ciates when taken internally into acetyl salicylate (aspirin) and ammonia. A five-grain tablet of this compound will yield on complete dissociation in the intestines 4.5 grains of aspirin and 0.5 grains of free ammonia or 1.0 grain of ammonium hydroxide. The ammonium ion acts as a counterirritant and as a stimulant. By the liberation of this volatile and diffusible ammonia the action of this ammonium compound extends deeply. The ammonia is absorbed by the system and is converted into urea.

Ammon-Aspirin is non-poisonous. It can be taken internally with the same ease as ordinary aspirin. The proper dose is in 5-grain tablets. It has the same effect on the human system as a mixture of ordinary aspirin and aromatic spirits of ammonia, or free ammonium hydroxide in the same proportions. It has the advantage of being a simple white crystalline compound containing both aspirin and ammonia in combination. If desired to increase the ammonia content of each tablet for greater effect on the central nervous system a little ammonium carbonate can be added to the ammonium acetyl salicylate before the tablets are made up.

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## BIOASSAY OF ACONITE AND ITS PREPARATIONS. I. LETHAL DOSE OF ACONITINE TO RATS.*

J. C. MUNCH[1] AND G. S. GITTINGER.[2]

The bioassay of aconite and its galenical preparations has been proposed by a number of investigators since Squibb (8), in 1882, proposed the determination of the minimum concentration just capable of producing the characteristic tingling of the human tongue when held in the mouth for 5 to 10 minutes. Taylor (12) showed the quantitative possibilities of the method, but other investigators have not been favorably impressed, claiming that the personal equation plays too great a part in the assay results. A series of publications from Haskell's laboratory (2) discuss the seasonal variation of guinea-pigs and develop a modification of the Hatcher cat method used for the assay of digitalis, which is claimed to give consistent results in the biosasay of aconite. Jauregui (4), in a critical study of various bioassay methods for aconite, presents a few data to substantiate his belief that more consistent results may be obtained with cats under artificial respiration, although the guinea-pig method of bioassay, originally proposed by Githens and Vanderkleed (1), is also considered reliable. Roth (6) found that the guinea-pig method was more satisfactory than the Squibb test. Various factors affecting the

* A preliminary report of the results obtained in this investigation was presented at the meeting of the Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION held in St. Louis, Mo., in August 1927.

[1] Resigned from the Government service January 1, 1928.

[2] Resigned from the Government service August 1928.

accuracy of the guniea-pig method were studied by Swanson and coworkers (9, 10, 11), and data which indicate that there is no definite seasonal variation of guinea-pig susceptibility to aconitine were presented. U. S. Pharmacopœia X officially adopted the guinea-pig method of bioassay for aconite and its preparations (13). This method may be summarized to read: "Two out of three guinea-pigs weighing between 275 and 325 Gm. will die within 12 hours after the subcutaneous injection of a specified quantity of aconite preparation." For aconitine the specified fatal dose is 0.060 mg./Kg.

Because of the cost of guinea-pigs and the occasional difficulty of obtaining an adequate supply of animals of the proper weight, Rowe (7) developed an assay method by which intraperitoneal injections of solutions are given to white mice weighing about 20 Gm. The fatal dose of aconitine for mice was 0.5 mg./Kg., and 0.080 mg./Kg. for guinea-pigs by the U. S. Pharmacopœia X method; 6.25 times as much aconitine was required to kill mice as to kill guinea-pigs, and Rowe recommended this figure as a factor for comparison of results by these methods. In a later study of this method, Swanson and Hargreaves (11) found that aconite preparations complying with the pharmacopœial requirements for potency gave comparable results by the two methods, but that the factor 10 was indicated. With weaker preparations the factor decreased progressively to 3. Swanson and Hargreaves suggest that this may be due to the greater susceptibility of mice to the alcohol in the test solution.

White rats are cheap and readily available and are commonly employed in pharmacological laboratories for a number of tests. We have studied the possibility of using them for the assay of aconite preparations, believing that they possess advantages over mice. To avoid various complications these experiments were conducted with a solution of U. S. P. aconitine, crystalline, Merck. Tested by the U. S. P. X guinea-pig method, the fatal dose was found to be 0.060 mg./Kg.

All rats used in these experiments had been held in the laboratory for at least a month. They were fed upon the same diet and were stored under the same conditions. Most of them weighed between 150 and 200 Gm. Some had received other drugs prior to use; others had not. No differences in reaction could be noted, and no other drug had been given within a period of several weeks. Subcutaneous injections were made by the U. S. P. X guinea-pig technic. For intraperitoneal injections 0.3 to 0.6 cc. of solution was injected from a 1-cc. Luer syringe through a small needle, care being taken to prevent loss of solution at the site of injection. Observations were made at frequent intervals after injection, to determine the rapidity of onset of symptoms and the time until death. All deaths occurring after typical aconite symptoms, and within 12 hours of injection, were attributed to aconitine.

The results obtained in the several series of tests are reported in Tables I, II and III. The numerator of the fraction in each entry represents the number of animals that died; the denominator represents the total number of animals injected with the indicated dose. As no consistent differences were detectable in the several series of experiments conducted at various periods throughout the year, the results obtained in each series have been consolidated, and the percentage of injected animals dying after each dose has been determined.

TABLE I.—FATAL DOSE OF ACONITINE TO WHITE RATS—SUBCUTANEOUS SINGLE INJECTION.

| Series. | Dose injected (mg./Kg.). 0.08. | 0.10. | 0.12. | 0.15. | 0.175. | 0.20. | 0.25. | 0.30. |
|---|---|---|---|---|---|---|---|---|
| 1 | .. | 0/1 | ... | 0/2 | ... | 0/2 | 3/3 | 2/2 |
| 2–7 | .. | 9/106 | 4/10 | 9/24 | ... | ... | .. | ... |
| 14–21 | 0/25 | 2/50 | 17/50 | 17/25 | 16/25 | 24/25 | ... | ... |
| 22 | .. | 3/5 | 4/5 | 5/5 | 5/5 | 5/5 | ... | ... |
| 23–25 | .. | 4/56 | ... | ... | ... | ... | ... | ... |
| Total | 0/25 | 18/218 | 25/65 | 31/56 | 21/30 | 29/32 | 3/3 | 2/2 |
| Deaths (%) | 0 | 8 | 39 | 55 | 70 | 91 | 100 | 100 |

TABLE II.—FATAL DOSE OF ACONITINE TO WILD RATS—SUBCUTANEOUS SINGLE INJECTION.

| Series. | Dose injected (mg./Kg.). 0.14. | 0.16. | 0.18. | 0.20. | 0.22. | 0.24. |
|---|---|---|---|---|---|---|
| 26 | 3/9 | 9/15 | 3/6 | 5/8 | 19/20 | 2/2 |
| Deaths (%) | 33 | 60 | 50 | 62 | 95 | 100 |

TABLE III.—FATAL DOSE OF ACONITINE TO WHITE RATS—INTRAPERITONEAL SINGLE INJECTION.

| Series. | Dose injected (mg./Kg.). 0.05. | 0.06. | 0.075. | 0.10. |
|---|---|---|---|---|
| 8–10 | 0/6 | 0/11 | ... | 4/6 |
| 11–13 | 0/6 | .. | 1/3 | 2/2 |
| Total | 0.12 | 0/11 | 1/3 | 6/8 |
| Deaths (%) | 0 | 0 | 33 | 75 |

The reactions of 431 white rats following a single injection of aconitine subcutaneously are given in Table I. The great variation in susceptibility of individual rats is readily apparent. Eighteen rats were killed by a dose of 0.10 mg./Kg., while 3 rats survived a dose twice as large. To kill all rats 0.25 mg./Kg. was required. Using the U. S. P. X criterion (that dose which kills 2 out of 3 animals within 12 hours), however, the fatal dose for white rats was found to be 0.175 mg./Kg. The fatal dose for guinea-pigs having been found to be 0.060 mg./Kg. our experiments show that 3 times as much aconitine is required to kill white rats as to kill guinea-pigs, when the aconitine is subcutaneously injected into each species.

The reactions of 59 wild (gray) rats, weighing 100 to 400 Gm., to subcutaneous injections of aconitine, are given in Table II. The fatal dose appears to be about 0.20 mg./Kg., which is somewhat greater than the fatal dose for the white rats. The small number of animals studied, however, does not warrant too close a comparison of the susceptibilities of the two species. Wild rats were not obtainable in sufficient numbers to determine whether this observed difference in susceptibility is apparent or real. The dose killing all wild rats was essentially identical with the dose killing all white rats.

The intraperitoneal injection of aconitine in 34 rats gave the results in Table III. Too few animals were employed to permit great accuracy, but it appears that the fatal dose of aconitine is approximately 0.10 mg./Kg., somewhat more than half the fatal dose by subcutaneous injection.

### VARIATIONS IN SUSCEPTIBILITY OF INDIVIDUAL RATS.

The results in Tables I, II and III show the variation in susceptibility of rats to a single injection of aconitine. The percentage of deaths increases as the dose

is increased. The most resistant rats required more than twice as large a dose to kill as the most susceptible ones. A further study of the variation in sensitivity was undertaken by a different method. All the rats of a given series were injected with the same dose of aconitine. After an interval of 2 days or 4 days all of the survivors were injected with a larger and larger dose, until all the rats in a given series were killed.

TABLE IV.—FATAL DOSE OF ACONITINE TO WHITE RATS—SUBCUTANEOUS REPEATED INJECTIONS.

| Series. | No. rats. | Increment. | Dose causing death (mg./Kg.). 0.08. | 0.10. | 0.125. | 0.15. | 0.175. | 0.20. | 0.22. | 0.25. |
|---|---|---|---|---|---|---|---|---|---|---|
| (Interval between injections, 4 days.) | | | | | | | | | | |
| 2 | 23 | 0.025 | .. | .. | .. | 9 | 11 | 3 | ... | ... |
| 3 | 10 | 0.025 | .. | .. | 4 | 4 | .. | 2 | ... | ... |
| 4 | 13 | 0.025 | .. | 1 | 1 | 6 | 1 | 2 | 2 | ... |
| 5 | 27 | 0.025 | .. | 3 | 9 | 6 | 4 | 2 | 3 | ... |
| 6 | 19 | 0.025 | .. | 2 | 3 | 2 | 8 | 3 | 1 | ... |
| 7 | 46 | 0.025 | .. | 3 | 12 | 8 | 17 | 6 | ... | ... |
| Total | 138 | ... | .. | 9 | 29 | 35 | 41 | 18 | 6 | ... |
| Deaths (%) | | | .. | 7 | 28 | 53 | 83 | 96 | 100 | ... |
| (Interval between injections, 2 days.) | | | | | | | | | | |
| 14 | 25 | 0.050 | .. | .. | .. | .. | .. | 24 | ... | 1 |
| 15 | 25 | 0.025 | .. | .. | .. | .. | 16 | 3 | ... | 6 |
| 16 | 25 | 0.075 | .. | .. | 5 | .. | .. | 19 | ... | 1 |
| 17 | 25 | 0.075 | .. | 1 | .. | .. | 24 | .. | ... | ... |
| 18 | 25 | 0.075 | .. | 1 | .. | .. | 24 | .. | ... | ... |
| 19 | 25 | 0.075 | 0 | .. | .. | 22 | 3 | .. | ... | ... |
| 20 | 25 | 0.025 | .. | .. | .. | 17 | 6 | 2 | ... | ... |
| 21 | 25 | 0.025 | .. | .. | 12 | 5 | 8 | .. | ... | ... |
| Total | 200 | | 0 | 2 | 17 | 44 | 81 | 48 | ... | 8 |
| Deaths (%) | | | 0 | 1 | 10 | 32 | 72 | 96 | ... | 100 |

TABLE V.—FATAL DOSE OF ACONITINE TO WHITE RATS—INTRAPERITONEAL REPEATED INJECTIONS.

| Series. | No. rats. | Increment. | Dose causing death (mg./Kg.). 0.05. | 0.06. | 0.07. | 0.08. | 0.09. | 0.10. | 0.11. | 0.12. | 0.16. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Interval between injections, 4 days.) | | | | | | | | | | | |
| 8 | 6 | 0.02 | ... | ... | ... | ... | ... | 4 | ... | 2 | ... |
| 9 | 6 | 0.01 | 0 | ... | 2 | 2 | 0 | 0 | 1 | .. | 1 |
| 10 | 11 | 0.01 | ... | 0 | 8 | 0 | 2 | 1 | ... | ... | ... |

Subcutaneous injections in Series 2–7 (Table IV) were made with an increment of 0.025 mg./Kg. of aconitine in successive doses. The lowest dose given, 0.10 mg./Kg., was fatal to 9, or 7 per cent, of the 138 rats used. After 4 days the remaining 129 rats were injected with a dose of 0.125 mg./Kg., causing the death of 29 more rats, or 21 per cent of the original 138 rats of the series. As the rats which died at the 0.10 mg./Kg. dose would also have been killed if they had received 0.125 mg./Kg., the cumulated mortality is taken as the sum of 7 per cent and 21 per cent, or 28 per cent. At 0.175 mg./Kg., the dose selected as the fatal dose (killing two-thirds of the animals injected), it was found that 83 per cent of the series had died.

Reducing the time-interval between injections to 2 days, 200 rats in Series 14–21 were injected (Table IV). The increments were varied from 0.025 to 0.075

mg./Kg. in the different series. Somewhat smaller perentages of animals were killed by the lower doses than in the group with 4-day intervals. However, 0.175 mg./Kg. killed 72 per cent of all the rats injected.

Using a 4-day interval, 23 rats in Series 8–10 were given intraperitoneal injections with similar increases in dosages (Table V). The same results were evident. The dose of 0.10 mg./Kg., which killed 75 per cent of the rats after a single injection, killed 83 per cent in this series of increased dosages. Too close comparisons are not justified on account of the small number of animals studied.

TABLE VI.—CUMULATIVE TOXICITY OF ACONITINE TO WHITE RATS—PERCENTAGE OF DEATHS.

| Method of injection. | No. rats. | Dose injected (mg./Kg.). 0.08. | 0.10. | 0.125. | 0.15. | 0.175. | 0.20. | 0.22. | 0.25. |
|---|---|---|---|---|---|---|---|---|---|
| Subcutaneous: | | | | | | | | | |
| Single | 431 | 0 | 8 | 39 | 55 | 70 | 91 | ... | 100 |
| 4-day interval | 138 | .. | 7 | 28 | 53 | 83 | 96 | 100 | ... |
| 2-day interval | 200 | 0 | 1 | 10 | 32 | 72 | 96 | ... | 100 |
| Intraperitoneal: | | | | | | | | | |
| | | 0.05. | 0.06. | 0.07. | 0.075. | 0.08. | 0.09. | 0.10. | 0.11. | 0.12. | 0.16. |
| Single | 34 | 0 | 0 | .. | 33 | .. | .. | 75 | .. | .. | ... |
| 4-day interval | 23 | 0 | 0 | 44 | .. | 52 | 61 | 83 | 87 | 96 | 100 |

TABLE VII.—DETOXICATION OF ACONITINE BY WHITE RATS—SUBCUTANEOUS INJECTIONS AT 8-HOUR INTERVALS.

| Series. | No. rats. | No. doses. | Total aconitine to kill, mg./Kg. | Detoxicating power (mg./Kg./8 hours.) |
|---|---|---|---|---|
| 23 | 3 | 1 | 0.10 | None |
| | 5 | 2 | 0.15 | None |
| | 2 | 3 | 0.20 | 0.0125 |
| | 3 | 4 | 0.25 | 0.025 |
| | 11 | 5 | 0.30 | 0.031 |
| 24 | 2 | 3 | 0.30 | 0.0625 |
| | 1 | 4 | 0.40 | 0.075 |
| | 5 | 5 | 0.52 | 0.086 |
| 25 | 1 | 1 | 0.10 | None |
| | 23 | 2 | 0.22 | 0.045 |
| Weighted average | | | | 0.037 |

TABLE VIII.—DETOXICATION OF ACONTINE BY WHITE RATS—INTRAPERITONEAL INJECTIONS AT 1-DAY INTERVALS.

| Series. | No. rats. | No. doses. | Total amount aconitine to kill, mg./Kg. | Detoxicating power (mg./Kg./day). |
|---|---|---|---|---|
| 11 | 1 | 3 | 0.15 | 0.025 |
| | 1 | 8 | 0.40 | 0.043 |
| | 1 | 11 | 0.575 | 0.0475 |
| | 1 | 16 | 1.00 | 0.060 |
| | 1 | 18 | 1.20 | 0.065 |
| 12 | 1 | 1 | 0.075 | None |
| | 2 | Over 4 | Over 0.30 | Over 0.067 |
| 13 | 1 | 2 | 0.20 | None |
| | 1 | 4 | 0.40 | None |
| Weighted average | | | | 0.037 |

The results of single and of repeated injections of aconitine subcutaneously and intraperitoneally to white rats are consolidated in Table VI. Although slight differences in the percentages of animals killed at the increasing dosages may be noted, the fatal doses by each method of injection are found to be in substantial agreement. An ogee curve (Fig. 1) shows the results obtained in the subcutaneous injections. From this curve the relative susceptibility for the animals used may be discerned. The 67 per cent axis intersects the three ogee curves at 0.162, 0.170 and 0.172, values in substantial agreement with the fatal dose of 0.175 which was selected. As the experimental results did not correspond to the usual types of frequency curves, further mathematical analyis was not attempted.

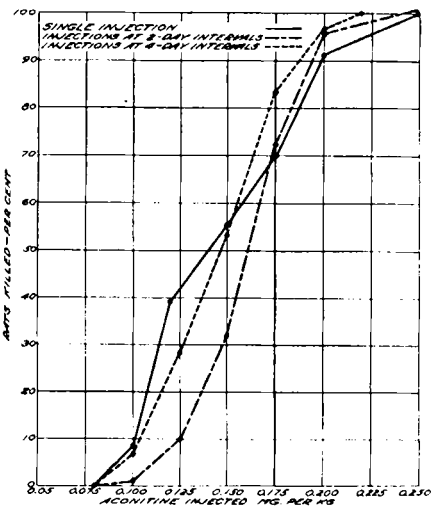


Fig. 1.

RATE OF DETOXICATION OF ACONITINE BY WHITE RATS.

As the fatal dose of aconitine was essentially the same in rats receiving injections at 4-day and at 2-day intervals and in rats which had not received aconitine previously, it was decided to follow the rate of destruction (or elimination) of aconitine in the living rat by changes in the fatal dose. Palet (5) found that aconitine is qualitatively detectable several months after death, when chemical analyses are made of the rat's tissues. Hottinger (3), injecting aconitine solutions intravenously into cats, demonstrated that aconitine action was the result of the two variables, time and concentration, and estimated the destruction in the cat's tissues to be between 0.004 and 0.0008 mg./Kg./min. Assuming a constant rate, intravenously injected aconitine is detoxicated by the cat at the rate of between 0.24 and 0.048 mg./Kg./hour.

Three series of subcutaneous injections were made, and the detoxicating power of the living rat's tissues was calculated. Injections were made at 8-hour intervals into all survivors of a series. To calculate the detoxicating power, it was assumed that the fatal dose would be 0.175 mg./Kg. for each rat, and that a rat would die as soon as that quantity of aconitine was actively present. In Series 23, 3 rats died at 0.10, and 5 at 0.15 mg./Kg., doses below the fatal dose. These animals apparently were overly sensitive, although some deaths at doses below 0.175 mg./Kg. are to be expected. Two rats were killed by a total quantity of 0.20 mg./Kg., given in 3 injections. As 0.175 mg./Kg. is necessary to kill, the rats must not have detoxicated more than the difference between the total quantity injected, 0.20 mg./Kg., and the fatal dose, 0.175 mg./Kg., or a total of 0.025 mg./Kg., during the two 8-hour periods between the original and the final injection. The detoxicating power of these two rats, then, was 0.025/2, or 0.0125 mg./Kg. in 8 hours. Of course, it is recognized that there might be more than 0.175 mg./Kg. of aconitine in the rat at the time of death, so that this figure for detoxication is probably a maximal value. Using this method, the values for detoxicating power given in the last column of

Table VII were calculated. The weighted mean value for detoxicating power was found to be 0.0376 mg./Kg. during 8 hours, which is approximately 22 per cent of the fatal dose. Assuming a constant rate, subcutaneously injected aconitine is detoxicated by the rat at the rate of 0.0047 mg./Kg./hour.

In a similar series of intraperitoneal injections (Table VIII) the same variations in sensitivity were obtained. Two rats were injected with 0.10 mg./Kg., the fatal dose by this method of injection. One rat died on the second, the other on the fourth day, after daily injections. The other animals showing detoxicating powers ranging from 0.025 mg./Kg. to 0.067 mg./Kg. per day. The weighted average value was found to be 0.037 mg./Kg./day, or 37 per cent of the fatal dose. Assuming a constant rate, intraperitoneally injected aconitine is detoxicated by the rat at the rate of 0.0015 mg./Kg./hour.

These results indicate that the rat is capable of detoxicating a fatal dose of aconitine in 3 to 4 days, and that survivors from the injection of an aconitine solution may be used safely for the assay of another preparation after that time. As a precaution, however, it would appear advisable to include a number of unused rats with the ones that are used again.

## CONCLUSIONS.

The fatal dose of aconitine (killing 2 out of 3 animals), when injected subcutaneously into white rats by the U. S. P. X method, is 0.175 mg./Kg. Wild rats are equally or somewhat less susceptible.

Injected intraperitoneally the fatal dose is 0.10 mg./Kg. Subcutaneous injections appear more reliable than intraperitoneal injections. Great variations in susceptibility of individual rats make it necessary to use a number of test animals. Surviving rats may be used again after 4 days.

Assuming a constant rate of detoxication of aconitine in terms of mg./Kg. per hour, white rats detoxicated 0.0047 mg. after subcutaneous injection, and 0.0015 mg. after intraperitoneal injection.

For the preliminary assay of aconitine, and presumably of aconite preparations, readily available white rats may be used to conserve the more expensive guinea-pigs.

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# A CHEMICAL STUDY OF THE RIND OF CALIFORNIA ORANGES.*,[1]

BY MARION BROOKS MATLACK.

The material used in the following investigation was collected daily from the Wisconsin General Hospital during a period covering about six weeks. The material consisted of the residue left after halved orange had been extracted with a revolving bur. The oranges were all California Valencias. The inner membranous material or "rag" was removed thus leaving only the rind. The rind was then dried over a radiator until brittle and ground in a food chopper. It was then placed in a large galvanized iron percolator and immediately covered with 95 per cent alcohol. Thus it was allowed to stand for some time and then the alcohol was drawn off; 33–36 liters at a time. The alcohol was recovered by distillation using a copper steam jacketed still. The residue remaining was drawn off into a flask and kept until all had been collected. The alcohol was returned to the percolator, and the process repeated until the residue from about two hundred liters of alcoholic percolate had thus been obtained.

The material was a dark brown to black liquid from which some solid matter separated out. This material was then steam distilled to remove volatile oil. The fixed oil separated on top of the aqueous layer in the distillation flask and was colored black by pigment.

The fixed oil which had thus separated out was removed by dissolving it in petroleum ether. After the petroleum ether had been evaporated off there remained a very dark almost black liquid residue.

The aqueous material remaining after removal of both volatile and fatty oil, was evaporated to a thick viscous consistency over a radiator and set aside until desired for use.

In this manner the following materials for further work were obtained:

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* Part of a thesis submitted for the degree of Doctor of Philosophy, University of Wisconsin, 1928.

[1] Presented before Scientific Section, A. PH. A., Portland meeting, 1928.