depression changes, and sodium bromide produced the least. It was interesting to note that the sodium bromide-fed cat showed many cells which were near the normal, whereas the other cells were greatly depressed. These two types of cells were gathered in groups.

It is therefore apparent that the cerebellum was affected in all three experiments, yet only the bromides had showed any disturbances in physiological equilibrium. It seems then that the cells of the cerebellum are not necessarily responsible for the loss of equilibrium and motor incoordination, but that the incoordination of the legs is probably produced by a depression of function elsewhere in the nervous complex, possibly in the cortex itself.

My acknowledgments are due to Dr. C. W. Greene and Dr. D. H. Dolley for the many helpful suggestions in the course of this research.

IV. Summary.

The results of the work outlined in this paper may be summarized in the following statements.

1. Sodium bromide in a pure solution produces essentially the same changes in the frog heart as are produced by sodium chloride in a pure solution. There is, however, at first, a slightly greater increase in rate and amplitude followed by a relative decrease with the bromide salt.

2. Sodium bromide equimolecular with .73% sodium chloride may be substituted in a Ringer's solution without producing any marked changes in rate or amplitude of a frog heart. The variation that occurs consists of a slight increase at first followed by a later decrease in rate and amplitude of the contractions.

3. A Ringer's solution in which the chlorides have been wholly replaced by the bromides will maintain rhythmical contractions in the frog heart at least two hours, and more than eight hours with an alternate Ringer's perfusion. There is a slight increase, at first, followed by a decrease in the rate. There may or may not be an increase in the amplitude.

4. A Ringer's mixture containing a one third bromide Ringer's solution and three fourths chloride Ringer's solution tends to increase the rate without after depression.

5. Potassium bromide and sodium bromide reduce functional activity of the nervous system of the cat, ~~of which~~ the former acts with greater intensity.

6. The general symptoms of the bromide intoxication are general depression, reduced reflexes, progressive paralysis commencing in the hind legs, vomiting, diarrhoea, drowsiness and death.

7. There is a loss in weight in a potassium bromide-fed animal, sharper than in either the sodium bromide fed or a fasted animal.

8. The Purkinje cells of the cerebellum show a marked depression, judged histologically, upon bromide feeding and upon fasting. Potassium bromide produced the most marked changes whereas sodium bromide produced the least.

TABLE I.

EXPERIMENT 3/14 b. SHOWING THE REACTION OF THE HEART OF THE FROG TO PERFUSION WITH PHYSIOLOGICAL SALINE IN COMPARISON WITH ISOTONIC SOLUTIONS OF SODIUM BROMIDE.

Test No. and Drug Strength.	Before the Drug.		During the Drug.		After the Drug.	
	Rate per minute.	Ampl'de in mm.	Rate per minute.	Ampl'de in mm.	Rate per minute.	Ampl'de in mm.
1. NaCl .73%	39	5	40 to 43	4	41	4
2. NaCl .73%	40	4	41 to 40	3	40	4
3. NaBr 1.29%	40	4	42 to 36	3	37	3

TABLE II.

EXPERIMENT 2/25 a. SHOWING THE REACTION OF THE HEART OF THE FROG TO PERFUSION WITH A SODIUM BROMIDE SUBSTITUTED RINGER'S SOLUTION WITH THE STANDARD RINGER'S SOLUTION.

Test No. and Drug Strength.	Before the Drug.		During the Drug.				After the Drug.	
	Rate per minute.	Ampl'de in mm.	Rate per minute.		Ampl'de in mm.		Rate per minute.	Ampl'de in mm.
			Early.	Late.	Early.	Late.		
1. NaBr 1.29% NaCl .03% $CaCl_2$ .026%	48	14	42	42	13	13	42	13
2. " "	42	13	43	36	15	13	40	12
3. " "	40	16	40	35	17	14	35	12

TABLE III.

EXPERIMENT 1/29. SHOWING TABULATED RESULTS OF THE CHANGES IN RATE AND AMPLITUDE BEFORE, DURING AND AFTER THE DRUG WAS PERFUSED. THE DRUG USED WAS NaBr 1.29%, KBr .048%, $CaCl_2$ .026%. NORMAL SOLUTION WAS RINGER'S.

		Rate per Minute.				Amplitude in mm.			
Test No.	Time of Drug in Sec.	Before the Drug.	During the Drug. Early.	Late.	After the Drug.	Before the Drug.	During the Drug. Early.	Late.	After the Drug.
1	80	54	54	54	49	15	15	15	16
2	60	49	52	52	53	16	16	16	17
3	500	53	53	42	41	17	17	17	17
4	450	36	37.5	31.5	29.5	17	20	18	16
5	90	28.5	30	30	28	17	20	20	16
6	80	28.5	30	30	28.5	16	20	20	16
7	180	29	33	33	35	16	19	19	16
8	700	28	31	30	27	16	19	14	15
9	650	26	28	28	26	14	14	10	11
10	200	21	20.5	29.5	17	8	5	5	2
11	250	17	18	18	16	8	7	2	1
12	600	16	17.4	15	13	10	11	2	2
	Recovery				18				11
Average		32.1	33.6	31.9	29.3	14.8	15.2	14	12.1
Percentage Change.			+4.6%	-.6%	-8.7%		+2.7%	-5.4%	-18.%

TABLE IV.

EXPERIMENT 4/19. SHOWING TABULATED RESULTS OF THE CHANGES IN RATE AND AMPLITUDE BEFORE, DURING AND AFTER A BROMIDE RINGER'S PERFUSION. NORMAL SOLUTION WAS RINGER'S.

		Rate per Minute				Amplitude in mm.			
Test No.	Time of Drug in Sec.	Before the Drug.	During the Drug. Early.	During the Drug. Late.	After the Drug.	Before the Drug.	During the Drug. Early.	During the Drug. Late.	After the Drug.
1	540	54	54	54	51	15.5	16	14	17
2	1000	48	48	42	42	19	16	14	16
3	2100	48	48	54	51	19	15	14	18
4	1480	51	48	48	41	17	16	13	14
5	5300	41	42	48	51	16	15	12	14
6	2200	42	42	48	45	18	16	10	11
7	2500	40	48	46	51	15	15	9	11
8	650	48	51	63	63	12	12	5	6
9	3800	51	51	60	65	10	7	2.5	2
10	500	69	69	69	72	2	2	.5	.5
Average		49.2	50.1	53.2	53.2	14.6	13.4	12.8	13.9
Percentage Change.			+1.8	+8.1	+8.1		-8.2	-12.1	-11.6

TABLE V.

TABULATED RESULTS OF EXPERIMENTS WITH A BROMIDE RINGER'S SOLUTION SHOWING THE AVERAGE PERCENTAGE CHANGES IN RATE AND AMPLITUDE UPON THE FROG HEART, BEFORE, DURING AND AFTER ITS PERFUSION.

Experiment No.		I	II	III	IV	V	
Date.		4/11/13	4/12/13	4/15/13	4/19	4/22	Average.
Rate Before the Drug per minute.		31.8	43.6	36.3	49.2	52.7	42.7
Rate During the Drug.	Early	31.4	44.6	37.2	50.1	55.7	43.8
	Late	29.1	36.8	27.7	53.2	59.2	41.2
Rate After the Drug.	Early	32	42.6	35.8	53.2	55.	43.7
	Late	31.5	36.9	36.3	51.1	51.6	41.5
Ampl'de Before the Drug in mm.		18.4	14.9	14	14.6	12	14.8
Ampl'de During the Drug.	Early	18.2	13.2	13.7	13.4	12.2	14.1
	Late	12.6	9.5	10.1	12.8	10.2	11
Ampl'de After the Drug.	Early	14.	10.8	12.9	12.9	11	12.3
	Late	17	13.3	13.6	13.9	12	13.9
% Change in Rate During.	Early	+1.2	+2.2	+2.4	+1.8	+5.7	+2.2
	Late	-8.4	-15.5	-23.7	+8.1	+12.3	-5.4
% Change in Rate After.	Early	+.6	-2.2	-1.3	+8.1	+4.3	+1.9
	Late	-.9	-15.3	0	+3.9	-2.	- 2.8
% Change in Ampl'de. During.	Early	-1.	-1.	-2.1	-8.2	+1.6	-2.1
	Late	-31.5	-36.2	- 28.	-12.1	-15.	-24.5
After	Early	-25.9	-27.5	-8.	-11.6	-8.3	-15.8
	Late	-7.9	-10.7	-4.3	- 4.8	0	- 5.5

TABLE VI.

SHOWING GENERAL AVERAGE CHANGES ON RATE AND AMPLITUDE PRODUCED BY VARIOUS BROMIDE SUBSTUTUTIONS IN A RINGER'S SOLUTION ON THE FROG HEART.

Heart Rate per Minute.

Drug Used.	Before the Drug.	During the Drug.	After the Drug.	During % Change.	After % Change.
NaBr 1.29%	40.6	40.8	36.9	+ .7	-9.1
NaCl .73%	45.5	44.8	43.8	-1.5	-3.9
KBr 1.48%	32.3	34-0	0-13	+5.2 to-100	-100 to-59
KCl .93%	49.	45_0	0-46.5-8	to-100	-100 to -7.1
NaBr 1.29% KCl .03% $CaCl_2$ .026%	31.7	32.7	26.6	+3.5	-8.9
NaBr 1.29% KBr .048% $CaCl_2$ .026%	32.1	33.6	29.3	+4.6	-8.7
NaBr 1.29% KBr .048% $CaBr_2$ .042%	42.7	43.8	43.7	+2.2	+1.9
NaBr 1.29% KCl .03%	32.2	29.2	31.5	-9	-2.1
NaCl .73% KCl .03%	34	35.3	34.3	+ 4	+.8

TABLE VI (CON'T)

Amplitude in mm.

Drug Used.		Before the Drug.	During the Drug.	After the Drug.	During % Change.	After % Change.
NaBr	1.29%	11.1	10.8	10.6	-2.7	-4.5
NaCl	.73%	13.1	11.1	8.6	-15.	- 34.
KBr	1.48%	15.	13.5-0	0-13.5	-10to100	-100to10
KCl	.93%	12.5	11.5-0	0-11.5	-8to100	-100to 8
NaBr KCl $CaCl_2$	1.29% .03% .026%	10.3	10.7	8.5	+3.5	-15.7
NaBr KBr $CaCl_2$	1.29% .048% .026%	12.7	10.5	6.8	-17.3	-46.4
NaBr KBr $CaCl_2$	1.29% .048% .042%	14.8	14.1	12.3	-2.1	-15.8
NaBr KCl	1.29% .03%	18.	12.5	16.5	-30.5	-8.3
NaCl KCl	.73% .03%	12	11.3	10.8	-9	-10.

TABLE VII.

COMPARATIVE LOSS OF WEIGHT, AS OBTAINED BY BROMIDE FEEDING AND FASTING.

Day of Experiment.	Percentage Loss During KBr Feeding.	Percentage Loss During NaBr Feeding.	Percentage Loss During Fasting.
5th Day.	31.	3.3	9.
6th Day.	46.	8.	12.
10th Day.	64.	-	19.

PLATE I.

Figures 1 and 2.

Experiment 3/14. Time trace marks seconds. Arrow pointing upward shows where the drug was put on and the arrow pointing downward shows where the drug was removed. To illustrate the gradual decrease in amplitude with a sodium bromide perfusion isotonic with .73% sodium chloride solution.

Figure 2. Illustrates the recovery four minutes later.

Figure 2.

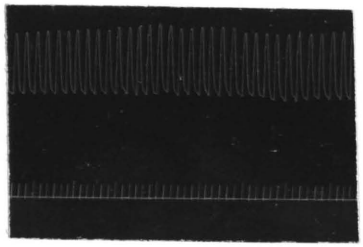

PLATE II.

Figure 1. Experiment 4/22.

To illustrate the gradual decrease in the amplitude due to the bromide perfusion, and recovery in a normal Ringer's perfusion. It also illustrates a slight increase in in rate. The last twenty seconds of the tracing show recovery seven minutes after the bromide solution was removed.

Time trace in seconds. The mark at Br indicates where the bromide Ringer's perfusion was commenced. The mark at Cl indicates where the chloride Ringer's solution was put on. The figures above the tracing indicate minute intervals.

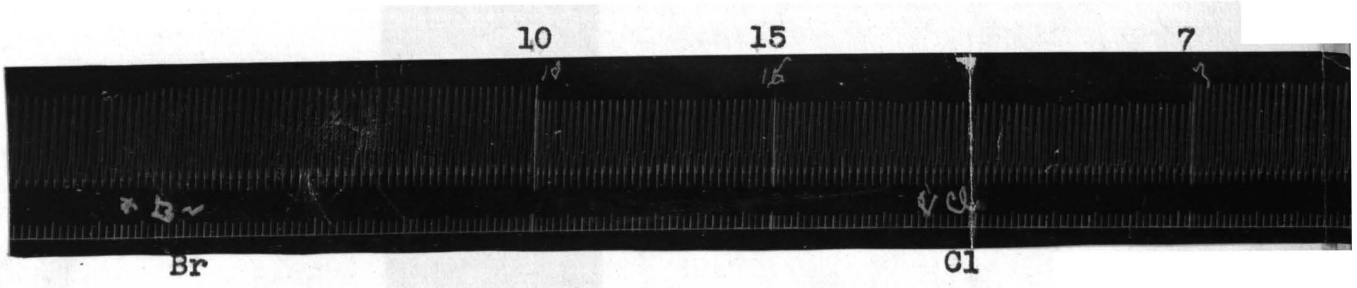

PLATE III.

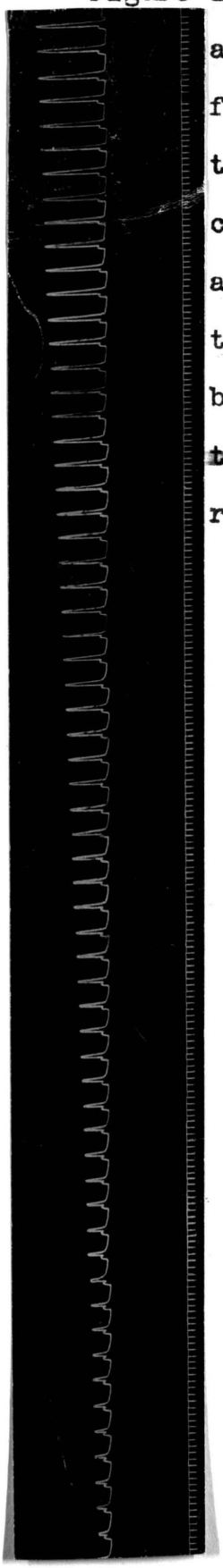

Figure 1. Shows the rapid recovery of amplitude in a frog heart when perfused with a normal Ringer's solution after a perfusion of a solution containing NaBr 1.29%, KBr .048% and $CaCl_2$ .026%. The time of this tracing was eight hours after the beginning of the perfusion. Note the strong contraction in the recovery.

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