SCIENTIFIC SECTION

A QUANTITIVE STUDY OF THE TOXICITY AND EFFICIENCY OF PICROTOXIN.

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Picrotoxin, known for a long time to the pharmacologist as an extremely potent drug, was considered too dangerous by the clinician for human use. It is a merit of Tatum and his co-workers (1, 2, 3) to have demonstrated in recent years that this drug represents an efficient respiratory stimulant and possesses a powerful analeptic action, especially against barbiturate depression, which makes it useful clinically. Koppanyi (4, 5) and others have extended this work experimentally and several favorable clinical reports have been published in the meantime (6, 7, 8), proving its usefulness in acute barbiturate poisoning.

At this time it seems desirable to report in brief our studies concerning the assay of Picrotoxin solutions for toxicity and efficiency and to give some data about stability and other properties of the solution.

Picrotoxin is not very soluble in water but a 0.3% solution is stable. Such a solution with addition of 0.5% chlorbutanol was used in all our experiments. The following investigations were conducted on rabbits. The average weight of the animals was 1.3 to 1.5 Kg.; albinos were used only in the first experiments but later discarded since they seem to be more sensitive than the colored races. The sex did not influence the results. All animals were kept in an air-conditioned building on a diet consisting of oats, alfalfa and water.

I—TOXICITY.

The determination of the lethal dose was conducted on 182 rabbits in a period of about twenty months. This gave an opportunity to compensate for an eventual fluctuation in the sensitivity of the animal material. All rabbits were injected intravenously. Table I gives the number of animals used on each dose and the number and percentage of death. Fifty-seven per cent of the rabbits injected with 1.0 mg./Kg.[1] showed one or more attacks of convulsions. Convul-

TABLE I.—TOXICITY OF PICROTOXIN IN RABBITS; INTRAVENOUS INJECTION.

No. of Animals.	Dose, Mg./Kg.	No. of Deaths.	Per Cent.
26	1.0	2	7.7
30	1.1	11	36.8
30	1.2	12	40.0
24	1.3	16	66.7
16	1.4	13	81.5
34	1.5	29	85.4
16	1.6	16	100.0

TABLE II.—DAILY INTRAMUSCULAR INJECTIONS OF PICROTOXIN.

Rabbit No.	Daily Dose, Mg./Kg.	Number of Days Given.	I. V. Dose Given Day after Last I. M. Injection.	Result.
682	1	7	1.5 mg./kg.	Death
623	1	7	" " "	"
577	1	7	" " "	"
598	1	10	" " "	"
578	1	10	" " "	"
596	1	10	" " "	"
441	1	13	" " "	"
603	1	7	" " "	Surv. (Died 1 day later with 1.6 mg.)

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[1] Mg./Kg. = mg. per Kg. body weight.

sions occurred in all animals if 1.3 mg./Kg. was given. As it is well known, a period of several minutes elapses between the injection of the drug and the appearance of convulsions, but there is no strict relationship between the size of the dose in the range given on Table I and the latent period. Similarly, the time until death varied greatly from approximately fifteen minutes to two hours.

The results show a rather sharp increment in toxicity from 1.0 to 1.1 mg./Kg. and again from 1.2 to 1.3 mg./Kg. According to the rules of statistics a smoother curve would be obtained from an even larger number of animals. However, from the present results the M. L. D. for fifty per cent of the animals can be calculated as between 1.2 and 1.3 mg./Kg. As already mentioned these results represent the summary of experiments carried out at different times. Since we have found that there really exists a change in the sensitivity in different groups of animals we

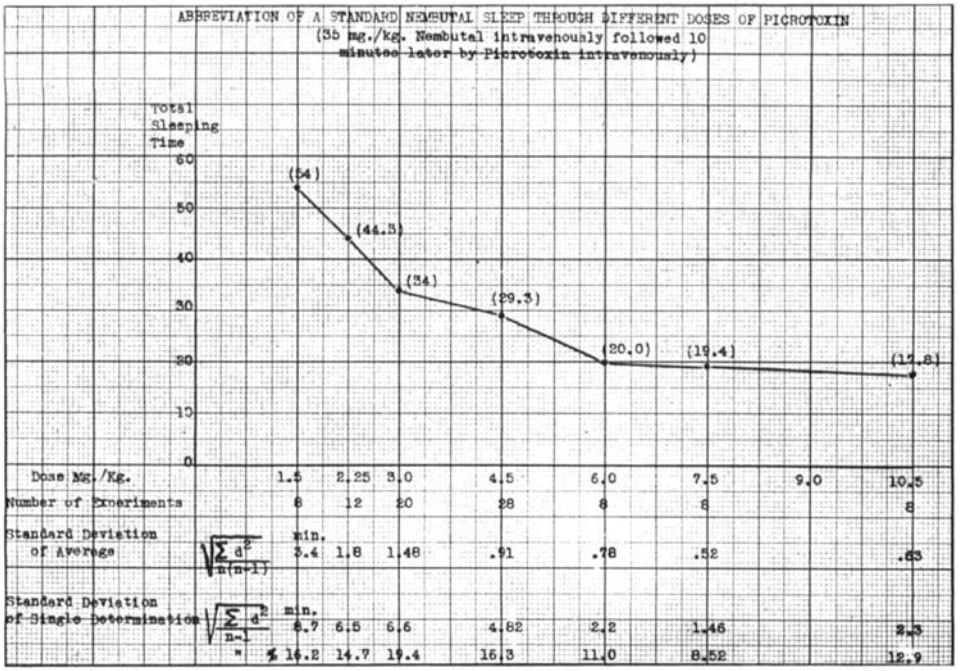

Fig. 1.

thought it practical to base the assay for toxicity upon the limits rather than upon the M. L. D. for fifty per cent. We define, therefore, a solution as of standard strength if of five rabbits (1.3 to 2.2 Kg. weight) injected with 1.0 mg. Kg. intravenously not more than one dies and not more than one survives of five animals injected with 1.5 mg./Kg. If the results do not check with this standard the experiment should be repeated with another set of animals.

II—TOLERANCE.

The older literature (9) gives very little information on the question whether or not a tolerance is formed by a continued administration of Picrotoxin. We have therefore injected a number of rabbits over a period of several days with a sub-lethal dose of Picrotoxin daily. Finally a dose of 1.5 mg./Kg. was given, which amount has been used by us as a standard lethal dose. The results are illustrated by Table II.

In addition, Picrotoxin was injected intramuscularly in one rabbit beginning with a dose of 1.0 mg./Kg. which was slowly increased up to 1.6 mg./Kg. After eleven such injections this animal survived 1.5 mg./Kg. intravenously. The injections were then continued and the rabbit tolerated later 1.6 and 1.7 mg./Kg. The twenty-fifth injection of 1.8 mg./Kg. proved fatal. Two other rabbits similarly treated died following the second injection of 1.5 or 1.6 mg./Kg. This demonstrates that there is only a small chance to produce tolerance and its degree remains slight. It seems, therefore, permissible to use rabbits repeatedly for Picrotoxin experiments within limits. A rest period of one week should be granted, as a rule.

III—TESTING FOR ANALEPTIC EFFICIENCY.

The testing for analeptic efficiency was performed by observing the abbreviation of a standard Nembutal sleep through increasing doses of Picrotoxin. All rabbits received 35 mg./Kg. Nembutal intravenously. The injection of Picrotoxin followed ten minutes later. We used also on this test 1.5 mg./Kg. as a unit dose for Picrotoxin. The animals were then observed and from time to time a stimulus was applied by pinching of the ears or tail. If the rabbits were able, following such a stimulus, to raise their heads and to keep them from falling to the side the animal was considered awake. This is, of course, a deliberate definition but proved to be a satisfactory criterion, after the investigator had gathered some experience with this method. The time which elapsed from the injection of the Nembutal until the awakening was called total sleeping time. Figure 1 on page 287 illustrates the relation between Picrotoxin dose and sleeping time. While the duration of sleep following an intravenous injection of 35 mg./Kg. Nembutal varies between 70 and 110 minutes, the injection of Picrotoxin abbreviates not only this period but also tends to equalize the sleeping time of different animals injected with the same dose of Picrotoxin. For the sake of brevity we refrain from giving the individual results in each series and reproduce in Table III only those with the dose of 2.25 mg./Kg. of Picrotoxin as an example.

TABLE III.—INDIVIDUAL SLEEPING TIME OF RABBITS INJECTED WITH 35 MG./KG. NEMBUTAL I. V. AND 2.25 MG./KG. PICROTOXIN I. V. 10 MINUTES LATER.

Sleeping Time in Minutes.	
52	41
44	40
40	39
41	34
53	46
44	55

Average of above 12 experiments 44.3 minutes.

TABLE IV.—ASSAY OF UNKNOWN PICROTOXIN SOLUTIONS.

Dose Given, Mg./Kg.	Dose Found, Mg./Kg.
1.5	2.25
1.5	2.30
1.5	1.80
2.0	2.45
2.5	2.50
3.5	2.50

The graph shows the number of experiments in each series. In addition the standard error of the average is given and also the standard error of the single determination which indicates how much the single results deviate from the average. These criteria indicate a satisfactory accuracy of the results. As can easily be seen from the shape of the curve the greatest steepness lies between 1.5 and 3.0 mg. Picrotoxin. The sleeping time between these two doses falls from approximately 60 to 24 minutes. We have therefore tried to use this part of the curve for an assaying of the Picrotoxin content of unknown solutions. Five rabbits were injected with Nembutal as described above and then with an amount of Picrotoxin unknown to the observer but being in the range of 1.5 to 3.0 mg./Kg. The results were plotted against the curve and compared with the dose injected. Table IV shows such experiments.

The results indicated that in this way a good indication can be obtained and furthermore that mostly the error tends to be in the direction of finding a too great dose as compared with the given one. We feel that this test is a useful supplement to the toxicity test in spite of the fact that the latter is probably more accurate in detecting small differences in dosage. With increasing doses the relative abbre-

viation of the sleeping time becomes less which makes this part of the curve less useful for assaying. With the extremely high dose of 15 mg./Kg. we found the total sleeping time cut down to fourteen minutes which means that the animal was awake four minutes after the injection of Picrotoxin.

Animals which received as much as 10.5 mg./Kg. Picrotoxin after Nembutal died later from convulsions, while 7.5 mg./Kg. were tolerated in the majority of cases. This means that the lethal dose for Picrotoxin under the influence of Nembutal as used in our experiments increases five times as compared with the unnarcotized animal. This fact illustrates the considerable therapeutic range if used as an analeptic in barbiturate poisoning.

IV—STABILITY OF PICROTOXIN SOLUTION.

Using these two methods we determined whether or not a Picrotoxin solution deteriorates while standing over a long period of time. For this purpose a solution was used which was prepared in January 1935, containing 0.3 mg. per cc. and 0.5% chlorbutanol as a preservative. It was kept at room temperature in a glass bottle with cork stopper. The reassay was done in December 1937, at which time the solution appeared completely clear and colorless. The test for toxicity and efficiency showed no deviation from the standard. Similar results were obtained with other samples tested for stability. We conclude, therefore, that no significant deterioration occurs in such solutions under ordinary circumstances within two years.

We have furthermore started a study concerning the cumulative action of Picrotoxin results of which we plan to report later. We wish, therefore, only to mention that if the dose of 1.5 mg./Kg. is given in two parts of 0.75 mg./Kg. each, convulsions and death follow in somewhat more than half of the cases, while the animals survived if the interval between the injection was one hour.

V—PICROTOXININ.

It is known that Picrotoxin can be split into two compounds, Picrotoxinin and Picrotin. The former represents the active part. Picrotoxinin is even less soluble in water, but a 0.1% solution is stable. We found its lethal dose to be 1.0 mg./Kg. intravenously. We have run a number of experiments with Picrotoxinin, counteracting Nembutal sleep with the same technique as described for Picrotoxin. The influence upon the sleeping time is principally the same as Picrotoxin. Death followed a dose of 5.0 mg./Kg. Picrotoxinin if given in Nembutal sleep. So far Picrotoxinin did not seem to offer advantages as compared with Picrotoxin.

SUMMARY.

1. Determination of the intravenous toxicity of Picrotoxin was performed on a large number of rabbits at different periods of time. The M. L. D. for fifty per cent lies between 1.2 and 1.3 mg./Kg. Approximately eighty-five per cent of the animals were killed by 1.5 mg./Kg.

2. A curve is presented showing the abbreviation of a standard Nembutal sleep by increasing doses of Picrotoxin.

3. This curve and determination of toxicity represents a satisfactory method for assaying Picrotoxin solutions.

4. No tolerance or only a slight degree, followed the repeated administration of Picrotoxin.

5. No deterioration in Picrotoxin solutions was noted within two years.

6. Some data regarding Picrotoxinin are presented.

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DRUG EXTRACTION. XVIII. MODIFIED DIACOLATION.*[1]

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Experiments have been carried out to determine the efficiency of a modified diacolation process in the preparation of fluidextract of belladonna root.

HISTORICAL REVIEW.

In 1935 and 1936, H. Breddin (1), (2) was granted patents in several countries on an "Apparatus for Extracting Drugs and the Like," commonly called the diacolation apparatus. In this apparatus, the menstruum is forced from a storage bottle, by means of air pressure obtained by pressing on a rubber bulb by hand, through a throttle device and drip chamber arranged for the purpose of controlling the rate of flow of menstruum into the drug. The menstruum is slowly forced through one or more cylindrical glass tubes packed with the drug, the tubes being connected by glass tubing running from the top of one tube to the bottom of the other so that the menstruum flows upward through the drug. The object of the invention is to produce highly concentrated percolates without use of heat, and using only relatively small quantities of extraction fluid (1).

In the preparation of fluidextract of cinchona by diacolation, Breddin (3) used a battery of nine tubes, each tube being 80 cm. long and 1.7 to 1.8 cm. wide. Six hundred grams of cinchona were moistened, macerated and packed in the tubes. By means of the visible drip chamber, the menstruum was allowed to enter the drug at the rate of one and one-half drops per minute and was forced through the drug by air pressure not exceeding one and one-half atmospheres. The receiver was evacuated to facilitate penetration of the drug by the menstruum and to aid in establishing the correct rate of flow. The preparation of 600 Gm. of fluidextract from 600 Gm. of drug was completed in about twenty days.

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