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THE BIO ASSAY OF ACONITE.*

BY L. W. ROWE.

A few years ago all aconite preparations were being standardized chemically by determining the amount of alkaloid contained in them. A comparison of clinical activity with the chemical showed many discrepancies and a search was begun for a more satisfactory means of determining aconite activity. A physiological method was adopted—M.L.D. guinea-pig method (1)—which has proved to be reasonably accurate and its results are really proportional to the therapeutic value. From a theoretical standpoint the present method is satisfactory and the work of Swanson (2), (3) on this subject has done much to standardize the technique of the test and to render the results obtained in different laboratories correspondingly more uniform.

When considered from the practical viewpoint of a commercial laboratory, however, the guinea-pig method has one disadvantage, namely, that it is more expensive to conduct than the importance of small lots of the drug really warrants. To be reasonably certain of results, from 8 to 15 healthy, normal adult guinea-pigs must be used and the pigs alone are worth usually $10 or more for a test. Sometimes an assay requires an even larger number of pigs.

The high initial cost for test animals alone, led to a desire to reduce it in some way without decreasing the accuracy of the results and experiments were begun using the white mouse as the test animal. The cost of white mice is only about one-tenth that of normal adult guinea-pigs. A technique was developed similar to that using guinea-pigs except that the mice are injected intraperitoneally instead of subcutaneously. This method of injecting gives more rapid and satisfactory results. The volume of the dose injected should never be over 1 cc. with 0.5 cc. as the average volume. The absorption following intraperitoneal injection is certainly more uniform from a quantitative standpoint than is true with subcutaneous administration and the work of Zeigler (4) is corroborative of these facts. Also, the possibility of leakage is negligible, if a fine needle is used; subcutaneous injection into so small an animal as a mouse being often attended by an appreciable loss through leakage at the site of injection.

The following table gives a summary of about twenty tests and presents comparative data for analysis:

* Scientific Section, A. PH. A., Des Moines meeting, 1925.

Sample.	M.L.D. to guinea pigs.	M.L.D. to white mice.	Ratio of M.L.D.'s.
F.E.	.000030 cc. per Gm.	.00030 cc.	1 to 10
Rx 744221	9 used pigs; 10 normal	6 used mice; 6 normal	
F.E.	.00005 cc. per Gm.	.0005 cc.	1 to 10
Rx 153682	3 used; 7 normal	9 used; 7 normal	
Tr.	.0002 cc. per Gm.	.0012 cc.	1 to 6
Rx 749816	3 used; 7 normal	8 used; 10 normal	
Tr.	.0004 cc.	.0025 cc.	1 to 6.25
Rx 749816 (Adjusted)	3 used; 7 normal	4 used; 8 normal	
S.E.	.0000030 Gm.	.000030 Gm.	1 to 10
Rx 757149	4 used; 12 normal	12 normal	
F.E.	.000020 cc.	.00015 cc.	1 to 7.5
Rx 760507	5 normal	4 used; 6 normal	
S.E.	.0000050 Gm.	.000020 Gm.	1 to 4
Rx 749212	9 used; 10 normal	6 used; 12 normal	
Tr.	.00010 cc.	.0005 cc.	1 to 5
Rx 760319	10 normal	5 used; 8 normal	
Trit.	.000030 Gm.	.00015 Gm.	1 to 5
Rx 760580	3 used; 6 normal	5 used; 6 normal	
S.E.	.0000060 Gm.	.000050 Gm.	1 to 8.3
Rx 762868	6 used; 4 normal	6 used; 6 normal	
F.E. Unknown "A"	.000065 cc. Calc. from .000020 cc.	.00040 cc. Theoretical .00048 6 used; 12 normal	1 to 6.1
F.E. Unknown "B"	.000032 cc. Calc. from .00002	.00025 cc. 6 used; 12 normal Theoretical .00024 cc. Error 4% high	1 to 7.8
F.E.	.000025 cc.	.00013 cc.	1 to 5.2
Rx 157146	8 used; 4 normal	5 used; 5 normal	
F.E.	.000030 cc.	.00020	1 to 6.7
Rx 157146 (Expt.)	5 used; 5 normal	10 used; 16 normal	
F.E.	.000030 cc.	.00015 cc.	1 to 5
Rx 157146	5 used; 5 normal	5 used; 6 normal	
Trit.	.00004 Gm.	.0002 Gm.	1 to 5
Rx 766354	4 used; 4 normal	3 used; 4 normal	
Aconitine	.00000008 Gm.	.0000005 Gm.	1 to 6.2
297684	4 used; 4 normal	7 used; 5 normal	
Trit.	.00003 Gm.	.00025 Gm.	1 to 8.3
Rx 767921	8 used; 10 normal	10 used; 8 normal	
Aconitine Sol. Unknown "A"	.00008 cc. 5 used; 6 normal Theoretical .00006 cc. Error 33% high	.0025 6 used; 6 normal Theoretical .0019 cc. Error 32% high	1 to 6.25
Aconitine Sol. Unknown "B"	.00040 cc. 4 used; 8 normal Theoretical .00030 cc. Error 33% high	.0025 6 used; 6 normal Theoretical .0019 cc. Error 32% high	1 to 6.25
F.E. Rx 768482	.000060 cc. 6 used; 5 normal Theoretical .000067 cc. Error 11% low	.00035 cc. 5 used; 7 normal Theoretical .00045 cc. Error 28% low	1 to 5.8

These data furnish several interesting comparisons.

First, as to the average number of each species of animal for a complete test. An average of 11.5 guinea-pigs were required for each test and 14.0 white mice for each test. This shows that very few more mice are needed for a test based on this series and part of this excess was, no doubt, due to absence of data as to the ratio between the doses for guinea-pigs and mice.

Second, as to the ratio between doses for the guinea-pig and white mouse in the various tests. The extremes in the 22 tests are 4 and 10 and the average of all the ratios is 6.7. If the four extremes (4, 10, 10, 10) are eliminated, the average is 6.25 which will be used in arriving at the following suggested standard doses for white mice when injected intraperitoneally.

	Standard M.L.D. for pigs.	Ratio.	Standard M.L.D. for mice.
F. E. Aconite	.000040 cc.	1 to 6.25	.00025 cc.
S. E. Aconite	.000010 Gm.	1 to 6.25	.00006 Gm.
Tr. Aconite	.00040 cc.	1 to 6.25	.0025 cc.
Aconitine	.00000008 Gm.	1 to 6.25	.00000050 Gm.

Third, as to the relative degree of accuracy between the pig and mouse methods. In the adjustment of Rx 749816 from 200% to standard the mouse test indicated the same accuracy as the guinea-pig test. The unknown solutions were known dilutions of previously tested Aconite preparations but were unknown to the person making the test. In five such tests by the mouse method two of the results were almost exactly correct while the other three were from 20% to 32% off. The average error was 18%. Only three such tests were conducted on guinea-pigs and the lowest error was 11%. The average error was 26%. These results on unknowns were not indicative of great accuracy and dependability by either method but the results on mice were even more accurate than those on pigs and the guinea-pig test has already been accepted as being the most dependable test available for aconite so the mouse test is apparently fully as accurate in this series of tests.

The data submitted should be of interest from an experimental standpoint and the greater economy which is evident in the new mouse method should commend it for practical commercial testing purposes where the cost of a guinea-pig assay is quite an item, especially if the lot is small.

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LOCAL ANESTHETICS OF THE AMINO ALKYL BENZOATES.*

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In spite of the vast amount of work which has been done on the physiological action of chemical compounds, there has been very little systematic grouping of facts and still less possibility of drawing general conclusions. Each experimenter

* Scientific Section, A. PH. A., Des Moines meeting, 1925.