STUDIES ON STRYCHNINE.

1. The Relative Sensitivity of Certain Chemical and Physiological Tests.

BY JUSTUS C. WARD* AND JAMES C. MUNCH.[1]

As the result of an intensive study of the efficiency of various substances in the control of predatory animals and rodents a great deal of attention has been directed to the use of strychnine alkaloid and its salts. A large number of mixtures have been prepared containing various proportions of strychnine in combination with acids, alkalies, sugars, oils, proteins and albumins. The results obtained by investigators of the Bureau of Biological Survey since its inception in 1905 have been consolidated in this series of papers. In this communication the relative sensitivity of a number of chemical tests is reported, as well as experiments to determine the minimum concentrations possessing just detectable bitterness to the human taste.

CHEMICAL TESTS.

A search of the literature failed to reveal any extensive investigation of the relative sensitivity of the various chemical tests which have been reported from time to time (1, 2, 4, 10).

A number of samples of commercial strychnine alkaloid, sulphate and hydrochloride obtained on the open market were analyzed quantitatively by the method outlined in U. S. P. X (11) and found to contain ninety-nine per cent or more of the estimated amount of free alkaloid. Brucine was not detected in any sample. In making tests upon the solid material a small amount was placed upon a porcelain crucible cover and the liquid reagents added drop-wise. In testing liquids, freshly prepared solutions were used. The developments of various colors were noted over a period of ten to fifteen minutes. The technic employed in the chemical tests is as follows:

A. Potassium Dichromate: One drop of a solution (0.05 cc.) of known concentration was measured from a 1-cc. Mohr pipette. In other tests, one cc. of a solution of known concentration was carefully evaporated to dryness in a drying oven at 50° C. Two drops of concentrated sulphuric acid were then added and stirred with a glass rod until solution was complete. A minute crystal of potassium dichromate was placed *near* the solution and streaked through it by a fine-tipped glass rod. A test was not considered positive unless the entire play of colors (violet, purple, blue, reddish brown and finally green) was obtained.

B. Lead Dioxide: The same technic was used as in *A*. Powdered lead oxide was used. The same play of colors was obtained except that the purple and blue were less vivid because of the masking effect of the lead oxide.

C. Potassium Permanganate: The same technic was used as in *A*. The purple and blue colors were overly intensified. Some degree of color was always developed from the interaction of the reagents employed. Accordingly the authors feel this test is not sufficiently distinct to be of much service.

D. Manganese Dioxide: The same technic was employed and the same difficulties developed as in *C*, due to the black color caused by the oxidizing agent.

E. Mandelin's reagent: (One per cent ammonium metavanadate in concentrated sulphuric acid.) The reagent was always freshly prepared. The sequence of development of colors

* Associate Pharmacologist.

[1] Consulting Pharmacologist, Control Methods Research, Bureau of Biological Survey, U. S. Dept. of Agriculture.

was identical with *A*. The violet color lasted much longer than with potassium dichromate, but the other colors were less persistent.

F. Potassium Iodate: The same technic was used and an identical play of colors observed as in *A*. However, the test was the least sensitive of those employing oxidizing agents in sulphuric acid.

G. Malaquin's reagent (6): To one cc. of solution, two cc. of dilute hydrochloric acid and approximately 0.2 Gm. of granular zinc were added. After standing one minute the mixture was rapidly brought to a boil, cooled and carefully stratified on two cc. of concentrated sulphuric acid. With concentrated solutions of strychnine a rose ring developed immediately at the juncture of the two layers; with dilute solutions an interval of five to ten minutes was usually required. This color diffused throughout the solution on standing. Buc[1] has published a modification of this test with the claim that it will detect 1 microgram of strychnine; this has not been confirmed by the authors.

The results obtained in testing a series of solutions of gradually decreasing concentration are given in Table I.

TABLE I.—CHEMICAL TESTS FOR STRYCHNINE.

Reagents used.	Threshold detected micrograms.
1. Sulphuric Acid + Potassium Dichromate	1.0
2. Sulphuric Acid + Lead Dioxide	1.7
3. Sulphuric Acid + Potassium Permanganate	1.7
4. Sulphuric Acid + Manganese Dioxide	1.7
5. Mandelin's Reagent	1.7
6. Potassium Iodate	5.0
7. Malaquin's Test	6.7

It is evident that the potassium dichromate test is the most delicate, serving to detect 1 microgram (0.001 milligram) of strychnine. Tests *B*, *C*, *D* and *E* were equally sensitive, having a threshold at 1.7 micrograms. The test F had a threshold at 5 micrograms. The test *G* was the least sensitive, having a threshold at 6.7 micrograms. For quantitative purposes it is concluded that tests with potassium dichromate when properly conducted are the most useful and will serve to detect one microgram of the alkaloid.

PHYSIOLOGICAL TESTS.

For the physiological determination of strychnine, several methods have been suggested in the literature. The most sensitive was that developed by Newman (5, 9, 12) and extensively employed in studying the elimination of strychnine. Strychnine solution injected into the ventral lymph sac of frogs produced perceptible convulsions (5, 12). The very small amount of one microgram of strychnine was determined quantitatively by intra-spinal injection into frogs. Falck (3) demonstrated the presence of 2 micrograms of strychnine by the effect upon immature mice. Tests have also been made upon plants, crabs, rats, cats, dogs and other animals (8). A comparative study of the detection and determination of strychnine using animals will be reported subsequently.

In this comparative study, the technic developed for the bio-assay of capsicum was used (7). Stock solutions of strychnine base and of its salts were prepared containing one part of strychnine alkaloid in ten thousand parts of distilled water (1 cc. contains 100 micrograms). Solutions were prepared for taste tests by dilut-

[1] *J. A. O. A. C.*, 3 (1919), 193.

ing measured volumes of the stock solution immediately before use. Five cc. of the test solution were retained in the mouth for exactly one minute, being flooded back and forth over the taste buds, then ejected. Great variation was noted in the time interval until bitterness was evident. In some instances bitterness was detected first at the tip of the tongue, in others on the sides or base. In some individuals a response was obtained within five seconds even with the threshold dilutions; in others an interval as long as one minute was required. For the sake of accuracy a recovery period of at least thirty minutes elapsed between tests on the same individual. The results obtained are reported in Table II.

TABLE II.—THRESHOLD TASTE LIMEN OF HUMANS FOR STRYCHNINE.

Results in Micrograms of Alkaloid.

Solvent.	Strychnine in Form of: Alkaloid.	Sulphate.	Hydrochloride.
Distilled Water	5.6	5.4	5.8
City Water	6.1	6.4	6.3
Sodium Chloride—0.6%	7.1	7.0	8.5
Sodium Chloride—0.9%	7.2	7.2	9.5
Sucrose—1%	8.9	7.2	7.2
Sucrose—5%	11.1	8.5	9.5
Sucrose—10%	17.5	14.8	14.3

Detailed study of the various thresholds show that it is possible to mask the bitter taste of strychnine by a number of products. In general a solution of strychnine in the form of the alkaloid was less bitter than in the form of the salt. This may be due to a slower rate of solution of strychnine within the cells of the taste buds around the terminations of the glosso-pharyngeal nerve. Solutions were less bitter in tap water than in distilled water, and a progressive decrease in apparent bitterness was noted in 0.6 per cent and 0.9 per cent sodium chloride, respectively. With the alkaloid and the sulphate, dilutions in 1 per cent sucrose gave a greater masking effect than 0.9 per cent sodium chloride. The sulphate gave practically the same bitterness and the hydrochloride was more bitter. Increasing the concentration of sucrose to 5 per cent and 10 per cent progressively decreased the apparent bitterness.

TABLE III.—INDIVIDUAL VARIATION IN THRESHOLD TASTE LIMEN OF FIVE HUMANS TO STRYCHNINE.

Solvent.	Strychnine as alkaloid: Individual No. 1.	2.	3.	4.	5.	Average.	Strychnine as sulphate: Individual No. 1.	2.	3.	4.	5.	Average.
Distilled Water	5.5	5.0	6.3	6.3	5.0	5.6	5.0	6.3	...	5.0	5.5	5.4
City Water	5.5	5.5	6.3	7.2	6.3	6.1	6.3	6.3	...	7.2	6.3	6.5
Sodium Chloride—0.6%	6.7	6.7	....	7.7	7.2	7.1	6.7	6.3	7.7	6.7	7.7	7.0
Sodium Chloride—0.9%	7.5	7.2	....	7.2	7.2	7.2	6.7	....	7.7	6.7	7.7	7.2
Sucrose—1%	7.7	8.3	10.0	10.0	8.5	8.9	7.2	7.2	...	7.2	7.2	7.2
Sucrose—5%	10.0	10.0	10.0	11.1	14.3	11.1	8.3	8.3	...	9.1	8.3	8.5
Sucrose—10%	16.7	14.3	16.7	20.0	20.0	17.5	14.3	14.3	...	14.3	16.7	14.7

The agreement of thresholds of five humans, tasting the same dilutions at the same time, are shown in Table III. In general, results of tests by the same individual agreed within 5 per cent.

CONCLUSIONS.

1. Chemical tests for the qualitative and quantitative determination of strychnine have shown that potassium dichromate–sulphuric acid is the most

sensitive, giving a positive reaction with a quantity of one microgram (0.001 milligram).

2. The average limit of perceptibility of bitterness of strychnine dissolved in distilled water to human taste was found to be five micrograms.

3. The addition of sodium chloride, or sucrose, decreased the apparent bitterness.

4. Marked differences in reaction time and place of detection on the tongue have been found in different individuals.

5. When each individual is standardized, it is possible to detect differences of five per cent without trouble.

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FOOD AS A PREVENTIVE MEDICINE.*

BY EDSEL A. RUDDIMAN.

Good health is the most important thing in life to have. Food largely influences good health. Therefore, food is the thing above all others which should receive our attention.

I have a little hesitancy in saying anything on the subject of food, because so much has been said and written; and yet in a way we have been more careless in this one thing than in anything else that we do. Of course if we all had perfect health there would be no need of pharmacy, but there is not one of us that would not gladly seek some other calling if that time should ever come.

Doctor Scoville (1), in an article entitled "Pharmacy, a Review and a Forecast," says: "The presence of the lunch counter in the drug store, which the old-time pharmacist views with misgivings, may be a real step in advance." I quite agree with him provided the pharmacist puts over the idea of what are the proper

* Scientific Section, A. PH. A., Rapid City meeting, 1929.