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# THE INFLUENCE OF CERTAIN DRUGS UPON THE TOXICITY OF ACETANILIDE AND ANTIPYRINE

By

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# THE INFLUENCE OF CERTAIN DRUGS UPON THE TOXICITY OF ACETANILIDE AND ANTIPYRINE.[a b]

By Worth Hale,

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*Summary.*—The results of the experiments which are recorded in this bulletin indicate that the deleterious effect of acetanilide upon the heart is very imperfectly antagonized by caffeine. They show that so far as the contractile power of the heart is concerned the antagonism is very weak or even not present at all, and in some cases that the two drugs seem to combine to depress the heart to a greater degree than acetanilide does when given alone. The heart rate, on the other hand, is not slowed after a mixture of acetanilide and caffeine are given, as is the case when acetanilide is given alone, and the decreased heart rate following the exhibition of the former alone tends to become normal upon the subsequent injection of caffeine. Caffeine is further shown to increase the toxicity of acetanilide mixtures when given to the intact animal, and in certain experiments this is not only a summation effect but even some synergistic action is to be observed.

Sodium bicarbonate, quite in contrast, appears to markedly lessen the poisonous effects of acetanilide upon the heart, which is shown to be less depressed than when the alkali is not given. The lessened toxicity also appears in the experiments upon the intact animal, in which case acetanilide when given alone proved to be far more toxic than in mixtures with the alkalies.

The combinations of the alkaloids of the morphine group also increase the toxic effects of acetanilide, while mixtures containing salicylic acid and the bromides seem not to alter its poisonous effects in any way.

[a] Submitted for publication April 29, 1909.

[b] The experiments recorded in this bulletin were suggested by some preliminary experiments carried out by Doctor Hunt, in February, 1908, his results indicating that caffeine increased the toxicity of both acetanilide and antipyrine very materially.

These experiments indicate, therefore, that caffeine increases the danger of acetanilide mixtures, as do also the opium alkaloids. On the other hand, in these experiments upon the lower animals sodium bicarbonate appears to be a fairly good antagonist and would possibly be of use in acetanilide poisoning in man.

In Part II it is shown that caffeine is not materially antagonistic to the circulatory depression following antipyrine, but that it prevents the slowing in the heart rate. In the experiments upon the intact animal the mixtures of the above drugs were invariably more poisonous than antipyrine alone. In contrast, and as in the acetanilide experiments, sodium bicarbonate was somewhat antagonistic to the heart effect of antipyrine, but when given to the intact animal it did not seem to lessen the toxicity of the antipyrine in any degree.

## HISTORICAL.

The year 1884 has become notable in the history of therapeutics on account of the introduction of antipyrine, the first of a large series of drugs as agents for the reduction of excessive temperature. This was followed very shortly (in 1886) by the second member of the antipyretic group, acetanilide, and in quick succession a large number of similar bodies appeared, all of which possessed in a general way the same action. The powerful influence of these drugs upon high temperature made them all extremely popular, and especially so since up to this time the other drugs used in the relief of fever were not only uncertain in their action but were very much less powerful.

Although having been preceded by antipyrine by two years, acetanilide quickly outranked it in popularity, and it has never been supplanted to any great extent by any of the large number of antipyretics appearing later. Many cases of poisoning appeared almost from the first, due not only to the enormous doses that were prescribed, but also to the inherent poisonous properties of these drugs. This, however, did not seem to detract from its popularity, which seems to have been based partly upon the idea that it was less poisonous than antipyrine,[a] and certainly less poisonous than other drugs of the series excepting acetphenetidin and partly upon the fact that it was somewhat more efficient,[b] at least in the doses used, and thought to be necessary. The chief reason for its greater popularity, however, seems to have been neither its comparative smaller degree of toxicity nor its greater efficiency, but its comparative cheapness.[c]

The immediate popularity of this group of compounds arose from their decided antipyretic action, but it was quickly observed that they were also very efficacious in relieving various more or less

[a] Barr, Pharm. Jour. and Tr., Lond., 1887, XVIII, 170.
[b] Faust, Deutsch. med. Wchnschr., 1887, XIII, 575.
[c] Hinzelmann, Munch. med. Wchnschr., 1887, XXXIV, 36.

obscure pains, generally neuralgic in character, as facial neuralgia, hemicrania,[a] and the lancinating pains of tabes dorsalis. At the present time enormously large amounts are used for the relief of symptoms of this sort, and comparatively very little as a means of reducing fever.

Drugs to relieve pain have always been especially popular with the general public, who prescribe for themselves all sorts of preparations with absolutely no idea of their poisonous properties. Hence the legitimate use of the antipyretics quickly was made subservient to an indiscriminate use, especially in the treatment of headache, so that Siefert,[b] as early as 1888, pointed out the great danger of allowing the apothecaries to dispense these preparations directly to the general public. Despite this early recognition of the danger of their promiscuous use in this class of disorders their use has become almost universal, and at the present time they are dispensed directly to the laity over the counters of every drug store and at almost every soda fountain with no warning as to their danger and with meager directions as to dosage.

The early history of this popular use of the antipyretics, especially of acetanilide, is closely connected with their exploitation in proprietary remedies. Appearing as one of the first, if not the first of these, were the notorious "Antikamnia" preparations. The promoters of these products claimed to have discovered a new and wonderful member of the antipyretic series which was far more efficacious than those in common use and without their deleterious effects. These claims were such that chemists both in this country and abroad became interested and a large number of analyses were made. These showed that instead of a new and harmless remedy Antikamnia was really a mixture which sometimes contained one thing, sometimes another, but always the already well-known aniline compound, acetanilide. Among the first analyses was that of Hall,[c] who found 77.5 per cent acetanilide and 19.3 per cent sodium bicarbonate. In the same year Goldman[d] reported acetanilide 70 per cent, sodium carbonate 20 per cent, and caffeine 10 per cent.

There seems to be no literature bearing directly upon the exhibition of caffeine and the alkaline carbonates with the antipyretics and it will probably never be known just why caffeine was introduced into the general type acetanilide prescription. Two reasons may be suggested, however. It had been known for a very long time that caf-

[a] Chomjäkow u. Ljwow, Wratsch., 1885, VI, 887. White, N. Y. Med. Rec., 1886, XXX, 293. Ungar, Centralbl. f. klin. Med., 1886, VII, 777. Secretan, Revue med. de la Suisse rom., 1887, VII, 29.

[b] Siefert, Munch. med. Wchnschr., 1888, XXXV, 850, 867.

[c] Hall, Druggists Circular, 1891, XXXV, 99.

[d] Goldman, Pharm. Ztg., 1891, XXXVI, 255.

feine in itself was useful in certain forms of headache, and it appeared in several formulæ combined with the bromides a number of years before the introduction of antipyrine and acetanilide. The natural inference, therefore, is that it was a direct transfer of caffeine from the old to the new type of headache remedies. The other explanation of its presence is to be found in the literature relating to the treatment of cases of acetanilide poisoning. Lepine [a] seems to have first suggested caffeine as an antidote, having reported that the cyanosis of acetanilide poisoning disappeared after large doses of this drug. In 1889 Mahnert [b] suggested that the excitants be used, and in treating three cases made use of ether injections, wine, and powdered caffeine, the latter being especially recommended by him. Hartge [c] treated a case of poisoning with coffee and brandy and later with camphor and ether injections. Falk [d] used caffeine, but thought that alcohol was distinctly contraindicated, owing to the increased solubility of acetanilide in this menstruum and therefore its more rapid absorption. Such a course of treatment for acetanilide poisoning might easily have suggested the addition of one of the above drugs to acetanilide mixtures with the idea that poisoning would be prevented. Whether caffeine was introduced into them as an active agent in the cure of headache or merely to give an additional safety to a drug with known poisonous properties it is of course impossible to say, and both factors may have played some part. At any rate caffeine thus introduced has been almost invariably a constituent of all prescriptions or proprietary formulæ containing acetanilide.

The generally prevailing idea at the present time is that caffeine is added to prevent the deleterious effects of the coal-tar drugs upon the heart,[e] although this does not seem to have been the original reason for its administration. No direct observations concerning its antidotal value seem to have been made, but on purely theoretical grounds it would apparently be useful as a stimulant to the respiratory center, which becomes markedly embarassed from the formation of methæmoglobin and to a lesser degree to the heart. It does not seem probable that it would have any special influence upon the cyanosis unless indirectly through increased respiratory activity.

The combination of alkali carbonates with acetanilide also became popular about 1890, but the reason for their presence in acetanilide

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[a] Lepine, Rev. de med. Par., 1887, VII, 531.

[b] Mahnert, Memorabilien, Heilbr., 1889, XXXIV, 321.

[c] Hartge, St. Petersb. med. Wchnschr., 1890, VII, 69.

[d] Falk, Therap. Monatsh., 1890, IV, 257.

[e] McFarline, Canad. Pharm. Jour., Toronto, 1906, XXXIX, 360, says in speaking of headache powders: "It will be noted that in most cases the depressant effect upon the heart is sought to be counteracted by the addition of caffeine, bicarbonate of soda, or other drugs of like character."

mixtures is largely a matter of conjecture. Herczel[a] in 1887 carried out some experiments upon dogs and was able to show that the exhibition of acetanilide definitely decreased the alkalinity of the blood. Although all methods for determining the alkalinity of the blood are unreliable this fact would afford an experimental basis for their presence, but the small amount present in the usual type of acetanilide mixture would probably be insufficient to make the blood materially less acid.

In this connection it is important to remember that acetanilide causes the formation of methæmaglobin in the blood and that this may be hastened by a decrease in the alkalinity of the blood has been pointed out by Kobert[b] who states that the alkalies prevent the breaking up of the blood cells and the formation of methæmaglobin. The exhibition of alkalies, according to Kobert, also serves to aid the regeneration of the oxyhæmaglobin, an alkali-methæmaglobin being formed as an intermediary product which changes the blood from a chocolate brown to a red and from this oxyhæmaglobin is formed.

Another reason for the combination of alkalies with acetanilide has to do with increasing the solubility of the drug, the idea formerly prevailing that acetanilide was made more soluble and hence more easily absorbed when thus prescribed. In 1891 Hall[c] gave as a reason for the greater activity that had been claimed for antikamnia its finely divided state and the presence of sodium bicarbonate, which he said made it more soluble. The idea of greater solubility when given with alkalies seems to have been held as late as 1906 for Ritter,[d] in commenting on the introduction of a compound acetanilide powder into the eighth decennial revision of the U. S. Pharmacopœia, wrote that it mattered very little whether the alkaline salt be a carbonate of ammonium or sodium or a bicarbonate, as it was only added to increase the solubility of the acetanilide. Puckner[e] could find no experimental basis for this, however, and was able to show in direct contradiction that no increased solubility occurred in alkaline solutions. Acids, on the other hand, especially strong solutions, increased the solubility to some extent, and he concluded therefore that acetanilide is probably even less soluble when taken with an alkalie because of the partial neutralization of the acid gastric contents. A further reason for the presence of alkalies in mixtures with the antipyretics, more particularly antipyrine, is found in the occasional gastric irritation that results from their administration,

[a] Herczel, Wien. med. Wchnschr., 1887 XXXVII, 1022.
[b] Kobert, Lehrbuch der Intoxikationen, 1902, 73–74.
[c] Hall, Druggist Circular, 1891, XXXV, 99.
[d] Ritter, Jour. Am. Med. Ass., 1906, XLVII, 683.
[e] Puckner, Ibid., 1206.

both sodium bicarbonate and seltzer water having been suggested as a means of lessening these disagreeable symptoms.[a] Looked at from a purely pharmaceutical standpoint the presence of carbonates in such mixtures, when dispensed in tablet form, would aid in the disintegration of the tablet because of the chemical action of the acids of the gastric juice.

No branch in the manufacture of proprietary medicine has offered such inducements for the introduction of special formulæ or special nomenclature as has that dealing with the preparation of headache remedies. And almost invariably this has meant acetanilide mixtures. The universal use of such drugs in the relief of such a common symptom has led to multiplication and remultiplication of the different preparations until they are numbered by the hundreds. These have in a general way followed the general type of formula as illustrated by antikamnia, containing acetanilide as a basis and occasionally antipyrine or acetphenetidin, although the cheapness of the former drug made it by far the most popular with the manufacturers. The other ingredients of these mixtures have usually included caffeine and an alkaline carbonate and less often the salicylates, the bromides, morphine, and codeine.

The fact that many of these preparations were advertised and sold to the physician on the one hand and directly to a drug-addicted public on the other, that they and similar proprietaries were often frauduently advertised as panaceas of unusual and wonderful virtue, and finally that their composition was shown to be notoriously variable, was sufficient to arouse a sentiment against all such preparations. In line with this, in the last revision of the United States Pharmacopœia, certain formulæ were introduced, the purpose of which was to give the physician official preparations to take the place of the many similar ones which he had previously been prescribing. This accounts for a number of formulæ which are now pharmacopœial, and especially for Pulvis Acetanilide Compositus. Although there may be some reasons for criticising this step, it certainly was desirable that the physician should be able to order an acetanilide mixture the composition of which was known and which contained a definite and constant proportion of the several ingredients.

It is the purpose of this investigation to determine through experiments upon animals to what extent the presence of such a combination of drugs is justifiable upon the basis of a lessened or altered toxicity of the contained acetanilide; also to determine to what extent the toxicity of other coal-tar combinations is altered by the addition of the various other drugs most frequently found with them in the various formulæ.

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[a] Am. Jour. Pharmacy, 1888, XVIII, 180.

## DETERMINATION OF HEART ACTION.

The popular belief in the value of caffeine in preventing the poisonous heart effects of the coal-tar products suggested the experimental determination of any modifications in the action upon this organ which might occur when it was exhibited in combination with acetanilide. Experiments were carried out upon both warm and cold blooded animals, using the myocardiograph method to record the changes in the dog's heart, the perfusion method in estimating the changes in the frog's heart.

*Action upon the frog's heart.*—The perfusion method was adopted as being most suitable for determining the changes occurring in the frog's heart after acetanilide and combinations of this drug with caffeine citrate or an alkaline carbonate. This method was believed to be especially suitable for this purpose on account of the relative insolubility of acetanilide in an aqueous solvent, since comparatively small amounts are sufficient to produce profound changes in the isolated heart. It is also of advantage because by its use secondary and extraneous effects are excluded, any action being limited to the intrinsic nerves of the heart or to the cardiac muscle substance.

In all cases frogs of the same variety (Rana pipiens) captured at the same time and kept under the same conditions were used. As far as possible those of the same weight were chosen and this was always done when comparisons between the relative effects produced on several frogs were to be noted. In most instances each heart was made its own control, so that these precautions were usually unnecessary. The frogs were pithed (both brain and cord) and the heart exposed in the usual manner. After removal of the pericardial sac the right branch of the aortic arch was dissected out and ligated, and a cannula inserted into the left branch as far from the heart as possible. By gentle traction upward the heart may be separated from the œsophagus and other tissues, best accomplished by running a blunt dissecting needle between these structures and the sinus venosus. If the animal is small it is probably easier to insert the inflow cannula into the posterior vena cava by turning the heart over with the base downward (frogs weighing 10 grams have been successfully employed using this procedure). If the animal is large, 25 to 50 grams, the venous cannula is usually inserted with the heart lying in the normal position.

Two Mariotte bottles of about 150 c. c. capacity were used to hold the perfusing fluid. They were mounted on a stand and into the upper opening of each bottle a glass tube reaching to within one-half c. m. of the bottom was inserted through a tightly fitting stopper in order to allow ingress of air and thus preserve a constant pressure no matter what the level of the fluids in the bottles might be. The outlets of the two bottles were connected through a Y tube held

immovably by a clamp. Ringer's solution, (sodium chloride 0.7, potassium chloride 0.03, and calcium chloride 0.026 per cent), was used as a perfusing fluid. The venous cannula was connected with the outlet of the Y tube, while the aortic cannula was supported in such a manner that the perfused fluid would flow back over the heart, thus keeping it moist and in good condition. The heart with certain exceptions as noted below was started by perfusing it with Ringer's solution at a temperature of 20 to 22° and the rate and output per five-minute periods were recorded. When a normal had been ascertained acetanilide in varying per cent in Ringer's perfusing fluid was substituted, and after the effect of this solution had been determined this was replaced by acetanilide of the same strength, but to which had been added either caffeine citrate or sodium bicarbonate and the output and rate again recorded as before.

In estimating the effect of acetanilide alone and in combination with other drugs, it was first planned to use a sufficient concentration to poison the heart to such an extent that it would stop beating after twenty or thirty minutes. The drug in large doses being of itself a depressant of the heart, it was thought that the rate and output would both grow gradually less and less, and that finally the heart would stop beating. Such a course of progressive poisoning, however, does not seem to follow. After perfusing with strong solutions (one-half to one-fifth per cent) the heart is found to cease beating almost instantly; slightly weaker solutions (one-sixth to one-tenth per cent) would stop the heart in some cases either at once or after a few minutes, or again would not stop it although perfused through it for an hour or more. Protocols illustrating this action of the drug are given in Table I.

TABLE I.—*Perfusion of the isolated frog's heart with Ringer's solution and acetanilide.*

| Protocol 20, October 12, 1908. | | | Protocol 25, October 13, 1908. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | *C. c.* |
| 9. 50 | 26 | ..... | 2. 00 | 28 | ..... |
| 9. 55 | 26 | 21 | 2. 05 | 29 | 16 |
| 10. 00 | 25 | 25 | Acetanilide, $\frac{1}{5}$ per cent. | | |
| Acetanilide, $\frac{1}{8}$ per cent. | | | 2. 10 | 12 | 5 |
| 10. 02 | 13 | ..... | 2. 14 | 0 | ..... |
| 10. 05 | 13 | 9 | | | |
| 10. 10 | 10 | 7 | | | |
| 10. 15 | 10 | 7 | | | |
| 10. 20 | 9 | 7 | | | |
| 10. 30 | 11 | 8 | | | |
| 10. 40 | 12 | 9 | | | |
| 10. 50 | 12 | 10 | | | |
| 11. 10 | 14 | ..... | | | |

In other respects the effect on the rate is very regular. Almost immediately after the introduction of the drug the rate decreases to

about half, although in some cases the slowing is more progressive and drops by degrees to a certain point or in certain cases rapidly to zero. The rate in both auricle and ventricle usually remains the same until late in the poisoning, when the auricles are observed to be less affected than the ventricle and continue to beat for some time after the latter has ceased to contract.

Another rather peculiar feature of the poisoning is that after the heart has been exposed to the action of the drug for some time the heart muscle seems to gain some tolerance for the poison, for although never regaining its normal rhythm, the toxic effect appears to become less and often the rate and output are secondarily augmented. The same thing is shown in other instances by the stoppage of the heart following the first introduction of the drug. After a short time it is allowed to regain its original rhythm by perfusing it with Ringer's solution, whereupon a second introduction of the drug, although slowing the rate, shows no tendency to check it entirely. Cahn and Hepp [a] in perfusing the hearts of frogs (species not recorded) reported some slowing but no change in the energy, but their work may be questioned because of an insufficient range in the per cent of acetanilide used. Weil [b] noted a primary increase in both rate and energy, but this was followed by a secondary decrease in both. Lepine [c] reported that acetanilide increased the energy but decreased the heart rate. My own observation was that the energy was sometimes considerably augmented, but this effect was always of very short duration. Less often there appeared also a momentary increase in heart rate, but in the case of all my experiments this was quickly followed by a very marked slowing.

The next problem was to determine what strength of caffeine citrate could be used to the greatest advantage in perfusing the heart of Rana pipiens. It is well known that rigor is produced in the skeletal muscles and the heart of frogs, although the latter is not so readily affected, and small doses cause definite stimulation, both rate and output being increased. It is obvious, of course, that an amount of the drug should be used too small to produce rigor and yet sufficiently large to cause stimulation. By experiment this was found to be between one-fifteen hundredth per cent and one-five thousandth per cent. Below one-fifteen hundredth per cent the heart rate was most often slowed, and even at this dilution the output was very materially lessened. In stronger solutions both rate and output rapidly fell to zero. Examples of the effects produced are shown in Table II.

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[a] Cahn and Hepp, Berl. klin. Wchnschr., 1887, XXIV, 27.
[b] Weil, Thesis, Paris. De l'Acetanilide, 1887, 47 pp.
[c] Lepine, Revue de med., Par., 1887, VII, 310.

TABLE II.—*Perfusion of the isolated frog's heart with caffeine citrate in Ringer's solution.*

| Protocol 13, October 7, 1908. | | | Protocol 14, October 8, 1908. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | *C. c.* |
| 11. 05 | 24 | ..... | 10. 10 | 27 | ..... |
| 11. 10 | 24 | 18 | 10. 15 | 27 | 19 |
| Caffeine citrate $\frac{1}{800}$ per cent. | | | 10. 20 | 28 | 21 |
| 11. 11 | 26 | ..... | 10. 25 | 27 | 20 |
| 11. 15 | 20 | 10 | 10. 30 | 27 | 20 |
| 11. 18 | 0 | ..... | Caffeine citrate $\frac{1}{2000}$ per cent. | | |
| 11. 20 | ..... | 4 | 10. 32 | 29 | ..... |
| | | | 10. 35 | 30 | 22 |
| | | | 10. 40 | 27 | 23 |
| | | | 10. 45 | 28 | 21 |
| | | | 11. 05 | 28 | ..... |

Having thus determined the manner in which the frog's heart reacts when perfused with definite percentages of caffeine or acetanilide, a third series of experiments were carried out to determine to what extent and in what manner the action of acetanilide in solutions of various strengths could be modified by the addition of such amounts of caffeine as had been shown to possess a definite stimulant action. Two methods of determining these effects were employed, one based upon the differences in the time necessary to stop the heart, the other upon changes in rate and output. It had been shown by a number of experiments, using one-seventh of one per cent solutions of acetanilide alone, that the heart was stopped by the poisonous action of the drug at the following intervals: 37, 17, 9, and 30 minutes, or on the average 23.1 minutes after the perfusion of the drug was begun. Accordingly one-seventh per cent acetanilide solutions to which one two-thousandth per cent caffeine citrate had been added were perfused at the same temperature through another series using frogs of approximately the same weight. The intervals between the introduction of the combined drugs and the stoppage of the heart were as follows: 25, 3, 15, and 8 minutes, or on the average 12.7 minutes after the drug was started. In both series marked irregularities will be noted. This is probably in great part due to the irregularity in the course of acetanilide poisoning and because of the great differences in the same series it becomes impossible to draw absolute conclusions. The results as they stand, however, indicate considerably more toxicity to the heart from the drugs when exhibited in combination than from acetanilide alone.

The second method of experimenting consisted in poisoning the heart with smaller quantities of acetanilide with the idea of keeping it beating for some time and then noting the changes in rate and output upon the substitution of the acetanilide caffeine combination. These experiments are also somewhat disappointing because of their

failure to show striking modifications in action. It seems possible, however, in going carefully over the results obtained in this manner to point out certain definite changes. In five experiments there was clearly a lessened toxicity as a result of using the drugs in combination. In fourteen experiments the results were exactly the opposite. The rate was lessened in five and in the others there was no change, but in all cases there was a decrease in the output over that produced by acetanilide alone. Two protocols illustrating this increased toxicity are given in Table III.

TABLE III.—*Perfusion of the isolated frog's heart with acetanilide, and with acetanilide and caffeine citrate in combination.*

| Protocol 17, October 10, 1908. | | | Protocol 40, October 23, 1908. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | *C. c.* |
| 9.50 | 29 | ..... | 9.20 | 28 | ..... |
| 9.55 | 32 | 20 | 9.25 | 36 | 23 |
| 10.00 | 32 | 25 | 9.30 | 35 | 22 |
| 10.05 | 33 | 27 | Acetanilide $\frac{1}{8}$ per cent + caffeine citrate $\frac{1}{3000}$ per cent. | | |
| Acetanilide $\frac{1}{10}$ per cent. | | | 9.31 | 0 | ..... |
| 10.07 | 19 | ..... | 9.32 | Ringer's solution. | |
| 10.10 | 17 | 25 | 9.35 | 26 | 10 |
| 10.15 | 15 | 23 | 9.40 | 33 | 19 |
| Acetanilide $\frac{1}{10}$ per cent + $\frac{1}{2000}$ per cent caffeine citrate. | | | Acetanilide $\frac{1}{8}$ per cent. | | |
| 10.17 | 13 | ..... | 9.42 | 19 | ..... |
| 10.20 | 14 | 26 | 9.45 | 13 | 8 |
| 10.25 | 11 | 27 | Acetanilide $\frac{1}{8}$ per cent + caffeine citrate $\frac{1}{3000}$ per cent. | | |
| 10.30 | 11 | 25 | 9.46 | 0 | ..... |
| Acetanilide $\frac{1}{10}$ per cent. | | | | | |
| 10.32 | 13 | ..... | | | |

It must be admitted that the changes are not very definite, but as in the results obtained by the other method there is some indication of increased toxicity from the combination. The results at any rate are confirmatory of each other. It may be very clearly stated too that there is no lessened toxicity when using the drugs in the above strengths and this again substantiates the results shown by the first series of experiments.

In another series of experiments, carried out to determine changes in rate and output, smaller amounts of the drugs were used. In these such small amounts of the drugs were perfused that the perfusion was begun with solutions of acetanilide in Ringer's solution. The second perfusion bottle in addition to the acetanilide contained either caffeine citrate or sodium bicarbonate. After a series of readings using acetanilide had been taken, the combined drugs were perfused and the resulting changes in heart rate and output were noted. Acetanilide was used in one-fifteenth per cent strength and caffeine citrate in one three-thousandth per cent, and with few exceptions the

results in this series indicated that caffeine if sufficiently dilute would antagonize, to some extent at least, the poisonous effects of acetanilide. There were some experiments which still showed greater toxicity from the combination of drugs than from acetanilide alone, but these were only occasional. However, they were sufficiently frequent to illustrate very well how incomplete the antagonism between these drugs really is.

Protocols showing the results of this series of experiments are given in the following table:

TABLE IV.—*Effect upon the isolated frog's heart of an acetanilide Ringer's solution and a similar solution to which caffeine citrate had been added.*

| Protocol 53. | | | Protocol 50. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes, | Time. | Rate. | Output per 5 minutes, |
| Acetanilide $\frac{1}{15}$ per cent. | | C. c. | Acetanilide $\frac{1}{15}$ per cent. | | C. c. |
| 9.35 | 22 | | 11.00 | 20 | |
| 9.40 | 20 | 54 | 11.10 | 15 | 21 |
| 9.45 | 20 | 52 | 11.15 | 17 | 20 |
| 9.50 | 21 | 50 | 11.20 | 17 | 20 |
| 9.55 | 22 | 53 | 11.25 | 16 | 19 |
| 10.00 | 22 | 56 | Acetanilide $\frac{1}{15}$ per cent+caffeine citrate $\frac{1}{3000}$ per cent. | | |
| Acetanilide $\frac{1}{15}$ per cent+caffeine citrate $\frac{1}{3000}$ per cent. | | | 11.27 | 16 | |
| 10.02 | 27 | | 11.30 | 16 | 18 |
| 10.05 | 26 | 58 | 11.35 | 14 | 17 |
| 10.10 | 27 | 56 | 11.40 | 14 | 15 |
| 10.15 | 27 | 57 | 11.45 | 14 | 14 |
| 10.20 | 25 | 54 | Acetanilide $\frac{1}{15}$ per cent. | | |
| Acetanilide $\frac{1}{15}$ per cent. | | | 11.47 | 13 | |
| 10.22 | 23 | | 11.50 | 12 | 13 |
| 10.25 | 23 | 58 | 12.00 | 12 | 10 |
| 10.35 | 22 | 55 | | | |

To determine to what extent the presence of alkaline carbonates in the perfusion fluid would modify or prevent the toxic effects of acetanilide was then made the subject of a series of experiments. In this work no effort was made to determine the length of time necessary to produce stoppage of the heart, but as in the later caffeine-acetanilide experiments the comparison was made by noting the changes in rate and output. Acetanilide was used in only one-eighth per cent solution, but the amount of sodium bicarbonate and ammonium carbonate was varied from one three-hundredth to one-twentieth per cent. As far as could be determined there seemed to be no difference in the antagonistic value whether the base were sodium or ammonium. In all cases there proved to be a considerable degree of antagonism, but this was not at all sufficient to abolish or even prevent quite marked slowing and weakening from the toxic action of the acetanilide. The degree to which sodium bicarbonate is antidotal to acetanilide when perfused through the frog's heart is shown by the protocols given in Table V.

TABLE V.—*Perfusion of the isolated frog's heart with acetanilide and with acetanilide and sodium bicarbonate in Ringer's solution.*

| Protocol 35, October 15, 1908. | | | Protocol 38, October 16, 1908. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | |
| 3.20 | 34 | | 1.55 | 42 | |
| 3.25 | 46 | 27 | 2.00 | 38 | |
| 3.30 | 40 | 30 | 2.05 | 33 | |
| Acetanilide $\frac{1}{8}$ per cent+ $\frac{1}{100}$ $NaHCO_3$. | | | Acetanilide $\frac{1}{8}$ per cent+$\frac{1}{200}$ per cent $NaHCO_3$. | | |
| 3.32 | 32 | | 2.07 | 21 | Not recorded. |
| 3.35 | 18 | 19 | 2.10 | 22 | |
| 3.40 | 17 | 15 | 2.20 | 22 | |
| 3.45 | 17 | 12 | 2.28 | 21 | |
| 3.50 | 17 | 13 | Acetanilide $\frac{1}{8}$ per cent. | | |
| Acetanilide $\frac{1}{8}$ per cent. | | | 2.30 | 16 | |
| 3.52 | 14 | | 2.35 | 14 | |
| 3.55 | 10 | 11 | | | |
| 4.00 | 7 | 3 | | | |
| 4.03 | 0 | | | | |

*Action on the dog's heart.*—The effect of acetanilide upon the mammalian heart is considered such an important factor in the poisoning in man that the results of the control experiments done in determining the nature of the effect of acetanilide caffeine combinations will be described in detail. Evans,[a] in describing the effect of acetanilide upon the circulation, states that it caused a rise of pressure and a slight acceleration in the heart rate when given to rabbits in doses of 15 to 75 milligrams. Lepine[b] reported in experiments on dogs that there was an increased heart rate, increased energy, and greater tension in the arteries, but that this was followed by slowing and lessened tension. Hare[c] also worked with dogs and found a slight fall in pressure, and Osler[d] in clinical observations noted a decrease of the pulse of from 20 to 30 beats per minute. Weil[e] seems to have summed up the action of the drug in his experiments by stating that the first effect was an increase of rate and energy, but that this was followed by a decrease in both. It is generally concluded from experimental evidence, therefore, that small doses increase the heart action, but that larger amounts cause depression. No experiments seem to have been made in which the heart action of acetanilide was recorded by a myocardiograph. Accordingly it seems worth while to again report upon the action of this drug upon the circulatory organs as a means of emphasizing its dangers, especially as myocardiograph tracings demonstrate this action so clearly.

It is generally recognized that the mammalian heart reacts to drugs and poisons in much the same manner as does the heart of cold-

[a] Evans, Therap. Gazette, 1887, XI, 237.
[b] Lepine, Rev. de Med., Par., 1887, VII, 310.
[c] Hare, Therap. Gazette, 1887, XI, 382.
[d] Osler, Ibid., 165.
[e] Weil, Paris Thesis, 1887, De la Acetanilide.

blooded animals. This reaction is entirely qualitative and, aside from differences due to variation in the action of the vagi, is always very uniform. In estimating the effects of a drug upon the human heart, however, it is preferable to experiment upon warm-blooded animals, and to choose those in which the vagus action is well developed. In this way the experimental data may be said to represent not only the qualitative but also quite closely the quantitative effects in man. Therefore, to check the results obtained by perfusing the frog's heart with acetanilide and with mixtures of acetanilide and other drugs, a series of experiments were carried out, using dogs as experimental animals. These animals were anæsthetized by giving hypodermic injections of morphine sulphate, 0.010 gram per kilogram body weight, and this was followed in the course of a half hour to an hour by chloretone, 0.180 gram per kilogram dissolved in a small amount of alcohol (1 gram chloretone to 2 c. c. 95 per cent alcohol), which, after dilution with a small amount of water, was introduced into the stomach by means of a stomach tube. Following the appearance of complete anæsthesia a tracheal cannula was introduced to provide for artificial respiration, the air being properly warmed by passing it through a coil submerged in hot water. The heart was then exposed by a median incision reaching to the diaphragm, the pericardial sac removed, and the ventricle attached to a modified form of the Roy-Adami myocardiograph. Blood pressure tracings from the carotid were also taken, using the ordinary mercury manometer to record the changes in pressure produced by the drug. Cannulæ were placed in both the right and left saphenous veins—one for the injection of the acetanilide, the other for the injection of the caffeine citrate or sodium bicarbonate, which was used to determine if there were any antagonism between these drugs and acetanilide.

Acetanilide is so slightly soluble in physiological salt solution that it was necessary to inject it as an emulsion. This was formed with mucilage of acacia, the amount used being as small as possible, and then diluted with salt solution. To insure uniformity of dosage the emulsion was thoroughly shaken before each injection, since the suspension of the acetanilide was only temporary.

Injections of small amounts (0.200 to 0.600 gram) of acetanilide was usually followed by a momentary increase in the strength of the heart, the systolic phase being more complete and the relaxation only slightly lessened. This effect lasted for a few seconds only and was succeeded by a rapid and marked decrease in efficiency, the contractions growing quickly less complete, while the relaxations were scarcely affected at all. In some instances the amplitude (efficiency (?)) was slightly greater after recovery from or as a late effect of the drug. As in the case of the perfusion experiments it

was also noted that following the primary injection the later injections caused much less weakening of the heart. For example, a preliminary dose of 1 gram lessened the amplitude 70 millimeters, a

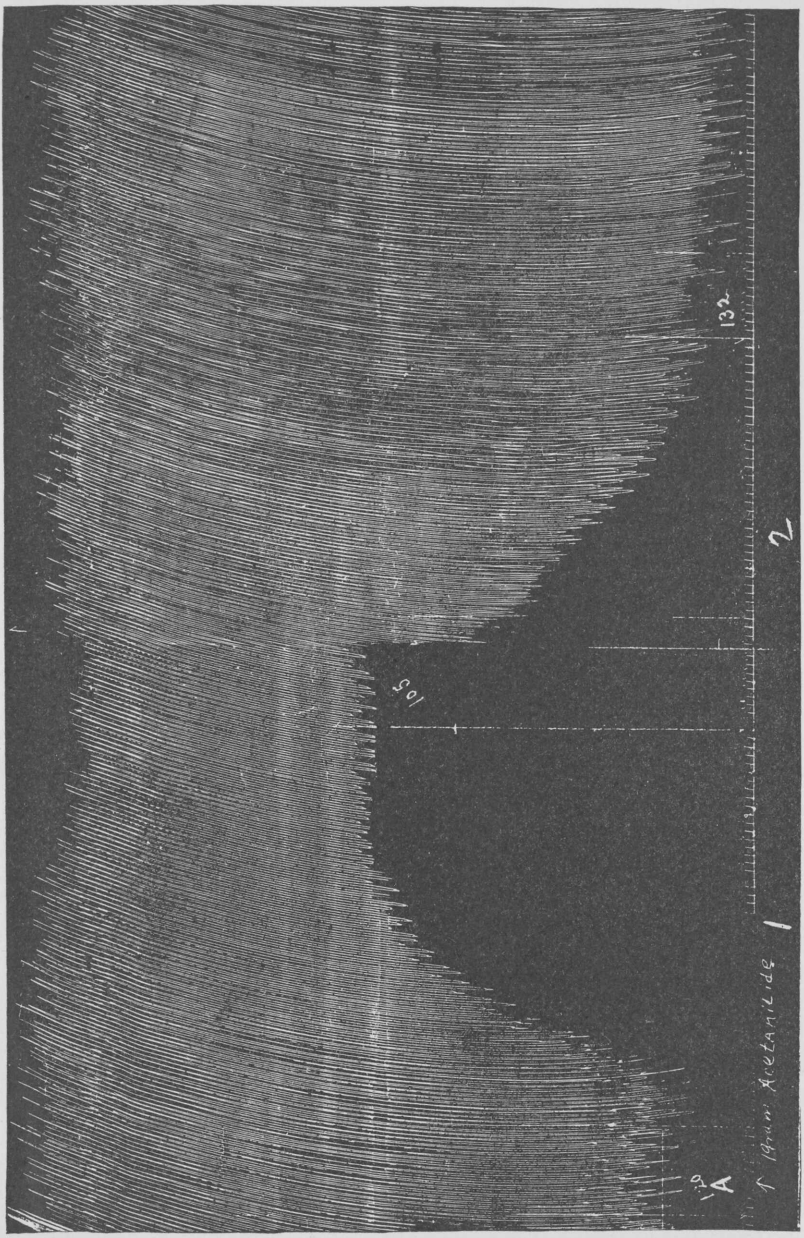



FIG. 1.—Tracing of the ventricular contractions under acetanilide. The lever moves downward during systole. "A" marks the point of injection. The rhythm of the heart is somewhat slower (120 to 105) after the drug and the lever does not descend so far, indicating a less complete systole. The diastole is also slightly less as is shown by the failure of the lever to reach so far upward. A late effect is the increased systole, indicated by the nearer approach of the lever to the base line. Between 1 and 2, one minute.

second injection only 38, a third injection of 1.5 grams 36, and a fourth also of 1.5 grams only 8. Figure No. 1 is given to illustrate

the deleterious effect on the dog's heart produced by a 1-gram dose of acetanilide.

The gradual decrease in the action of acetanilide makes the determination of possible antagonistic effects of caffeine and sodium bicarbonate somewhat difficult. As has been noted, the effect of

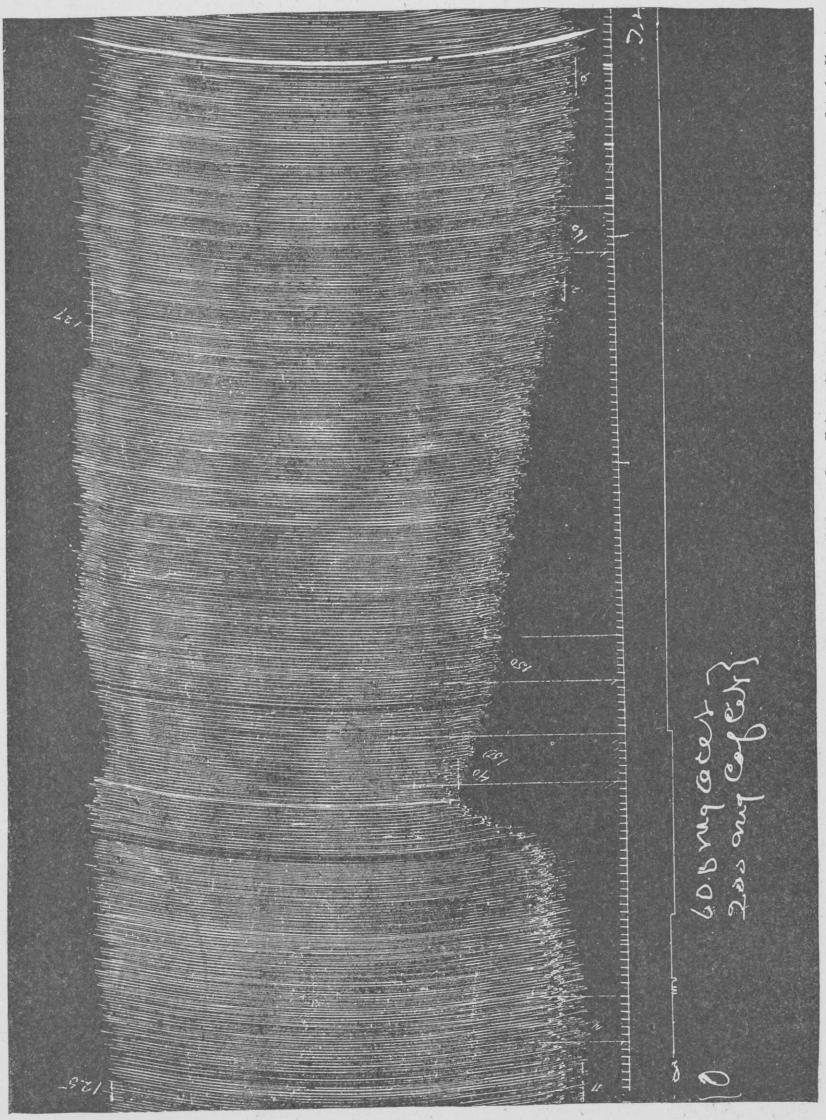


FIG. 2.—Tracing of the ventricular contractions under the acetanilide and caffeine citrate. A signal marker was used to indicate the time of injection. The failure of the heart to draw the lever downwards indicates a lessened systole. The diastole is little influenced as the upper line scarcely is changed by the drugs.

acetanilide is to lessen the contractile power of the heart, and caffeine should prevent this symptom if it acted as an antagonist. A mixture of the two drugs was injected, but in no case was the decrease in the systolic phase prevented—whether it may have been less pronounced is of course difficult to determine since, as has been

noted, the effect of the poison appears to become less at subsequent injections. This is well illustrated by the tracings given in figures 2 and 3.

Figure No. 2 shows the effect after 0.600 gram acetanilide and 0.200 gram caffeine citrate had been injected into the saphenous

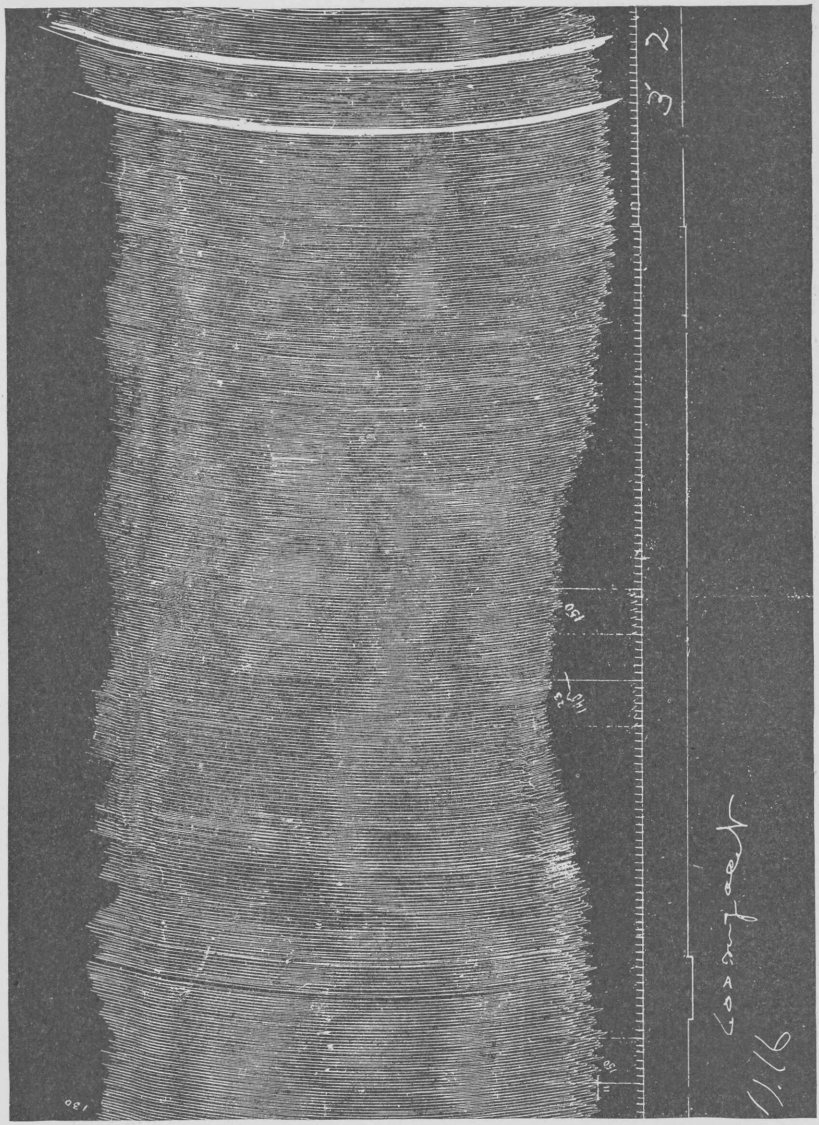


Fig. 3.—Tracing of the ventricular contractions under acetanilide, 0.600 gram dose. The lever moves downwards during systole. Compare with Fig. 2 and note the lessened contraction, although this tracing followed that in Fig. 2 after an interval of six minutes.

vein; figure No. 3, the effect after an injection of 0.600 gram acetanilide, this tracing immediately following that given in figure 2. It may also be inquired whether the caffeine is not responsible for the greater weakening shown in figure 2; but this is not likely, judging from other experiments where the reverse order of injection was used.

When the caffeine acetanilide mixture followed the plain acetanilide the effect of the combined drugs was less instead of greater as in the above case. Usually the heart does not become accustomed to the poison quite so quickly as in this instance, animals differing very widely in this respect.

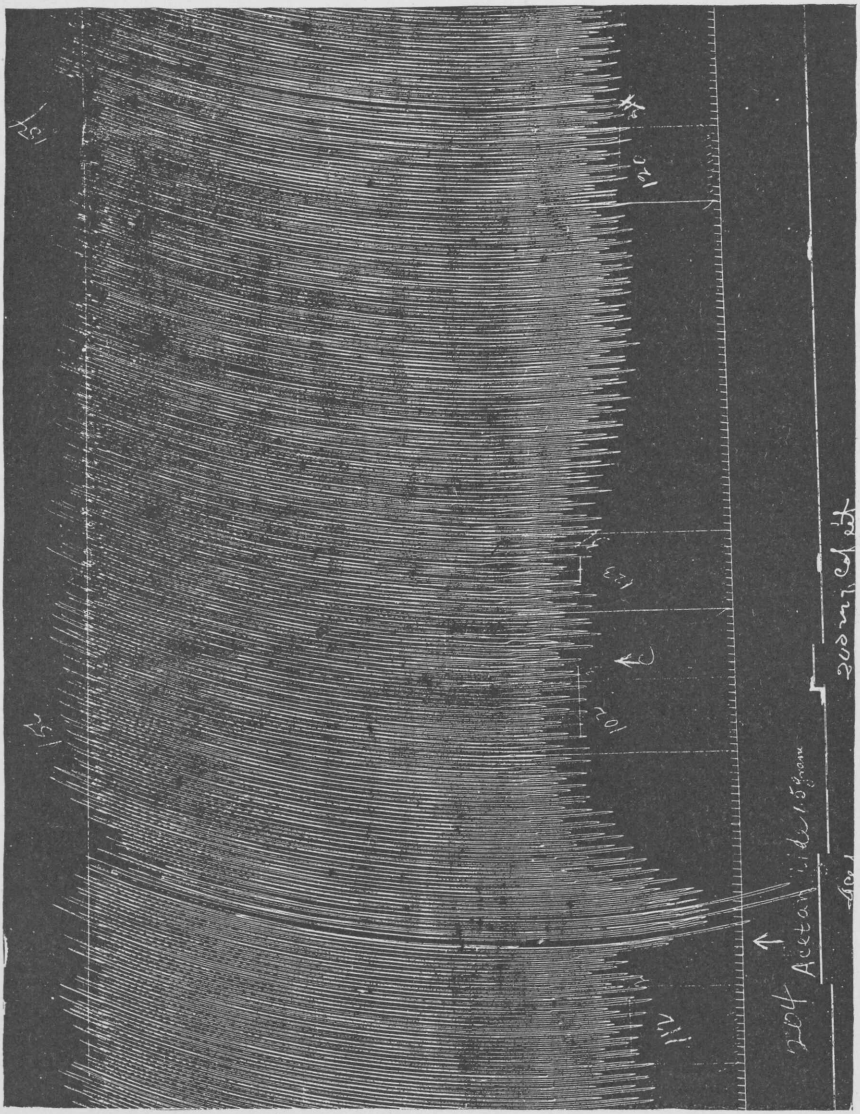


FIG. 4.—Tracing of the ventricular contractions under acetanilide and under caffeine citrate. The lever moves downward in systole. Note the momentary increase in the systole after the injection of acetanilide and the very slight lessened contraction after the injection of caffeine at "C."

Further experiments were carried out in which caffeine citrate was injected as soon as the acetanilide effect became pronounced. In some instances this procedure showed a possible slight increased contraction coincident with the caffeine injection, but almost equally

often such an injection of caffeine was followed by a decrease in the completeness of systole. In other cases there was a momentary slight increase in strength followed by a short decrease immediately following the caffeine injection. This is illustrated by figure 4.

No increase in heart rate was observed following the injection of acetanilide in the doses used in these experiments. On the other hand, there was generally a decrease of from 10 to 20 beats per minute, the slowing evidently being due to a direct action on the heart muscle, since paralysis of the vagi by atropine did not prevent it. As regards the rate, caffeine proved to be completely antagonistic, when injected at the same time usually preventing any slowing and when injected subsequent to an injection of acetanilide causing the heart rate to return at once to the normal or in some cases to a rate more rapid than normal.

There is a marked fall in blood pressure immediately following the injection of acetanilide, which is probably due in a great measure to the lessened efficiency of the heart. The injection of caffeine, although restoring the rate to normal, has hardly any effect upon the blood-pressure curve after injections of acetanilide. In general, there is a slight upward tendency, but in certain instances caffeine seemed to check the return to normal, agreeing in this respect to the occasional tendency of caffeine to lessen the completeness of the heart's contraction. Figure 5 illustrates the lack of antagonism between caffeine and acetanilide upon the blood pressure.

Sodium bicarbonate was used to antagonize the action of acetanilide only after the heart had become seriously poisoned. The immediate effect of large doses (2 grams) was to slightly increase the depression as marked by the systolic phase. This was quickly followed, however, by a rather marked and prolonged increase in the contractile power of the ventricle, as is shown by the nearer approach of the writing lever to the base line

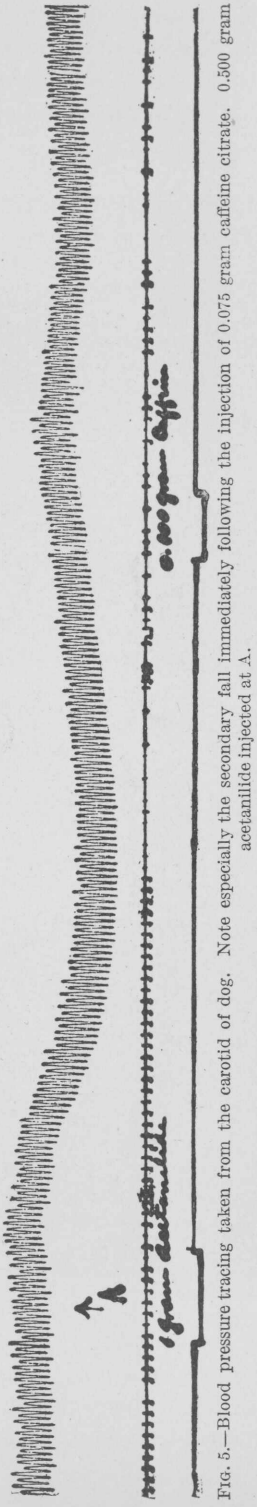


FIG. 5.—Blood pressure tracing taken from the carotid of dog. Note especially the secondary fall immediately following the injection of 0.075 gram caffeine citrate. 0.500 gram acetanilide injected at A.

in Figure 6. Note also the great dilation of the heart as a late effect of acetanilide poisoning, both diastole and systole being much less complete as compared with the normal, a portion of which is also included in the cut.

## DETERMINATION OF GENERAL TOXIC ACTION.

In view of the fact that the cases of acetanilide poisoning are due to an involvement of all the vital organs of the body and not on

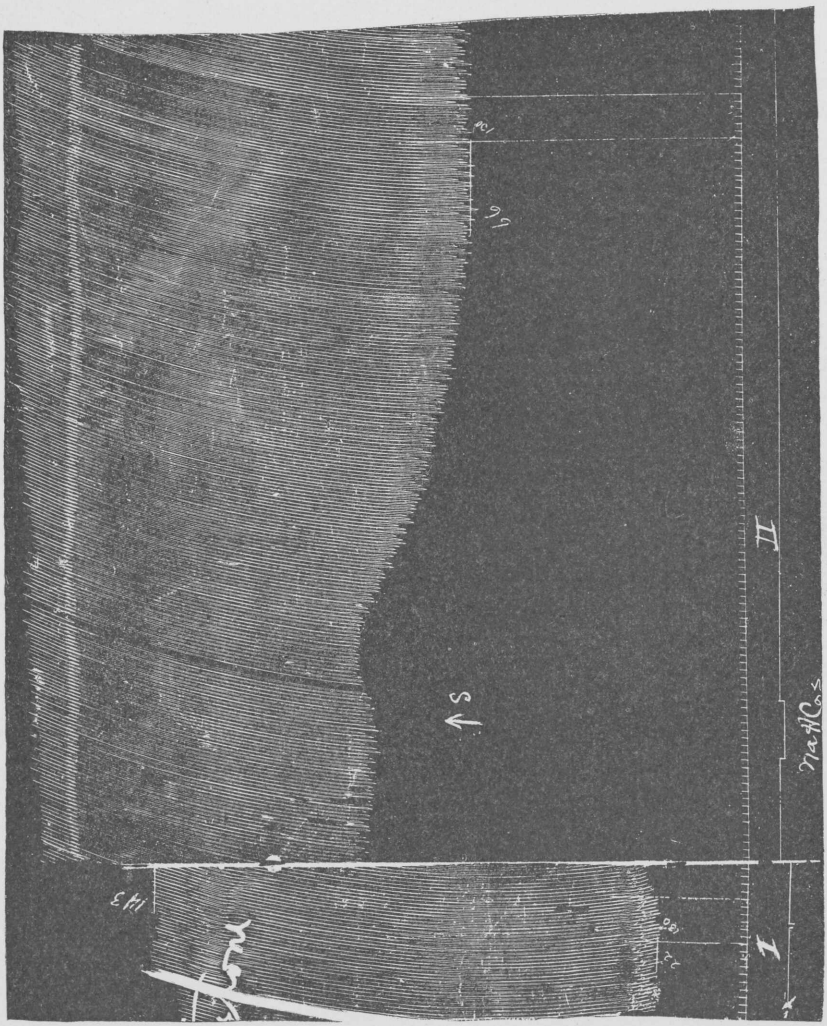



FIG. 6.—Tracing of the ventricular contractions of the normal heart and of the heart after the injection of acetanilide. Sodium bicarbonate was injected at S. Note the increased systole after the introduction of the carbonate. 1, normal heart; 2, the same heart one hour and a half later poisoned with acetanilide.

account of its heart action alone, a series of experiments was carried out to determine what modification of the general toxicity could be secured by administering it to intact animals with the drugs commonly found in acetanilide mixtures. In these experiments the drugs were administered to white mice by hypodermic injection and to mice and guinea pigs by the stomach.

In the first series of experiments acetanilide and an acetanilide-caffeine mixture were given to white mice by hypodermic injection. The mice were obtained from the same lot for each series of experiments and after being weighed they were placed in separate jars and the dose calculated in terms of grams of body weight. Acetanilide is so slightly soluble in water that it was found necessary to dissolve it in dilute alcohol (55 per cent) in order to make the hypodermic method available. This unfortunately introduces an additional factor, and to lessen the amount of alcohol injected as far as possible a supersaturated solution (at ordinary temperature) was formed, each cubic centimeter of the solution representing 200 milligrams of the drug. It was necessary to heat the solution to about 40° before the injections were made, and to prevent precipitation when drawn up in the syringe the latter was also kept in a warm place and the injection under the loose skin of the back made as quickly as possible. The minimum lethal dose of acetanilide when given in this way was found to be 0.0013 gram per gram of body weight. In control mice somewhat more than double the amount of alcohol was just sufficient to cause death, showing that the poisonous action of acetanilide was the principal toxic agent, although its effects were possibly modified to some extent by the solvent. The following table (Table VIII) gives the result of a determinative series of experiments:

TABLE VIII.—*Determination of the minimum lethal dose of acetanilide for white mice, hypodermic injection. Dose given represents grams of drug per gram body weight.*

[−=survived; +=death.]

| | Weight in grams. | Dose per gram body weight. | Result. | Hours till death. |
|---|---|---|---|---|
| Series I | 15.12 | 0.0010 | − | ..... |
| | 16.10 | .0012 | − | ..... |
| | 14.41 | .0014 | + | 23 |
| Series II | 24.18 | .0011 | − | ..... |
| | 19.24 | .0012 | + | 26 |
| | 23.66 | .0013 | + | 25 |
| Series III | 17.45 | .0012 | − | ..... |
| | 18.96 | .0012 | − | ..... |
| | 14.32 | .0013 | + | 30 |
| | 15.92 | .0013 | + | 14 |

As a means of control the following experiments were carried out to determine the fatal dose of 55 per cent alcohol:

TABLE IX.—*Determination of minimum lethal dose of 55 per cent alcohol for white mice. Dose calculated in terms of grams of acetanilide for sake of comparison.*

[−=survived; +=death.]

| | Weight in grams. | Dose per gram body weight. | Result. | Hours till death. |
|---|---|---|---|---|
| Series I | 30.93 | 0.0014 | − | ..... |
| | 25.72 | .0016 | − | ..... |
| Series II | 26.00 | .0020 | + | 12 |
| | 26.06 | .0024 | − | ..... |
| | 26.65 | .0028 | + | 3 |
| Series III | 20.60 | .0020 | − | ..... |
| | 21.99 | .0024 | − | ..... |
| | 19.32 | .0028 | + | 6 |

The minimum lethal dose for caffeine citrate was determined in the same manner, excepting that the drug was dissolved in physiological salt solution. The dose just sufficient to cause death was found to be 0.0007 gram.

The results of this series of experiments are found in Table X.

TABLE X.—*Determination of the minimum lethal dose of caffeine citrate for white mice, hypodermic injection.*

[−=survived; +=death.]

| | Weight in grams. | Dose per gram body weight. | Result. | Minutes till death. |
|---|---|---|---|---|
| Series I | 13.72 | 0.0005 | − | ..... |
| | 14.39 | .0008 | + | 127 |
| | 13.28 | .0010 | + | 70 |
| Series II | 12.51 | .0006 | − | ..... |
| | 16.92 | .0007 | + | 25 |
| | 12.52 | .0008 | − | ..... |
| Series III | 14.46 | .0006 | − | ..... |
| | 14.02 | .00075 | + | 17 |
| | 12.82 | .00085 | + | 50 |

Having thus determined the minimum fatal dose for acetanilide and caffeine citrate, both drugs were mixed in varying proportions, dissolved in 55 per cent alcohol, and injected in the manner as in the previous experiments. A series of mice were injected with mixtures containing 0.0012 of acetanilide and 0.0001 and 0.0002 gram of caffeine per gram of body weight, but this dosage proved to be invariably fatal, thus proving the absence of any antidotal properties for caffeine in the above amounts. A further series of experiments was then carried out, using smaller amounts of acetanilide to determine whether the effect might not represent a summation of the toxic action of the two drugs. In estimating the doses to be given the fatal doses of each drug, as already determined, were added

together, it being believed that if there were a decrease in toxicity the half of this sum or any proportion representing half this sum would surely not cause death. The sum of the minimum fatal doses being 0.0020, the initial doses were estimated as about one-half of this amount, or 0.0010. But when given in proportions the sum of which equaled 0.0010 the mixture also proved fatal, showing not only no antagonism but a summation effect even. Further experiments were then carried out in which smaller amounts than half the sums of the minimum lethal doses were injected. Likewise these doses, even when slightly less than half the amount of the minimum lethal dose, always proved fatal, thus indicating more emphatically not only a summation effect in the toxic action of the two drugs but even some synergistic action. The time after the injections were made until death of the animal was also shorter, and this served as further proof of an increased toxicity. Some of the results obtained in these experiments are given in Table XI.

TABLE XI.—*Determination of the minimum lethal dose for mice of mixtures of acetanilide and caffeine citrate, hypodermic injection.*

[−=survived; +=death; A=acetanilide, and C=caffeine citrate.]

| Weight. | Dose per gram body weight. | Result. | Hours till death. |
|---|---|---|---|
| 17.51 | A 0.0006<br>C .0001 | − | ..... |
| 14.15 | A .0006<br>C .0002 | − | ..... |
| 16.01 | A .0007<br>C .0001 | − | ..... |
| 12.77 | A .0007<br>C .0002 | + | 7.30 |
| 12.96 | A .0007<br>C .0002 | + | 9.00 |
| 14.62 | A .0008<br>C .0001 | + | 4.50 |
| 12.55 | A .0008<br>C .0001 | + | 2.30 |
| 14.34 | A .0009<br>C .0001 | + | 3.37 |
| 20.83 | A .0009<br>C .0001 | + | 3.12 |
| 16.73 | A .0007<br>C .0003 | + | 9.52 |
| 15.04 | A .0007<br>C .0004 | + | 4.40 |
| 15.73 | A .0009<br>C .0002 | + | 4.49 |
| 14.56 | A .0009<br>C .0002 | + | 2.20 |

## FEEDING EXPERIMENTS.

The hypodermic method of administering the antipyretics is rarely, if ever, used, at least in recent years, in the therapeutic application of these drugs. In order, therefore, to carry out experiments more closely simulating the ordinary methods of giving them, a series of feeding experiments was planned, using white mice and guinea pigs as experimental animals. In all cases the animals for

the same series were taken from the same lot and kept under the same conditions to lessen the chances for the individual variation in results.

The mice were weighed, placed in separate jars, and fed according to the method of Ehrlich upon cakes made up with cracker meal to which the drug or mixture of drugs to be tested had been added. Each cake represented 4 grams of the meal and constituted the daily ration for each mouse. Because the whole cake was not always eaten entirely up, the daily amount of drug ingested necessarily varied to some extent, being somewhat less than the amount computed as the daily dose. In some cases, too, the death of the animal was probably only in part due to the toxic action of the drugs, in part to a dislike for the medicated cakes, and consequent partial starvation. To lessen the chances for error from this cause and from individual variations in the susceptibility of the mice, etc., a number of different combinations of the drugs were fed and several series of mice (in different series mice of different lots but always of the same lot for the same series) were used in testing the various combinations.

Control experiments were always carried out for each series. In the first series unmedicated cakes were used as well as cakes containing the drugs which are commonly found in the ordinary type of acetanilide mixtures. In the later experiments the control with plain cakes was omitted as unnecessary, since the controls using the drugs exhibited in the above mixtures usually lived as long or approximately as long as the mice fed upon plain cakes, the controls with acetanilide being an exception. In certain cases three different drugs were mixed together and fed, but usually the simpler combination of acetanilide with only one other drug was used. Caffeine and sodium bicarbonate, the two drugs most commonly exhibited with acetanilide, were fed to a greater number of mice than the drugs appearing less frequently. The controls of caffeine were fed 0.040 gram, an amount from two to four times that used in the mixtures of this drug with acetanilide, although even in the above amount it appeared practically nontoxic, the controls living about the same time as the mice receiving plain cakes.

*Toxicity of acetanilide-caffeine mixtures.*—In the experiments recorded below white mice were fed on unmedicated cakes, on cakes containing acetanilide, caffeine, and a mixture of acetanilide and caffeine.

The three tables which follow show the results of feeding experiments carried out as controls to other experiments in which a mixture of caffeine citrate and acetanilide was used:

SERIES I.—*Controls.*

[Plain cakes: Feeding mice unmedicated cakes (4 grams cracker dust to 4 parts water, dried at 50–60° C.).]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1908. | | | | |
| December 3 | 20.38 | 18.04 | 18.40 | 20.94 |
| December 7 | 19.76 | 16.95 | 17.76 | 18.64 |
| December 11 | 19.75 | 16.38 | 17.78 | 18.29 |
| December 18 | 18.50 | 16.02 | 17.40 | 18.53 |
| December 23 | 18.14 | 15.52 | 17.24 | 15.12 |
| December 27 | 16.65 | 14.55 | 15.87 | 14.00 |
| December 30 | 16.28 | 14.01 | 15.47 | 13.16 |
| 1909. | | | | |
| January 4 | 15.02 | 13.33 | 14.97 | 14.47 |
| January 7 | ..... | 13.60 | 14.37 | 13.59 |
| January 11 | ..... | 11.97 | 14.49 | 12.41 |
| January 15 | ..... | ..... | 14.07 | 12.23 |
| January 19 | ..... | ..... | ..... | 11.45 |
| January 23 | ..... | ..... | ..... | 11.40 |

No. 1, dead January 7, 35 days; No. 2, dead January 12, 40 days; No. 3, dead January 18, 46 days; No. 4, dead January 25, 53 days.

[Caffeine: Feeding mice 0.040 gram caffeine citrate per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1908. | | | | |
| December 3 | 14.78 | 18.48 | 17.50 | 22.88 |
| December 7 | 13.12 | 17.32 | 15.06 | 21.52 |
| December 11 | 12.62 | 17.24 | 14.73 | 19.81 |
| December 18 | 11.45 | 16.71 | 13.70 | 20.05 |
| December 23 | 12.18 | 17.15 | 14.21 | 20.47 |
| December 27 | 10.90 | 15.87 | 13.30 | 20.82 |
| December 30 | 11.68 | 14.51 | 13.76 | 20.26 |
| 1909. | | | | |
| January 4 | 11.08 | 14.33 | 13.00 | 19.91 |
| January 7 | ..... | 14.51 | 12.69 | 15.60 |
| January 11 | ..... | 13.47 | 12.31 | 15.31 |
| January 15 | ..... | 11.99 | 11.99 | 15.20 |
| January 19 | ..... | ..... | 11.92 | 15.02 |
| January 23 | ..... | ..... | ..... | 14.96 |

No. 1, dead January 7, 35 days; No. 2, dead January 15, 43 days; No. 3, dead January 16, 44 days; No. 4, dead January 23, 51 days.

[Acetanilide: Feeding mice 0.050 gram acetanilide per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1908. | | | | |
| December 3 | 20.13 | 17.38 | 20.36 | 16.11 |
| December 5 | 17.10 | 13.47 | 16.65 | 14.20 |
| December 7 | ..... | 11.57 | 17.15 | 12.39 |
| December 9 | ..... | ..... | 17.96 | 11.62 |
| December 11 | ..... | ..... | ..... | 11.16 |
| December 15 | ..... | ..... | ..... | 9.91 |
| December 19 | ..... | ..... | ..... | 10.24 |
| December 21 | ..... | ..... | ..... | 9.30 |
| December 23 | ..... | ..... | ..... | 8.79 |

No. 1, dead December 6, 3 days; No. 2, dead December 8, 5 days; No. 3, dead December 10, 7 days; No. 4, dead December 23, 20 days.

Having determined the period which mice fed on the simple drugs, a series of mice were fed upon a mixture of these drugs with the following results:

SERIES I.—*Acetanilide-caffeine.*

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.020 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1908. | | | | |
| December 3 | 14.71 | 15.42 | 14.16 | 14.20 |
| December 5 | 11.70 | 12.64 | 11.97 | 11.77 |
| December 7 | ..... | 11.75 | 12.50 | 12.06 |
| December 9 | ..... | ..... | ..... | 11.51 |

No. 1, dead December 7, 4 days; No. 2, dead December 8, 5 days; No. 3, dead December 8, 5 days; No. 4, dead December 10, 7 days.

A somewhat decreased toxicity was observed when smaller amounts of caffeine were given. The results are tabulated below:

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.010 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1908. | | | | |
| December 3 | 16.29 | 16.53 | 17.41 | 14.42 |
| December 5 | 13.39 | 13.71 | 14.08 | 12.25 |
| December 7 | ..... | 13.09 | 13.08 | 11.02 |
| December 9 | ..... | 12.79 | 11.00 | 9.73 |

No. 1, dead December 7, 4 days; No. 2, dead December 10, 7 days; No. 3, dead December 10, 7 days; No. 4, dead December 11, 8 days.

In the following tables are given the results of a later series of experiments. Control mice fed caffeine citrate 0.040 gram lived a somewhat shorter time than in the previous series, namely, 13, 34, 35, and 40 days. The table showing this control may be omitted, since the relative small degree of toxicity of this drug in this dose is apparent in the above figures. The protocols of control mice fed on simple acetanilide cakes and mice fed on a mixture of acetanilide and caffeine are to be found in the following tables:

SERIES II.—*Control: Acetanilide.*

[Feeding mice acetanilide 0.050 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| February 11 | 18.34 | 20.78 | 18.92 | 20.00 |
| February 13 | 15.43 | 17.80 | 15.59 | 17.29 |
| February 15 | 13.67 | 15.80 | 14.06 | 14.44 |
| February 17 | 14.15 | 14.23 | 13.26 | 12.65 |
| February 20 | ..... | ..... | ..... | 10.80 |

No. 1, dead February 18, 7 days; No. 2, dead February 18, 7 days; No. 3, dead February 19, 8 days; No. 4, dead February 21, 10 days.

*Acetanilide-caffeine*

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.020 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| February 11 | 20.68 | 16.68 | 22.10 | 26.26 |
| February 13 | ..... | 14.00 | 17.97 | 22.78 |
| February 15 | ..... | 13.11 | 18.25 | 20.52 |
| February 17 | ..... | ..... | ..... | ..... |

No. 1, dead February 13, 2 days; No. 2, dead February 16, 5 days; No. 3, dead February 16, 5 days; No. 4, dead February 16, 5 days.

A still later series using acetanilide and caffeine mixtures showed the following results. The caffeine control mice lived 8, 34, 41, and 65 days.

SERIES III.—*Control: Acetanilide.*

[Feeding mice acetanilide 0.050 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| March 6 | 15.20 | 15.28 | 18.25 | 15.08 |
| March 8 | 13.99 | 15.12 | 16.61 | 13.23 |
| March 11 | 11.96 | 13.69 | 14.43 | 12.61 |
| March 13 | ..... | ..... | 13.32 | 11.94 |

No. 1, dead March 12, 6 days; No. 2, dead March 12, 6 days; No. 3, dead March 14, 8 days; No. 4, dead March 14, 8 days.

*Acetanilide-caffeine.*

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.020 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| March 6 | 15.95 | 13.44 | 15.21 | 15.88 |
| March 8 | 14.65 | 11.84 | 14.00 | 13.45 |
| March 11 | ..... | ..... | ..... | 12.19 |

No. 1, dead March 9, 3 days; No. 2, dead March 10, 4 days; No. 3, dead March 11, 5 days; No. 4, dead March 13, 7 days.

Another lot fed smaller amounts of caffeine gave results as follows:

[Feeding mice acetanilide 0.050 gram+caffeine citrate 0.010 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| March 6 | 15.43 | 16.39 | 14.99 | 16.02 |
| March 8 | 14.78 | 14.21 | 13.39 | 14.82 |
| March 11 | ..... | 13.63 | 12.42 | 14.10 |
| March 13 | ..... | ..... | ..... | 13.85 |

No. 1, dead March 10, 4 days; No. 2, dead March 11, 5 days; No. 3, dead March 12, 6 days; No. 4, dead March 15, 9 days.

A summary of the results given in the above tables is given here for the sake of comparison.

SUMMARY.

| Series. | Control. | | | Acetanilide mixtures. | |
|---|---|---|---|---|---|
| | Plain cakes. | Caffeine, 0.040. | Acetanilide, 0.050. | Acetanilide 0.050, caffeine 0.020. | Acetanilide 0.050, caffeine 0.010. |
| | *Days.* | *Days.* | *Days.* | *Days.* | *Days.* |
| I | 35 | 35 | 3 | 4 | 4 |
| | 40 | 43 | 5 | 5 | 7 |
| | 46 | 44 | 7 | 5 | 7 |
| | 53 | 51 | 20 | 7 | 8 |
| Average | 43.50 | 43.25 | 8.75 | 5.25 | 6.50 |
| II | ..... | 13 | 7 | 2 | ..... |
| | ..... | 34 | 7 | 5 | ..... |
| | ..... | 35 | 8 | 5 | ..... |
| | ..... | 40 | 10 | 5 | ..... |
| Average | ..... | 30.50 | 8.00 | 4.25 | ..... |
| III | ..... | ..... | 6 | 3 | 4 |
| | ..... | ..... | 6 | 4 | 5 |
| | ..... | ..... | 8 | 5 | 6 |
| | ..... | ..... | 8 | 7 | 9 |
| Average | ..... | ..... | 7.00 | 4.75 | 6.00 |
| General average | 43.50 | 36.87 | 7.91 | 4.75 | 6.25 |

NOTE.—The figures in this table refer to the number of days from the beginning of the experiment until the death of the animal.

As will be noted, the figures in the above summary show that mice fed on acetanilide lived almost two times as long as those fed on a mixture of acetanilide and caffeine. This indicates that instead of any antagonism, caffeine markedly increases the toxicity of acetanilide when given to white mice with their food, although caffeine itself is scarcely toxic at all even when given in doses from two to four times greater.

Unfortunately, in these and in all the other experiments the mice lost weight quite rapidly, so that starvation may be argued as a partial reason for the animal's death. This seems to be an insufficient reason, however, as there is no relation between the loss in weight and the death of the animal, some decreasing in weight as much as 50 per cent before death, others decreasing only 10 to 20 per cent. A more important argument is furnished by certain experiments in which a record was kept of the number of cakes each mouse ate. Excepting in those cases where the animal died within the first two or three days, there was approximately the same average amount of cake eaten per day, whether the cakes contained acetanilide alone or a mixture of acetanilide and caffeine. The figures covering this point are as follows: Average amount of cake eaten per day—acetanilide, 0.51 per cent; of mixture containing 0.020 gram caffeine citrate, 0.62 per cent; of mixture containing 0.010 gram caffeine citrate, 0.69 per cent. In other words, the animal eating the most cake per day, grouping the mixtures containing caffeine, died in the

shortest length of time, and if starvation were a factor it is evident that they should have lived the greater period of time.

Again, it may be argued that death naturally resulted more quickly from the toxic action of the greater amount of acetanilide consumed by those mice which ate the most cake. But the figures do not bear out this assumption. In the first place, of the two lots receiving caffeine that receiving the smaller amount lived the longer, and yet this lot consumed more cake, and therefore acetanilide in the ratio of 62 to 69. In the second place, the length of time until death of the acetanilide control mice and the acetanilide mixture (0.020 gram caffeine) mice are not in even an approximate ratio to the amount of cake eaten: Ratio of cake eaten, 100 to 119; ratio of days until death, 100 to 166. The conclusion seems unavoidable, therefore, that caffeine adds to the toxicity of acetanilide.

## TOXICITY OF ACETANILIDE AND SODIUM BICARBONATE MIXTURES.

Other experiments were carried out in which mice were fed cakes containing sodium bicarbonate mixed with acetanilide. In one series, also, caffeine citrate was added to this mixture, giving a compound acetanilide powder quite closely imitating the United States pharmacopœial preparation of this name. Control mice were given sodium bicarbonate 0.020 gram per cake, which amount showed no toxic properties. Other controls fed on sodium bicarbonate 0.020 gram plus caffeine citrate 0.020 gram appeared relatively nontoxic, the animals living for 25, 27, 35, and 36 days. The other controls for this series of experiments have already been given, this work being carried out at the same time and as a part of the series already tabulated. Accordingly they will not be recorded again, the only data to be repeated appearing in the summary of the following experiments:

SERIES I.—*Acetanilide—caffeine—sodium bicarbonate.*

[Feeding mice acetanilide 0.050 gram + caffeine citrate 0.020 gram + sodium bicarbonate 0.020 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | No. 1. | No. 2. | No. 3. | No. 4. |
| | *Days.* | *Days.* | *Days.* | *Days.* |
| December 3 | 16.92 | 21.26 | 18.15 | 15.84 |
| December 5 | 13.13 | 17.90 | 15.15 | 15.50 |
| December 7 | 12.39 | 14.58 | 12.89 | ..... |
| December 9 | 11.53 | ..... | ..... | ..... |

No. 1, dead December 10, 7 days; No. 2, dead December 9, 6 days; No. 3, dead December 9, 6 days; No. 4, dead December 6, 3 days.

The following table gives the results of giving smaller amounts of caffeine.

[Feeding mice acetanilide 0.050 gram + caffeine citrate 0.010 gram + sodium bicarbonate 0.020.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | No. 1. | No. 2. | No. 3. | No. 4. |
| | *Days.* | *Days.* | *Days.* | *Days.* |
| December 3 | 16.39 | 16.16 | 14.92 | 18.40 |
| December 5 | 14.06 | 13.47 | 12.06 | ..... |
| December 7 | 13.48 | 12.44 | 11.86 | ..... |
| December 9 | 12.95 | 13.80 | ..... | ..... |
| December 13 | 13.65 | ..... | ..... | ..... |
| December 15 | 12.54 | ..... | ..... | ..... |
| December 17 | 13.57 | ..... | ..... | ..... |

No. 1, dead December 17, 14 days; No. 2, dead December 11, 8 days; No. 3, dead December 9, 6 days; No. 4, dead December 5, 2 days.

In a later series of experiments sodium bicarbonate was mixed with acetanilide, the caffeine being omitted. The results of experiments of this sort are tabulated below:

SERIES II AND IV.—*Acetanilide and sodium bicarbonate.*

[Feeding mice acetanilide 0.050 gram + sodium bicarbonate 0.020 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | No. 1. | No. 2. | No. 3. | No. 4. |
| 1909. | *Days.* | *Days.* | *Days.* | *Days.* |
| February 11 | 21.50 | 21.61 | 22.68 | 18.52 |
| February 13 | 18.50 | 18.23 | 20.10 | 16.02 |
| February 15 | 16.95 | 16.10 | 16.84 | 14.50 |
| February 17 | 15.90 | 14.71 | 15.42 | 13.46 |
| February 20 | 14.50 | 13.80 | ..... | ..... |
| March 15 | 15.82 | 17.58 | 17.30 | 19.18 |
| March 18 | 13.66 | 16.51 | 16.00 | 16.18 |
| March 20 | 12.03 | 14.10 | 13.73 | 13.82 |
| March 22 | 10.73 | 12.15 | 12.50 | 13.43 |
| March 24 | 10.70 | 11.44 | 11.50 | ..... |
| March 26 | 10.41 | 10.55 | ..... | ..... |

No. 1, dead February 21, 10 days, and March 28, 13 days; No. 2, dead February 21, 10 days, and March 27, 12 days; No. 3, dead February 20, 9 days, and March 25, 10 days; No. 4, dead February 19, 8 days, and March 24, 9 days.

As a control for Series IV mice were fed on acetanilide 0.050 per cake and lived for 7, 7, 9, and 13 days, respectively.

To determine whether a larger amount of the carbonates would still further lessen the toxicity, a series of mice were fed cakes each containing 0.040 gram sodium bicarbonate. Again this mixture showed the antidotal action of an alkali carbonate, and while this is somewhat greater with the increase of alkali the figures do not indicate any special advantage. The averages are given in the following table:

| Series. | Acetanilide 0.050. | Acetanilide 0.050, $NaHCO_3$ 0.020. | Acetanilide 0.050, $NaHCO_3$ 0.040. |
|---|---|---|---|
| | *Days.* | *Days.* | *Days.* |
| II+IV | 8.50 | 10.10<br>Advantage 1.60 | ..... |
| III | 7.00 | ..... | 8.75<br>Advantage 1.75 |

The following summaries are arranged in order to bring the results of these experiments together for the sake of comparison:

SUMMARY.

| Series. | Control. | | Acetanilide mixture. |
|---|---|---|---|
| | Caffeine 0.020, $NaHCO_3$ 0.020. | Acetanilide 0.050. | Acetanilide 0.050, $NaHCO_3$ 0.020. |
| | *Days.* | *Days.* | *Days.* |
| II | 25 | 7 | 8 |
| | 27 | 7 | 9 |
| | 35 | 8 | 10 |
| | 36 | 10 | 10 |
| IV | ..... | 7 | 9 |
| | ..... | 7 | 10 |
| | ..... | 9 | 12 |
| | ..... | 13 | 13 |
| Average | 30.75 | 8.50 | 10.10 |

By comparing the results as given in the above summary it will be noted that in the case of mice sodium bicarbonate is antagonistic to acetanilide to a certain degree in the general average of the two series, as 8.50 is to 10.10. This same antagonism is also shown in the experiments in which caffeine was also a constituent of the mixture, a summary of which is given below:

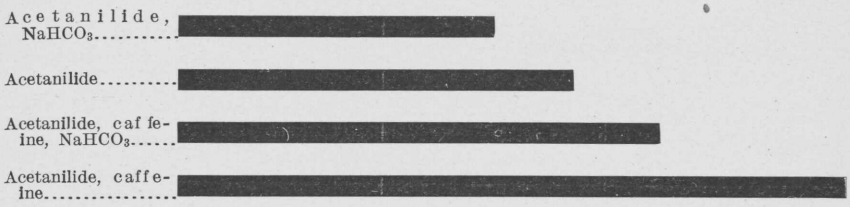
SUMMARY.

| Series. | Control. | | | Acetanilide mixture. | |
|---|---|---|---|---|---|
| | Acetanilide 0.050. | Acetanilide 0.050, caffeine 0.020. | Acetanilide 0.050, caffeine 0.010. | Acetanilide 0.050, caffeine 0.020, $NaHCO_3$ 0.020. | Acetanilide 0.050, caffeine 0.010, $NaHCO_3$ 0.020. |
| | *Days.* | *Days.* | *Days.* | *Days.* | *Days.* |
| I | 3 | 4 | 4 | 3 | 2 |
| | 5 | 5 | 7 | 6 | 6 |
| | 7 | 5 | 7 | 6 | 8 |
| | 20 | 7 | 8 | 7 | 14 |
| Average | 8.75 | 5.25 | 6.50 | 5.50 | 7.50 |

These results do not show quite so marked antagonism between acetanilide and sodium bicarbonate as is shown by the results given in the preceding summary. Their agreement serves, nevertheless, as very valuable contributory evidence of the lessened toxicity of a mixture of acetanilide containing sodium bicarbonate. It will be observed, however, that despite some antidotal action this is still insufficient to make acetanilide mixtures containing caffeine as non-toxic as those which omit it entirely. This is illustrated by the following data, the averages of series No. I, II, and III:

| | Acetanilide 0.05. | Acetanilide 0.05, caffeine 0.02. | Acetanilide 0.05, caffeine 0.02, $NaHCO_3$ 0.02. | Acetanilide 0.05, $NaHCO_3$ 0.02. |
|---|---|---|---|---|
| General average | 7.91 | 4.75 | 5.50 | 10.10 |

This may be represented graphically as follows:
The degree of toxicity being represented by the length of the bars.




## TOXICITY OF ACETANILIDE AND THE OPIUM ALKALOIDS.

Experiments were carried out upon white mice to determine the toxicity of mixtures composed of acetanilide and codeine and acetanilide and morphine. Control mice were fed morphine sulphate 0.002 gram per cake lived 20, 27, 36, and 45 days. Controls of codeine-fed mice lived 10, 29, 61, and 65 days. As in the previous experiments where the results of the same series have been recorded, the acetanilide controls will not be given again except as data to complete the summary of these experiments.

SERIES II.—*Acetanilide-codeine.*

[Feeding mice acetanilide 0.050, codeine 0.010 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| February 11 | 22.85 | 15.20 | 17.08 | 15.88 |
| February 13 | 20.34 | 12.86 | 14.47 | 13.25 |
| February 15 | 18.74 | 10.98 | 14.38 | 11.31 |
| February 17 | 16.38 | 9.59 | 12.16 | 9.30 |
| February 20 | 16.18 | 9.36 | 11.74 | 8.92 |
| February 24 | 14.69 | ..... | ..... | ..... |

No. 1, dead February 26, 15 days; No. 2, dead February 23, 12 days; No. 3, dead February 21, 10 days; No. 4, dead February 21, 10 days.

[Feeding mice acetanilide 0.050, codeine 0.005 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| February 11 | 15.48 | 19.34 | 17.06 | 14.48 |
| February 13 | 14.55 | 16.63 | 15.42 | 13.62 |
| February 15 | 12.02 | 13.88 | 13.10 | 12.34 |
| February 17 | 11.19 | 11.93 | 12.05 | ..... |
| February 20 | 10.38 | 10.48 | ..... | ..... |

No. 1, dead February 21, 10 days; No. 2, dead February 21, 10 days; No. 3, dead February 20, 9 days; No. 4, dead February 16, 5 days.

A summary of the results of this series is given in the following table to make the comparison of the toxicity easier.

SUMMARY.

| Series. | Control. | | Acetanilide mixtures. | |
|---|---|---|---|---|
| | Codeine, 0.010. | Acetanilide, 0.050. | Acetanilide, 0.050, codeine, 0.005. | Acetanilide, 0.050, codeine, 0.010. |
| | *Days.* | *Days.* | *Days.* | *Days.* |
| II | 10 | 7 | 5 | 10 |
| | 29 | 7 | 9 | 10 |
| | 61 | 8 | 10 | 12 |
| | 65 | 10 | 10 | 15 |
| Average | 41.25 | 8.00 | 8.50 | 11.75 |

The data shown in this summary indicates that codeine is antidotal to the general toxic effects of acetanilide. This result was rather unexpected, but the fact that the mice receiving the larger dose of codeine in combination with acetanilide lived the longer served to confirm these findings. The number of mice fed upon mixtures of this sort was too small however to give results from which to draw absolute conclusions and accordingly a further series of mice were fed, using heroine and morphine in addition to codeine. The results of these experiments are given in the following tables.

*Control[a] acetanilide.*

[Feeding mice acetanilide 0.050 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| April 17 | 17.17 | 19.39 | 15.19 | 19.30 |
| April 19 | 14.20 | 16.63 | 12.82 | 16.83 |
| April 21 | 13.83 | 15.04 | 12.55 | 15.05 |
| April 23 | 12.16 | 13.89 | 11.03 | 14.55 |
| April 26 | 11.27 | 12.44 | 10.56 | ..... |

[a] The control mice receiving 0.010 gram codeine, 0.002 gram morphine sulphate, and 0.00075 gram heroine hydrochloride are still alive at this date (April 29, 1909).

No. 1, dead April 28, 11 days; No. 2, dead April 27, 10 days; No. 3, dead April 27, 10 days; No. 4, dead April 24, 7 days.

In the following table the protocols of mice fed upon acetanilide and codeine are given:

SERIES V.—*Acetanilide-codeine.*

[Feeding mice acetanilide 0.050 gram plus codeine 0.010 gram per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| April 17 | 17.73 | 20.67 | 18.57 | 22.31 |
| April 19 | 14.75 | 16.63 | 15.71 | 17.85 |
| April 21 | 13.45 | 15.43 | 14.08 | 16.76 |
| April 23 | 12.62 | 14.24 | 12.28 | ..... |
| April 26 | 10.86 | ..... | ..... | ..... |

No. 1, dead April 27, 10 days; No. 2, dead April 24, 7 days; No. 3, dead April 23, 6 days; No. 4, dead April 23, 6 days.

The following table gives the results of feeding mice acetanilide 0.050 plus morphine sulphate 0.002 gram per cake:

*Acetanilide-morphine.*

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909 | | | | |
| April 17 | 18.23 | 18.09 | 14.26 | 16.48 |
| April 19 | 15.73 | 14.92 | 11.23 | 13.07 |
| April 21 | 15.29 | 13.27 | 9.78 | 12.30 |
| April 23 | 14.78 | 12.12 | ..... | ..... |

No. 1, dead April 24, 7 days; No. 2, dead April 24, 7 days; No. 3, dead April 23, 6 days; No. 4, dead April 22, 5 days.

Another series of mice were fed cakes containing a smaller amount of morphine, with the following results:

*Acetanilide-morphine.*

[Feeding mice acetanilide 0.050 gram plus 0.001 gram morphine sulphate per cake.]

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | 1. | 2. | 3. | 4. |
| 1909. | | | | |
| April 17 | 14.53 | 14.89 | 13.43 | 15.52 |
| April 19 | 12.22 | 12.60 | 12.43 | 12.91 |
| April 21 | 12.22 | 12.30 | 11.44 | 11.08 |
| April 23 | 12.16 | 11.26 | 10.16 | ..... |

No. 1, dead April 25, 8 days; No. 2, dead April 25, 8 days; No. 3, dead April 24, 7 days; No. 4, dead April 23, 6 days.

A series of mice were also fed acetanilide 0.050 gram and heroine 0.00075 gram per cake with the following results:

*Acetanilide-heroine.*

| Date. | Weight of mice in grams. | | | |
|---|---|---|---|---|
| | No. 1. | No. 2. | No. 3. | No. 4. |
| 1909. | | | | |
| April 17 | 19.10 | 15.88 | 17.10 | 14.32 |
| April 19 | 16.75 | 13.69 | 14.37 | 12.53 |
| April 21 | 15.56 | 11.42 | 16.23 | ..... |
| April 23 | 14.01 | ..... | ..... | ..... |

No. 1, dead April 24, 7 days; No. 2, dead April 23, 6 days; No. 3, dead April 22, 5 days; No. 4, dead April 21, 4 days.

A second series of mice fed upon codeine, acetanilide, and heroine and morphine gave results the general averages of which are as follows: Acetanilide 0.050 gram and codeine 0.010 gram, lived six days; acetanilide 0.050 gram and morphine 0.002 gram, three days; acetani-

lide 0.050 gram and heroine 0.00075 gram, 7 days. These results are brought together in the following summary for the sake of comparison:

| Series. | Control. | Acetanilide mixture. | | | |
|---|---|---|---|---|---|
| | Acetanilide, 0.050. | Acetanilide 0.050, morphine 0.002. | Acetanilide 0.050, morphine 0.001. | Acetanilide 0.050, codeine 0.010. | Acetanilide 0.050, heroine 0.00075. |
| V | 7 | 5 | 6 | 6 | 4 |
| | 10 | 6 | 7 | 6 | 5 |
| | 10 | 7 | 8 | 7 | 6 |
| | 11 | 7 | 8 | 10 | 7 |
| VI | 6 | 3 | ..... | 6 | 6 |
| | 9 | 8 | ..... | 5 | 8 |
| | 11 | 10 | ..... | 8 | 8 |
| | 11 | ..... | ..... | ..... | ..... |
| Average.. | 9.37 | 6.57 | 7.25 | 6.85 | 6.28 |

As will be noted, the figures given in this summary do not confirm those of Series III, but show exactly the opposite effect, the toxicity of acetanilide being increased by codeine, heroine, and morphine. The contributory evidence of morphine and heroine is of special value when it is remembered the similarity in the effects produced by the drugs of the opium series and serves to substantiate the findings with codeine. The much larger number of mice and the uniformity in the results of both Series V and VI make the results of Series III probably incorrect. The longer life of acetanilide-codeine mice in the earlier series is probably to be explained by some external variation in the condition of the mice which escaped the notice of the experimenter.[a]

The results of these experiments indicate, therefore, that the toxicity of acetanilide is considerably increased when combined with the above opium alkaloids.

### TOXICITY OF ACETANILIDE AND SALICYLIC ACID.

In a series of experiments mice were fed on cakes each containing 0.050 gram acetanilide and 0.020 gram salicylic acid. The control mice fed on acetanilide alone, 0.050 gram per cake, lived 7, 7, 8, and 10 days, or an average of 8 days. The mice receiving the two drugs mixed in the above proportions lived 6, 7, 9, and 10 days, an average of 8 days,[b] or, in other words, lived the same length of time as the acetanilide controls. Another series fed on slightly larger amounts of salicylic acid (0.030 gram with acetanilide 0.050 gram per cake)

[a] Mice are especially sensitive to changes in temperature, and the unusual results of this series may be explained upon these grounds. During the course of the experiments the mice were moved into a room where part were much closer to a steam radiator than others, and it is very likely that those kept in the warmest position lived the longer.

[b] The control mice fed on 0.030 gram salicylic acid lived for 50, 62, and 66 days.

lived 6, 6, 7, 10 days, an average of 7.25 days. The control mice fed on acetanilide alone, 0.050 gram per cake, lived 6, 6, 8, and 8 days, an average of 7 days. These results show that salicylic acid neither increases nor decreases the toxic effect of acetanilide.

### TOXICITY OF ACETANILIDE AND SODIUM BROMIDE.

Mice fed upon a mixture of acetanilide 0.050 and sodium bromide 0.030 gram per cake lived approximately as long as the control, an average of 8 days, the controls an average of 8.25 days. This indicates no alteration in toxicity.

The method of feeding mice upon cakes gives the results from long continued use of a drug, but the amount taken is entirely dependent upon the appetite of the animal. Therefore, to confirm the above results, to make more certain the exact amount of drug given, and to test the action of acetanilide mixtures when given in amounts sufficiently large to produce acute poisoning, the following experiments were carried out upon guinea pigs. These animals were all of about the same weight, obtained from the same lot, and were kept under the same conditions. The dose was estimated per gram of body weight, each dose being weighed separately and made into pills of suitable size with mucilage of acacia and arrow-root starch. These were dried until a slight crust was formed on the outside and then fed in such a manner that none of the drug was lost during administration. The protocols in the following table show the toxicity of acetanilide as determined by this method of administration:

[Acetanilide control: Determination of the minimum lethal dose of acetanilide for guinea pigs. —=survived; +=death.]

| Weight in grams. | Dose per gram weight in grams. | Result. | Hours till death. |
|---|---|---|---|
| 515 | 0.0008 | — | ..... |
| 490 | .0010 | — | ..... |
| 500 | .0010 | — | ..... |
| 450 | .0010 | — | ..... |
| 500 | .0010 | + | 14 |
| 465 | .0012 | — | ..... |
| 445 | .0012 | — | ..... |
| 422 | .0014 | + | 10¼ |
| 485 | .0014 | + | 12 |
| 406 | .0016 | + | 9¼ |
| 465 | .0016 | + | 32 |
| 402 | .0018 | + | 12½ |

Control experiments with caffeine citrate gave the following results:

[Caffeine control: Determination of the minimum lethal dose of caffeine citrate for guinea pigs. −= survived; += death.]

| Weight in grams. | Dose per gram weight in grams. | Result. | Hours till death. |
|---|---|---|---|
| 530 | 0.0003 | − | ..... |
| 480 | .0004 | − | ..... |
| 495 | .0004 | − | ..... |
| 405 | .0004 | + | 16 |
| 435 | .00045 | − | ..... |
| 415 | .0005 | − | ..... |
| 410 | .0005 | − | ..... |
| 565 | .0005 | + | 15 |
| 405 | .00055 | + | 15 |
| 415 | .0006 | + | 7 |

In like manner a series of guinea pigs were given pills containing acetanilide and caffeine citrate. The results of these experiments are tabulated below:

*Acetanilide-caffeine mixture.*—Determination of the minimum lethal dose of a mixture of acetanilide and caffeine citrate.

[−= survived; += death; A=acetanilide; C=caffeine citrate.]

| Weight in grams. | Dose per gram weight in grams. | Result. | Hours till death. |
|---|---|---|---|
| 555 | A 0.0006<br>C .0002 | − | ..... |
| 545 | A .0006<br>C .0003 | − | ..... |
| 580 | A .0008<br>C .0002 | − | ..... |
| 520 | A .0008<br>C .0003 | − | ..... |
| 600 | A .0010<br>C .0002 | + | 21 |
| 380 | A .0010<br>C .0002 | + | 13 |
| 460 | A .0010<br>C .0003 | + | 17 |
| 350 | A .0012<br>C .0002 | + | 20 |
| 425 | A .0012<br>C .0003 | + | 112 |
| 385 | A .0014<br>C .0002 | + | 23½ |
| 450 | A .0014<br>C .0003 | + | 19½ |

The minimum lethal dose of acetanilide for guinea pigs appears by these experiments to be approximately 0.0013 gram and that of caffeine citrate about 0.00055. The mixture of caffeine (caffeine about one-third of the least fatal dose) and acetanilide shows an increased toxicity, a dose of 0.0010 gram acetanilide in a mixture with caffeine being sufficient to invariably cause death instead of 0.0013 gram per gram of body weight, as is the case when given

alone. These results serve to confirm the results of the feeding experiments upon mice and show very clearly the absence of any antagonistic action.

### TOXICITY OF ACETANILIDE AND SODIUM BICARBONATE.

Experiments using equal parts of acetanilide and sodium bicarbonate were carried out in the same manner as the above experiments. The animals used in this series had been given acetanilide a week previously and had apparently recovered. In the controls for this series a smaller fatal dose showed that they were more susceptible, however, than in the earlier experiments, death invariably resulting from 0.0011 gram per gram of body weight. The pigs receiving the mixture containing sodium bicarbonate were somewhat more resistant, with one exception, the least fatal dose appearing to be approximately 0.0014 gram per gram of body weight, thus indicating that the carbonates are antagonistic to acetanilide to a certain extent. These experiments are tabulated below.

### ACETANILIDE-SODIUM BICARBONATE.

[Determination of the lethal dose of a mixture of acetanilide and sodium bicarbonate. − = survived; + = death; A = acetanilide; S = sodium bicarbonate.]

| Weight in grams. | Dose per gram weight in grams. | Result. | Hours till death. |
|---|---|---|---|
| 365 | A 0.0010<br>S .0010 | + | 36 |
| 380 | A .0012<br>S .0012 | − | ..... |
| 375 | A .0014<br>S .0014 | − | ..... |
| 340 | A .0014<br>S .0014 | + | 13½ |
| 485 | A .0015<br>S .0015 | + | 50 |
| 315 | A .0016<br>S .0016 | + | 7¾ |

The lessened toxicity of this mixture was further confirmed by comparing the duration of life under the same dosage either of the simple remedy or the mixture. The time guinea pigs lived after increasing doses of acetanilide alone was 20, 4¾, 21, 9⅓, 4½, 13, and 26⅓ hours. Pigs receiving corresponding doses of the mixture lived 36, 13½, 50, 7¾, 13½, 15, and 18 hours. The sum of the hours of duration of life after dosage therefore is 99½ for the simple remedy; 153¾ hours for the mixture. Rather little emphasis is intended for this point, however, because of the great irregularity in the time the animals lived, as will be noted in the above figures, which are arranged in order of the increase in amount of drug given.

# PART II.

## THE TOXICITY OF ANTIPYRINE MIXTURES.

Although it is now generally recognized that antipyrine is less toxic than acetanilide[a] it has never been so popular as an ingredient of headache mixtures. It is occasionally dispensed with other drugs, however (as in bromopyrine for example), and accordingly a series of experiments were made to determine whether these altered its toxicity.

### ACTION UPON THE FROG'S HEART.

The method used to determine the effect of antipyrine and mixtures of antipyrine upon the frog's heart was the same as that described for acetanilide, page 13. The species of frog and the various precautions used to obtain uniform results were also the same.

Although affecting the frog's heart when perfused through it in much the same way as acetanilide, antipyrine is much less toxic to it. This ratio is about 1 to 6 or 7. A 1 per cent solution of antipyrine usually stopped the heart at once; one-half per cent solutions decreased the rate quite markedly, but this effect was far more pronounced immediately after the introduction of the drug. Later the heart apparently became accustomed to the poisonous effects and little or no further slowing resulted, while in some cases actual increase in rate was observed after the primary slowing, a phenomenon which was also present after acetanilide, but less frequently. Solutions of 0.8 per cent were generally found to be strong enough to cause marked changes and only occasionally stop the heart within a short time. This latter reaction appeared so erratically, however, that no conclusions could be drawn from the results obtained. In one case the heart might continue to beat for a half hour or more after the primary slowing with absolutely no further changes in rate. In other instances in experiments carried out in exactly the same manner, the heart would stop as soon as the drug reached it, a variability in action which seems to be somewhat more marked with this drug than with acetanilide. The changes in output were also similarly irregular, so that any conclusions were necessarily indefinite.

The addition of caffeine citrate to an antipyrine solution caused no material change in the course of the poisoning, especially in regard to the efficiency of the heart. The primary slowing induced by 0.8 per cent solutions of antipyrine was not prevented, the experiments indicating on the other hand that the slowing was greater from the combined action of the two drugs than from antipyrine alone. This represents the general result from a large number of experiments, but exceptions occasionally occurred.

[a]According to Cushny, Pharmacology and Therapeutics, 1906, p. 371, antipyrine is more toxic than phenacetine and less toxic than acetanilide.

The amount of fluid perfused is so irregular that no conclusions can be drawn from changes in this factor. Experiments illustrative of these changes are given in table I.

TABLE I.—*Perfusion of the isolated frog's heart with antipyrine and with antipyrine and caffeine citrate in Ringer's solution.*

| Protocol 14. | | | Protocol 17. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | *C. c.* |
| 10. 45 | 39 | 30 | 9. 25 | 20 | 35 |
| 10. 50 | 38 | 32 | 9. 30 | 18 | 34 |
| Antipyrine 0.8 per cent + caffeine $\frac{1}{2000}$ per cent. | | | 9. 35 | 19 | 34 |
| 10. 52 | 19 | ..... | Antipyrine 0.8 per cent + caffeine $\frac{1}{2000}$ per cent. | | |
| 10. 55 | 15 | 17 | 9. 40 | 17 | 25 |
| 11. 00 | 16 | 13 | 9. 45 | 18 | 24 |
| 11. 10 | 20 | 5 | 9. 50 | 13 | 10 |
| 11. 30 | 18 | 6 | Antipyrine 0.8 per cent. | | |
| 11. 40 | 19 | 9 | 9. 55 | 17 | 5 |
| Antipyrine 0.8 per cent. | | | 10. 00 | 17 | 4 |
| 11. 42 | 21 | ..... | 10. 10 | 18 | 5 |
| 11. 45 | 21 | 10 | 10. 53 | Still beating. | |
| 11. 50 | 20 | 9 | | | |
| 11. 55 | 19 | 10 | | | |

As had been the result in the case of acetanilide it was hoped that more dilute solutions of the drugs might give more definite results. Accordingly in a series of experiments the perfusions were begun with antipyrine in 0.5 per cent solution, and after sufficient readings had been recorded to establish a normal a caffeine-antipyrine solution was turned on. It was shown by these experiments that caffeine quite consistently lessened the toxic effect of antipyrine to a slight extent, although occasional exceptions occurred. The protocols in Table II illustrate the results obtained.

TABLE II.—*Perfusion of the isolated frog's heart with antipyrine and antipyrine-caffeine dissolved in Ringer's solution.*

| Protocol 26. | | | Protocol 29. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | *C. c.* |
| Antipyrine 0.5 per cent. | | | Antipyrine 0.5 per cent. | | |
| 9. 55 | 25 | 53 | 3. 30 | 24 | 49 |
| 10. 00 | 22 | 51 | 3. 35 | 23 | 46 |
| 10. 05 | 22 | 46 | 3. 40 | 19 | 46 |
| 10. 10 | 22 | 45 | 3. 45 | 19 | 46 |
| 10. 15 | 20 | 42 | Antipyrine 0.5 per cent + caffeine $\frac{1}{3000}$ per cent. | | |
| Antipyrine 0.5 per cent + caffeine $\frac{1}{3000}$ per cent. | | | 3. 47 | 23 | ..... |
| 10. 17 | 22 | ..... | 3. 50 | 24 | 56 |
| 10. 20 | 23 | 45 | 3. 55 | 19 | 52 |
| 10. 25 | 22 | 44 | | | |
| 10. 30 | 22 | 44 | | | |
| 10. 35 | 20 | 44 | | | |
| 10. 40 | 19 | 41 | | | |

The use of sodium bicarbonate[a] has been suggested to relieve the gastric irritation which sometimes follows the use of antipyrine. Accordingly a series of perfusion experiments were carried out, using sodium bicarbonate in conjunction with antipyrine. The effect was to lessen but not abolish the poisonous action of antipyrine, both rate and output being increased to a considerable degree, although never to normal. The following protocols illustrate this antagonism:

TABLE III.—*Perfusion of the isolated frog's heart with antipyrine and with antipyrine and sodium bicarbonate in Ringer's solution.*

| Protocol 81. | | | Protocol 85. | | |
|---|---|---|---|---|---|
| Time. | Rate. | Output per 5 minutes. | Time. | Rate. | Output per 5 minutes. |
| | | *C. c.* | | | *C. c.* |
| 10. 35 | 35 | Antipyrine 0.8 per cent. | 3. 20 | 32 | Antipyrine ½ per cent. |
| 10. 40 | 24 | 20 | 3. 50 | 23 | 26 |
| 10. 45 | 24 | 13 | 3. 53 | 21 | 27 |
| 10. 50 | 24 | 8 | 4. 00 | 21 | 27 |
| Antipyrine 0.8 per cent + $NaHCO_3$ $\frac{1}{20}$ per cent. | | | Antipyrine ½ per cent + $NaHCO_3$ $\frac{1}{20}$ per cent. | | |
| 10. 52 | 28 | ..... | 4. 02 | 24 | ..... |
| 10. 53 | 32 | ..... | 4. 05 | 22 | 32 |
| 10. 55 | 25 | 14 | 4. 10 | 19 | 30 |
| 11. 00 | 28 | 17 | 4. 15 | 20 | 32 |
| 11. 15 | 27 | 16 | 4. 20 | 21 | 30 |
| | | | Antipyrine ½ per cent. | | |
| | | | 4. 22 | 17 | ..... |
| | | | 4. 25 | 16 | 21 |
| | | | 4. 29 | 0 | ..... |

## ACTION ON THE MAMMALIAN HEART.

The changes in the dog's heart induced by antipyrine injections were also determined, using the same methods as for acetanilide, except that the perfect solubility of antipyrine made its injection as an emulsion with acacia unnecessary. As in the perfusion experiments upon the frog's heart, antipyrine was found to be very much less poisonous to the dog's heart than acetanilide. Small doses (0.5 to 1 gram) were practically devoid of any depressant action, and a dose of 0.500 gram was actually stimulant, increasing the amplitude 15 millimeters through an increase in the completeness of the systole. Doses of 1 gram also increased the amount of contraction, but the diastole was less complete, so that the result was a slight decrease in the amplitude. The injections of still larger amounts produced a very definite decrease in the heart's action, but the change was not at all comparable to the depression following acetanilide in the same dose. Figure 7 is given to show the stimulant action of 1 gram antipyrine (compare this with the depressant action of 1 gram acetanilide. (Fig. 1, p. 21.)

[a] Am. J. Pharm., 1888, XVIII, 180.

Antipyrine in 500 milligram doses has only a slight depressant action on the heart rate, but with the injection of larger amounts the slowing became quite pronounced. The blood pressure is also markedly affected, being lowered to such a degree that neither the slowing

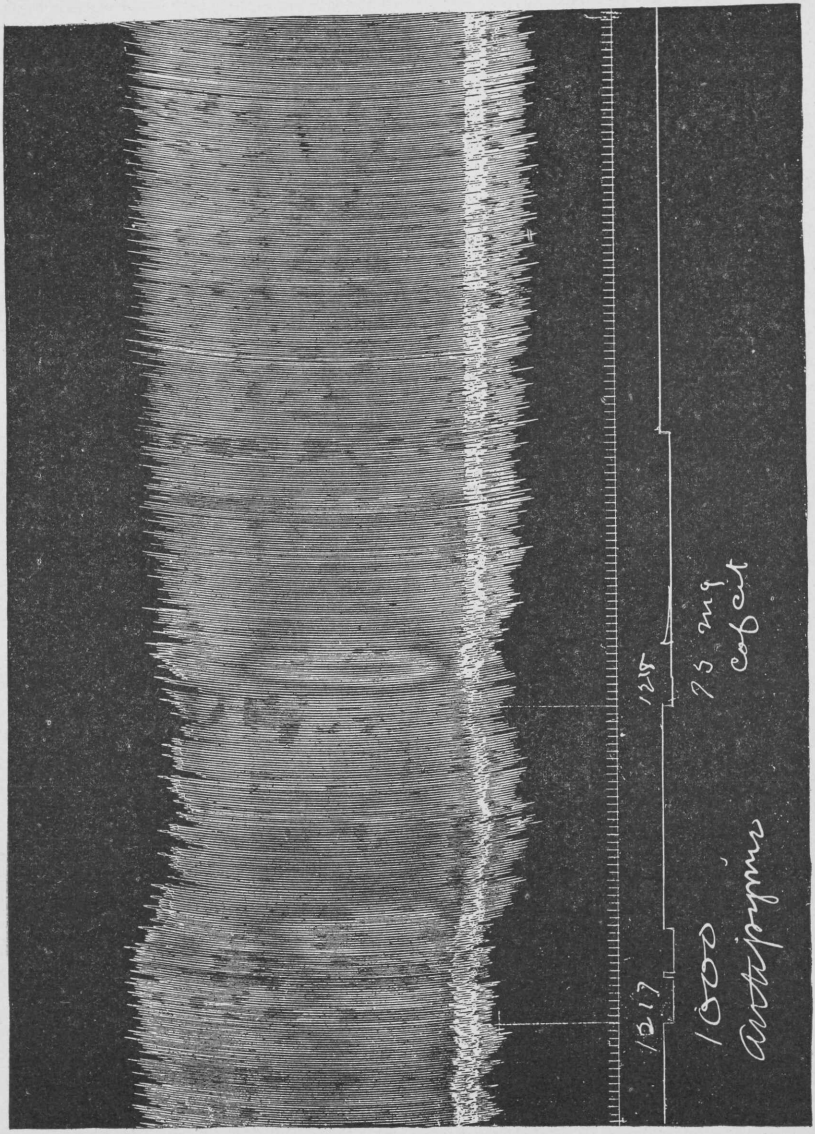



FIG. 7.—Tracing of ventricular contractions under antipyrine and caffeine citrate. The contraction of the heart pulls the lever downward. This tracing is given to show the difference between the heart effect of antipyrine and acetanilide, Fig. 1, page 21.

nor the changes in the efficiency of the heart afford a sufficient reason for its depression. For instance, with a lessened amplitude of 3 millimeters and a decrease in rate of only 10 beats per minute, the pressure fall after an injection of a 1 gram dose amounted to 58

millimeters mercury, or a fall of 42 per cent (fig. 8). The obvious conclusion, therefore, is that antipyrine either depresses the vasomotor center or dilates the peripheral vessels from a local action, and both factors may play some part in this marked fall in pressure. The decrease in the heart rate is not prevented by paralysis of the vagi, and would be due, therefore, to a direct depression of the heart muscle.

Caffeine was injected coincident with and also subsequent to the injection of antipyrine, but appeared to be devoid of any antagonistic action upon either the depressant effect of antipyrine on the strength of the heart or upon the blood pressure. As a matter of fact, when caffeine was injected at the time when the heart was most depressed or just beginning to recover from the antipyrine, caffeine appeared to delay the recovery or to cause a secondary weakening in both the heart strength and the blood pressure. Some antagonism was shown upon the heart rate which was restored to normal by the caffeine injections.

## GENERAL TOXIC ACTION.

The general toxic effect of antipyrine and mixtures containing antipyrine when given hypodermically to mice was the subject of a further series of experiments. The easy solubility of antipyrine in water obviated the introduction of the additional factor, alcohol, into the problem, as in the case of acetanilide. The drug was given by the method already described and the minimum lethal dose determined. This was found to be 0.0010 gram, which amount caused death in about half an hour. By comparison, it will be noted that this is not only a smaller dose than was required in the case of acetanilide, but also that the time the animal survived was much shorter. The reason for this apparently greater toxicity (upon the frog's heart about seven times less toxic) when compared with acetanilide is probably dependent upon the relative solubility of the two drugs in the fluids of the tissues, and hence upon the rate of their absorption. Table IV gives protocols showing the determination of the minimum lethal dose for this drug.

TABLE IV.—*Minimum lethal dose of antipyrine for white mice, hypodermic injection.*

[−=survived; +=death.]

| Weight. | Dose. | Result. | Minutes till death. |
|---|---|---|---|
| 14.33 | 0.0008 | − | ..... |
| 20.00 | .0008 | − | ..... |
| 19.70 | .0009 | − | ..... |
| 17.17 | .0010 | + | 30 |
| 19.92 | .0010 | + | 80 |
| 15.47 | .0011 | + | 25 |
| 18.89 | .0012 | + | 35 |

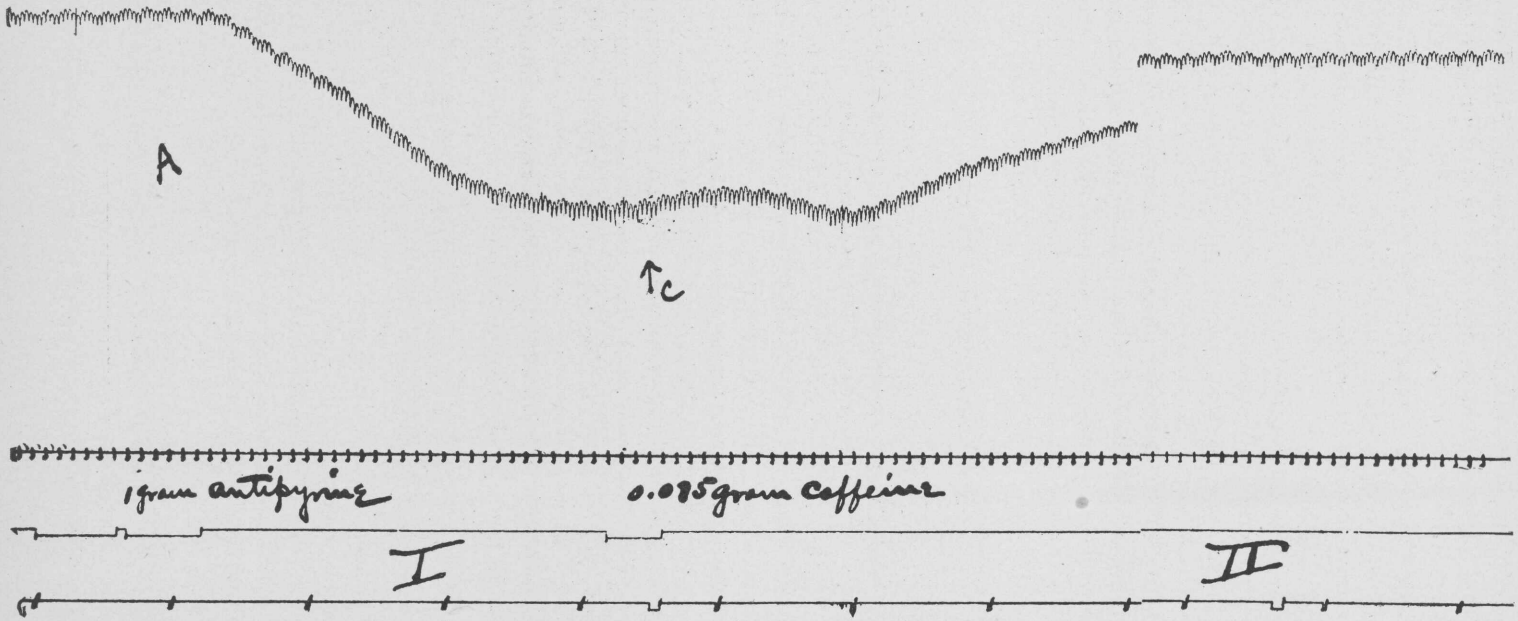



FIG. 8.—Blood pressure tracing taken from the dogs carotid. At "A" 1 gram antipyrine was injected causing a fall in pressure of 58 mm. mercury. The injection of caffeine at "C" indicates a very slight depressant action. II indicates the recovery from the drugs; taken after an interval of 3 minutes.

The mice being of the same lot as in the determination of the minimum lethal dose for caffeine, this was not redetermined in these experiments.

The experiments with acetanilide and caffeine having shown no antagonistic action, the experiments using a mixture of antipyrine and caffeine were begun with relatively small doses. Half the sums of the minimum lethal dose for each drug were used as a basis for estimating the probable amount of the mixture necessary to cause death. Antipyrine and caffeine were given in varying proportions, except that the relative amount of antipyrine was always the greater. The result of the whole series was to show that the two drugs were not antagonistic, death resulting invariably from half the sums of the doses of the two drugs, and when exhibited in smaller amounts failing to kill. This indicates mere summation, but no synergistic action, as in the case of acetanilide-caffeine mixtures. Protocols taken from this series of experiments are given in Table V.

TABLE V.—*Determination of the mimimum lethal dose of a mixture of antipyrine and caffeine citrate for white mice, hypodermic injection.*

[−=survival; +=death; A=antipyrine; C=caffeine.]

| Weight. | Dose per gram body weight. | Result. | Hours till death. |
|---|---|---|---|
| 13.49... | A 0.0007<br>C .0001 | − | ..... |
| 15.91... | A .0007<br>C .0001 | − | ..... |
| 15.14... | A .0007<br>C .0002 | + | 2.50 |
| 13.28... | A .0008<br>C .0001 | + | 2.35 |
| 13.17... | A .0008<br>C .0001 | + | 16.10 |
| 16.53... | A .0006<br>C .0003 | − | ..... |
| 14.82... | A .0006<br>C .0003 | + | 1.35 |
| 15.92... | A .0006<br>C .0003 | + | 2.47 |
| 13.20... | A .0006<br>C .0003 | + | 1.30 |

## FEEDING EXPERIMENTS.

Guinea pigs were given a mixture composed of antipyrine and caffeine citrate to determine whether any modification in the toxicity of the former resulted. The method used was practically the same as that used in the experiments on acetanilide, the only modification being in the method of administration. Since both drugs are easily soluble in water, they were given in solution by means of a stomach tube (a small sized, semielastic urethral catheter). No control experiments were carried out with caffeine citrate, the control experiments of the acetanilide series serving this purpose, as the animals in both

cases belonged to the same lot, and the experiments were carried out at the same time. Protocols of the control experiments using antipyrine and of the experiments using a mixture of antipyrine and caffeine citrate are given in the tables which follow.

*Control—antipyrine.*

[Determination of the minimum lethal dose of antipyrine for guinea pigs. — Survived; + death.]

| Weight in grams. | Dose per gram. | Result. | Hours till death. |
|---|---|---|---|
| 465 | 0.0011 | — | ..... |
| 415 | .0012 | — | ..... |
| 345 | .0012 | — | ..... |
| 395 | .0013 | — | ..... |
| 380 | .0014 | + | 7.30 |
| 365 | .0014 | + | 10.45 |
| 435 | .0015 | — | ..... |
| 435 | .0015 | + | 11.45 |
| 445 | .0016 | + | 12.45 |
| 470 | .0016 | + | 3.00 |

A series of guinea pigs belonging to the same lot were given a mixture of antipyrine and caffeine in the determination of the least fatal dose, with results as follows:

[Antipyrine and caffeine mixture: Determination of the least fatal dose of a mixture of antipyrine and caffeine citrate for guinea pigs. —survived; +death.]

| Weight in grams. | Dose per gram. | Result. | Hours till death. |
|---|---|---|---|
| 375 | A 0.0008<br>C .0002 | — | ..... |
| 395 | A .0008<br>C .0003 | — | ..... |
| 340 | A .0008<br>C .0003 | — | ..... |
| 490 | A .0010<br>C .0002 | + | 110 |
| 400 | A .0010<br>C .0003 | — | ..... |
| 400 | A .0012<br>C .0002 | + | 12 |
| 490 | A .0012<br>C .0002 | + | 8 |
| 475 | A .0012<br>C .0003 | + | 7 |
| 400 | A .0012<br>C .0003 | + | 7 |
| 495 | A .0014<br>C .0002 | + | 4 |

Estimating the minimum lethal dose of antipyrine for guinea pigs as 0.0014 gram per gram body weight, the experiments with a mixture of antipyrine and caffeine indicate an increased toxicity to the simple drug, the amount of antipyrine producing death in the mixture being 0.0012 gram per gram body weight, or an increased toxicity in the inverse ratio of 12 to 14.

## TOXICITY OF ANTIPYRINE AND SODIUM BICARBONATE.

Guinea pigs were given doses of equal parts of antipyrine and sodium bicarbonate to see whether any antagonism, as was apparent in the case of acetanilide, could be developed. The control experiments using antipyrine alone are tabulated on the preceding page. No controls using sodium bicarbonate were thought necessary on account of its nonpoisonous character. The results of the experiments using the mixture of antipyrine and sodium bicarbonate are tabulated below.

[Antipyrine and sodium bicarbonate: Determination of the minimum lethal dose for guinea pigs of a mixture of antipyrine and sodium bicarbonate. — Survived; + death; A, antipyrine; S, sodium bicarbonate.]

| Weight in grams. | Dose per gram. | Result. | Hours till death. |
|---|---|---|---|
| 315 | A 0.0012<br>S .0012 | — | ..... |
| 365 | A .0012<br>S .0012 | — | ..... |
| 485 | A .0014<br>S .0014 | + | 18 |
| 420 | A .0014<br>S .0014 | + | 15 |
| 445 | A .0016<br>S .0016 | + | 9 |
| 355 | A .0016<br>S .0016 | + | 8 |

These protocols indicate that a mixture containing sodium bicarbonate is of approximately the same toxicity as antipyrine given alone, the minimum lethal dose being 0.0014 gram per gram body weight in each case. A lessened toxicity is suggested by the longer period of life after equivalent doses of the mixture, but at best this is so slight as to be almost negligible.

## SALIPYRIN.

Salipyrin, a chemical combination of antipyrine and salicylic acid, was made the subject of a further series of experiments in order to compare its toxicity with a simple mixture of antipyrine and salicylic acid when given in the same proportions as occurs in the chemical compound. Special interest in this comparison was stimulated by the abstracts and reprints sent out by the American firm selling this product, since these invariably point out its nontoxic character. These may be abstracted as follows:

Lohman[a] reported that it was more active than its components and was free from the secondary action so often observed after anti-

[a] Lohman, Deutsch. med. Ztg., 1903, XXIV, 1142.

pyrine. Lubowski[a] stated that there was complete absence of secondary effects such as are common when its components are used. According to Buettner[b] the dangerous heart effect of antipyrine is avoided by its administration as salipyrin. Muhlbauer[c] reported no bad effects subsequent to a dose of 10 grams of the drug.

In entire disagreement to these reports a large number of others[d] have appeared reporting the deleterious effects from the use of salipyrin even in small doses. The symptoms generally present were various skin eruptions, burning in the region of the stomach, profuse sweating, dilated pupils, great air hunger, marked heart distress, and fear of impending death. In Dumstrey's cases these symptoms appeared after 1 gram of the drug had been taken.

As will be recognized, these are the ordinary symptoms associated with antipyrine or with salicylic acid poisoning, and they certainly do not bear out the statements found in the advertising literature as to the absence of toxic and secondary symptoms. From the comparatively large number of cases of poisoning it would seem probable also that the salipyrin was fully as toxic as a simple mixture of its components. Theoretically also this would seem probable, since this compound is broken up in the body into its constituents,[e] and it would therefore produce an effect in the body similar to that of the two drugs from which it is compounded.

In proof of this point the following experiments were carried out to determine the relative toxic values for animals of salipyrin and of a mixture composed of antipyrine and salicylic acid in the same proportions as occur in the chemical compound. In the first series of experiments the drugs were injected beneath the skin of the back of white mice. Mice belonging to the same lot and kept under the same conditions were weighed and the dose given was estimated upon the basis of grams of body weight. On account of the insolubility of salipyrin and salicylic acid in water it was found necessary to dissolve the drugs in 50 per cent alcohol in such amounts that 1 c. c. of the solution represented 100 milligrams of salipyrin in one case and 57.7 milligrams antipyrine and 42.3 milligrams of salicylic acid, the proportionate amount of these drugs entering into the compound, in the other. The amount of alcohol injected in this way is so small that the chief symptom from it was merely some unsteadiness in the animal's

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[a] Lubowski, Allg. med. Centr.-Ztg., 1903, LXXII, 682.

[b] Buettner, Cor. Bl. f. schweiz. Aerzte, 1900, XXX.

[c] Muhlbauer, Wien. med. Wochenschr., 1897, XLVII, 196.

[d] Schmey, Therap. Monatshefte, 1897, XI, 175. Dumstrey, Deutsche med. Wochenschr., 1903, XXIX, 461. Dittmer, Med. Woche, 1903, IV, 579. Scharfe, Therap. Monatshefte, 1903, XVII, 163. Ritter, Berl. klin. Wochenschr., 1908, XLV, 338.

[e] Cushny, Pharmacology and Therapeutics, 1906, p. 380.

movements. After about twenty minutes this was followed by lessened movements, the animal sitting quietly and acting as if cold. The fur was roughened and occasionally slight convulsions appeared. No difference in the symptoms of poisoning could be determined in the two series of experiments. The toxicities of the mixture and of the compound, salipyrin, were also approximately the same as is shown by the protocols given in the following table:

SERIES I.

[Effect of salipyrin and of an antipyrine-salicylic acid mixture upon white mice, hypodermic injection.]

| Dose per gram body weight, in grams. | Salipyrin. | A. and S. mixture. |
|---|---|---|
| | Hours till death. | Hours till death. |
| 0.0010 | 0 | 0 |
| .0011 | 0 | 3.39 |
| .0012 | 2.33 | 2.47 |
| .0014 | 1.25 | 1.03 |

SERIES II.

| Dose per gram body weight, in grams. | Salipyrin. | A. and S. mixture. |
|---|---|---|
| | Hours till death. | Hours till death. |
| 0.0013 | 2.12 | 1.44 |
| .0013 | 2.21 | 2.27 |
| .0013 | 3.02 | 2.50 |

Feeding experiments were also carried out to determine the toxic effect of salipyrin and of the antipyrine-salicylic acid mixture when given in a manner more closely simulating therapeutic administration. Guinea pigs of about the same weight and belonging to the same lot were used in the first series. The dose was estimated per gram body weight, each dose being weighed separately and made up into pills of suitable size and then fed in such a manner that none of the drug was lost in their administration. The results of the first series, using 0.0016 milligram per gram body weight, indicated that salipyrin was most toxic, two pigs receiving salipyrin dying after ten and twelve hours, respectively. Two days later a second dose was administered in the same manner to the surviving animals, using, however, 0.0018 milligram of the combination or of the mixture per gram body weight. In this series the toxicities appeared to be approximately the same,

as judged by the time the animals survived the introduction of the drug. The results are as follows:

SERIES I.

[Protocols of experiments to determine relative toxicity of salipyrin and of a mixture of antipyrine and salicylic acid when given to guinea pigs by the stomach. −=survived; +=death.]

[Salipyrin.]

| Weight. | Dose. | Result. | Hours till death. |
|---|---|---|---|
| 620 | 0.0016 | + | 12 |
| 480 | .0016 | + | 9 |
| 470 | .0016 | − | ..... |
| 450 | .0016 | − | ..... |
| 430 | .0016 | − | ..... |
| 470 | .0016 | − | ..... |

[Antipyrine 57.76+salicylic acid 42.3 per cent.]

| | | | |
|---|---|---|---|
| 530 | 0.0016 | − | ..... |
| 470 | .0016 | − | ..... |
| 610 | .0016 | − | ..... |
| 490 | .0016 | − | ..... |

SERIES II.

[Salipyrin.]

| Weight. | Dose. | Result. | Hours. till death. |
|---|---|---|---|
| 530 | 0.0018 | + | 3.30 |
| 405 | .0018 | + | 5 |
| 410 | .0018 | + | 6 |
| 420 | .0018 | + | 14 |

[A. and S. mixture.]

| | | | |
|---|---|---|---|
| 455 | 0.0018 | + | 2.30 |
| 405 | .0018 | + | 3.30 |
| 505 | .0018 | + | 5 |
| 430 | .0018 | + | 26 |

Feeding experiments were carried out on white mice, using the method already described for acetanilide, page 29. Each cake contained 0.100 gram salipyrin as a control; the cakes made up with the simple mixture each contained 0.0577 gram antipyrine and 0.0423 gram salicylic acid. In other words, the control cakes contained just the same proportionate amount of the two drugs as did those containing the mixture.

The feeding of these cakes to mice obtained from the same lot was followed by a slow but equal decrease in weight, and the duration of life in the case of the mice receiving the mixture and those receiving the same

amount of drugs chemically combined was approximately the same. The protocols taken from these experiments are as follows:

*Control, salipyrin.*

[Feeding mice salipyrin 0.100 gram per cake. − = survived; + = death.]

| Date. | Weight of mice, in grams. | | | |
|---|---|---|---|---|
| | No. 1. | No. 2. | No. 3. | No. 4. |
| 1909. | | | | |
| February 13 | 19.84 | 18.36 | 18.08 | 17.71 |
| February 17 | 19.19 | 17.16 | 16.70 | 17.05 |
| February 20 | 20.12 | 18.50 | 18.47 | 16.76 |
| February 24 | 19.30 | 17.74 | 18.08 | 16.10 |
| February 27 | 18.89 | 17.30 | 16.99 | 14.65 |
| March 1 | 17.40 | 16.34 | 15.45 | 14.30 |
| March 3 | 15.99 | 15.19 | 15.23 | 13.30 |
| March 6 | 13.95 | 14.85 | 14.76 | ..... |
| March 9 | 15.62 | 15.57 | ..... | ..... |
| March 11 | 14.02 | 14.14 | ..... | ..... |

No. 1, dead March 2, 27 days; No. 2, dead March 11, 26 days; No. 3, dead March 7, 22 days; No. 4, dead March 6, 21 days.

In like manner a series of mice were fed upon a mixture of antipyrine and salicylic acid given in the same proportions in which they combine and form salipyrin. The results of these experiments are given below.

*Antipyrine-salicylic acid mixture.*

[Feeding mice antipyrine, 0.0577 gram plus salicylic acid 0.0423 gram per cake.]

| Date. | Weight of mice, in grams. | | | |
|---|---|---|---|---|
| | No. 1. | No. 2. | No. 3. | No. 4. |
| 1909. | | | | |
| February 13 | 22.51 | 19.75 | 16.15 | 20.04 |
| February 17 | 21.19 | 18.92 | 15.71 | 19.70 |
| February 20 | 22.09 | 18.46 | 15.81 | 18.30 |
| February 24 | 21.54 | 16.86 | 14.50 | 18.38 |
| February 27 | 20.34 | 16.01 | 14.40 | 16.85 |
| March 3 | 18.36 | 14.28 | 13.39 | 16.30 |
| March 6 | 16.57 | 13.46 | 12.62 | ..... |
| March 9 | 16.08 | 13.92 | ..... | ..... |

No. 1, dead March 10, 25 days; No. 2, dead March 10, 25 days; No. 3, dead March 7, 22 days; No. 4, dead March 5, 20 days.

The average duration of life for mice fed salipyrin was twenty-four days; for mice receiving the mixture, twenty-three days—a difference that is quite negligible in experiments of this sort, on account of the small toxic action of the drugs used.

In conclusion, it may be said that the results of the whole series of experiments indicate no change in the toxicity of a mixture of antipyrine and salicylic acid when formed into a definite compound by chemical means.

## LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nama*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant, with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Frorida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations on two new parasitic trematode worms: *Homalogaster philippinensis* n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger.

4. A reexamination of the original specimen of *Tænia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

*No. 41. Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g. n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). By W. W. Miller.

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No. 48. The Physiological Standardization of Digitalis. By Charles Wallis Edmunds and Worth Hale.

No. 49.—Digests of comments on the United States Pharmacopœia. Eighth decennial revision for the period ending December 31, 1905. By Murray Galt Motter and Martin I. Wilbert.

No. 50.—Further studies upon the phenomenon of anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 51.—Chemical Tests for Blood. By Joseph H. Kastle.

No. 52.—Report No. 3 on the origin and prevalence of typhoid fever in the District of Columbia (1908). By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 53.—The influence of certain drugs upon the toxicity of acetanilide and antipyrine. By Worth Hale.

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