since the production of liver damage results in a higher concentration of ergotoxine and ergonovine at the end of selected intervals.

The method described for the determination of ergotoxine, ergotamine and ergonovine in blood and other tissues should be useful for toxicological purposes. The ordinary toxicological texts such as McNalley (9), Peterson, Haines and Webster (10), and Autenrieth and Warren (11) give no method for this purpose if the amount of alkaloid is small. Rosenbloom and Schildecker (12) report that they were able to isolate a crystalline material which gave color reactions and melting point of ergotinine from stomach, intestine, kidney and liver analyzed together. The method described here is sufficiently sensitive but is not specific for a particular ergot alkaloid. The method of drying the blood proved to be so effective that we suggest it for other alkaloids.

SUMMARY

The color reaction of ergot alkaloids with *p*-dimethylaminobenzaldehyde can be used to make quantitative estimation of ergotoxine, ergotamine and ergonovine in blood and muscle.

The three pharmacologically active ergot alkaloids, ergotoxine, ergotamine and ergonovine, disappear quite rapidly from the blood and muscle of guinea pigs after the administration of these drugs. Thus the late appearance of gangrene cannot be explained by the long-continued presence of these drugs in the body.

The alkaloids appear to be detoxicated rather than excreted and the liver appears to take part in this process.

We wish to thank Sandoz Chemical Works, Inc., for supplying ergotamine tartrate (Gynergen) and ergonovine tartrate (Basergin); and Burroughs Wellcome and Co., U. S. A., for supplying the ergotoxine ethanesulfonate used in these experiments.

REFERENCES

(1) Goodman, L., and Gilman, A., "The Pharmacological Basis of Therapeutics," The Macmillan Company, New York, N. Y., 1941.

(2) Van Urk, H. W., *Pharm. Weekblad*, 66 (1929), 473.

(3) Smith, M. I., *Pub. Health Rep.* (1930), p. 1466.

(4) Barger, G., *Hand. der Exper. Pharmakol.*, 6 (1938), 90.

(5) Burn, J. H., *Pharm. J.*, 118 (1927), 565.

(6) Ifuku, D., *Japan J. Med. Sci.*, 8 (1935), 148.

(7) McGrath, E. J., *Arch. Intern. Med.*, 55 (1935), 492.

(8) Rothlin, E., *Arch. intern. de pharmacodynamie*, 27 (1923), 459.

(9) McNalley, W. D., "Toxicology," Industrial Medicine, Chicago, Ill., 1937.

(10) Peterson, F., Haines, W. S., and Webster, R. W., "Legal Medicine and Toxicology," W. B. Saunders Company, Philadelphia, Pa., 1923.

(11) Autenrieth, W., and Warren, W. H., "Laboratory Manual for the Detection of Poisons and Powerful Drugs," The Blakiston Company, Philadelphia, Pa., 1928.

(12) Rosenbloom, J., and Schildecker, C. B., *J. Am. Med. Assoc.*, 63 (1914), 1203.

Strychnine X. Comparative Accuracies of Stomach Tube and Intraperitoneal Injection Methods of Bioassay*

By Justus C. Ward and D. Glen Crabtree

While carrying out the experiments with strychnine, upon which the earlier papers in this series[1] were based, we found it would be desirable to conduct adequate studies to compare our system of stomach tube bioassay with the better recognized intraperitoneal injection method. In using strychnine as an economic poison, it must be mixed with baits to be taken by mouth by predatory animals and rodents. Consequently, we have adopted the stomach tube bioassay procedure to approximate most closely the conditions under which the poison must act under field conditions.

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[1] "Strychnine I," JOUR. A. PH. A., 19 (1930), 954–957; "II," *Ibid.*, 19 (1930), 1057–1060; "III," *Ibid.*, 23 (1934), 984–988; "IV," *Ibid.*, 25 (1936), 422–426; "V," Thesis, University of Colorado: "VI," JOUR. A. PH. A., 25 (1936), 590–593; "VII," *Ibid.*, 26 (1937), 29–31; "VIII," *Ibid.*, 26 (1937), 129–134; "IX," Unpublished manuscript.

We feel that this is a most logical system for evaluating potent drugs and poisons which must be taken by mouth, since a bioassay should indicate definitely the results to be expected from the general use of the material assayed.

To investigate several aspects of strychnine bioassays, we have tested this poison in the form of the alkaloid and as the sulfate. We have administered it by stomach tube and by intraperitoneal injection to both male and female rats. In the present work from 18 to 36 rats have been used per dose. These experiments were completed between September, 1940, and early June, 1941, and a total of 918 mature adult white rats of one uniform strain were used. The technique of stomach tube bioassay employed varied only slightly from that previously reported (1). In the present experiments, the authors worked always together, one of us holding the rats without arousing in them undue nervous excitation, while the other administered the previously measured dose through a No. 8 soft rubber catheter which had been passed into the stomach through a perforated, wooden gag. The same holding technique was used when the intraperitoneal injections were given. In all strychnine alkaloid bioassays, the poison was suspended by first mixing the strychnine with a double quantity of U. S. P. powdered Gum Acacia in the dry condition; a few drops of water were added to this mixture and the mass stirred until all the strychnine particles were completely wetted. The resulting paste was then diluted to such a volume that 1 cc. of suspension contained exactly 1 mg. of strychnine alkaloid. This suspension was always stirred rapidly for a few seconds before each dose was removed. In the strychnine sulfate bioassays a true solution was prepared which carried the equivalent of 1 mg. of strychnine alkaloid per cubic centimeter of solution.

The poison samples used in these experiments were taken from laboratory reference lots which had been tested previously and all found to be equally toxic, and consisted of U. S. P. IX Strychnine Alkaloid and U. S. P. Strychnine Sulfate Crystals manufactured by the same firm.

Our experimental results are shown in Tables I, II, III, IV and V.

Table I.—Toxicity of Strychnine Alkaloid by Stomach Tube

Dose, Mg./Kg.[a]	Male Rats No. Used	Male Rats % Mortality	Female Rats No. Used	Female Rats % Mortality
15.00	18	89	18	100
10.00	18	83	18	100
7.50	18	89	18	100
6.00	18	33	18	100
5.00	18	22	18	100
4.00	..	..	18	95
3.00	..	..	27	74
2.50	..	..	27	70
2.25	..	..	18	33

[a] Milligrams of strychnine per kilogram of animal body weight.

Table II.—Toxicity of Strychnine as the Sulfate by Stomach Tube

Dose, Mg./Kg.[a]	Male Rats No. Used	Male Rats % Mortality	Female Rats No. Used	Female Rats % Mortality
15.00	18	78	18	100
10.00	18	94	18	100
7.50	18	72	18	100
6.00	36	31	18	100
5.00	18	17	18	100
4.00	..	..	18	94
3.00	..	..	18	94
2.50	..	..	18	39

[a] Milligrams of strychnine per kilogram of animal body weight.

Table III.—Toxicity of Strychnine as the Sulfate by Intraperitoneal Injection

Dose, Mg./Kg.[a]	Male Rats No. Used	Male Rats % Mortality	Female Rats No. Used	Female Rats % Mortality
15.00	18	100	18	100
10.00	18	100	18	100
7.50	18	100	18	100
6.00	18	100	18	100
5.00	18	100	18	100
4.00	18	95	18	100
3.00	18	67	18	95
2.50	18	22	18	89
2.25	..	..	18	83
2.00	..	..	18	89
1.75	..	..	18	83
1.60	..	..	18	56
1.50	..	..	18	61
1.40	..	..	18	39

[a] Milligrams of strychnine per kilogram of animal body weight.

Table IV.—Summary of Toxicity of Strychnine to Mature White Rats

	L.D.50% Computed from Mortality Curves	
	Male Rats	Female Rats
Strychnine alkaloid by stomach tube	6.50 mg./Kg.	2.35 mg./Kg.
Strychnine as the sulfate by stomach tube	6.50 mg./Kg.	2.60 mg./Kg.
Strychnine as the sulfate by intraperitoneal injection	2.80 mg./Kg.	1.45 mg./Kg.

In Table V are shown the comparative speeds of kill with strychnine at the different

dosage levels used. Obviously only those animals dying in each series can be listed in such a tabulation, so the per cent mortality at each dose is included to aid in evaluating the data.

Table V.—Speed of Strychnine Action

Dose, Mg./Kg.[a]	Average Time to Death in Minutes: Male Rats % Mortality	Male Rats Time	Female Rats % Mortality	Female Rats Time
A. As the alkaloid by stomach tube				
15.00	89	10.8	100	7.4
10.00	83	12.5	100	7.3
7.50	89	11.9	100	8.5
6.00	33	19.5	100	8.5
5.00	22	13.3	100	8.3
4.00	..	..	95	11.2
3.00	..	..	74	12.0
2.50	..	..	70	14.1
2.25	..	..	33	13.3
B. As the sulfate by stomach tube				
15.00	78	11.0	100	7.2
10.00	94	13.1	100	7.3
7.50	72	14.0	100	7.5
6.00	31	14.8	100	7.5
5.00	17	11.0	100	8.1
4.00	..	..	94	11.1
3.00	..	..	94	12.6
2.50	..	..	39	15.8
C. As the sulfate by intraperitoneal injection				
15.00	100	4.1	100	3.6
10.00	100	5.7	100	3.6
7.50	100	6.1	100	7.3
6.00	100	8.9	100	8.3
5.00	100	8.8	100	6.8
4.00	95	9.5	100	9.4
3.00	67	13.3	95	9.7
2.50	22	19.3	89	11.6
2.25	..	..	83	9.9
2.00	..	..	89	18.5
1.75	..	..	83	18.5
1.60	..	..	56	15.6
1.50	..	..	61	12.2
1.40	..	..	39	12.2

[a] Milligrams of strychnine per kilogram of animal body weight.

DISCUSSION

Tables I, II and III bring out quite forcibly the comparison between stomach tube and intraperitoneal injection methods of bioassay for strychnine. They show how difficult it is to obtain a 100% mortality of male rats with strychnine either as the alkaloid or as the sulfate when it is given by stomach tube at moderate doses. This observation is correlated with field reports of variable results found in using strychnine as an economic poison. They also show that strychnine toxicity follows the general pattern of the standard curve, with the susceptibility of the female rat approaching the ideal in most cases.

Table IV illustrates the differences in toxicity of strychnine to male and female rats. Although this characteristic is more marked for stomach tube administration, it is shown quite plainly on intraperitoneal injection also. Other workers have made similar observations on this sex variation (2).

Table V shows that there are smaller differences in the times required to kill susceptible individuals than the large variations in doses administered would indicate should be expected.

The results of these tests justify our selection of stomach tube bioassay on mature male rats for properly evaluating the efficiency to be expected from the use of strychnine under field conditions (1). They furthermore prove that reliable bioassays can be made using either suspensions of strychnine alkaloid or solutions of strychnine as the sulfate administered by stomach tube.

CONCLUSIONS

1. Strychnine given by stomach tube is uniformly less toxic than it is when given by intraperitoneal injection.

2. Strychnine as the alkaloid and as the sulfate is equally toxic when given by stomach tube to male rats, and it varies only slightly when tested on female animals.

3. Female rats are more than twice as susceptible to strychnine at the $L.D._{50\%}$ dose by stomach tube than are male rats of the same strain.

4. Female rats are slightly less than twice as susceptible as are males to strychnine by intraperitoneal injection.

5. Bioassay of strychnine alkaloid held in suspension by means of 0.2% gum acacia and administered to standardized male rats by stomach tube gives the most accurate indication of the field efficiency to be expected of the sample tested.

BIBLIOGRAPHY

(1) Ward, Munch and Garlough, JOUR. A. PH. A., 25 (1936), 590.

(2) Poe, Suchy and Witt, *J. Pharm. Exptl. Ther.*, 58 (1936), 239.