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STRYCHNINE VIII. THE RELATIONSHIP OF BORAX AND CERTAIN OTHER CHEMICALS TO TOXICITY.*

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Earlier papers in this series presented findings in the Control Methods Research Laboratories of the United States Biological Survey relating to the effects of certain chemicals in modifying the bitter taste of strychnine, (1, 2, 3). The present study grew out of the fact that it was known that certain procedures affecting the bitterness of this alkaloid greatly modifies its toxicity. One case in point is that the addition of a methyl group to the strychnine molecule to form methyl strychnine causes a great reduction in the bitterness and at the same time lowers the toxicity decidedly (4).

Several other theories regarding strychnine action were given more attention in the present study than the taste-toxicity relationship, however. We used chemically unrelated substances in this work, and as a consequence will be forced to report our findings in a rather haphazard manner.

The matter of variation in physiological action of chemically pure strychnine alkaloid might conceivably be mentioned in any study reporting variation in strychnine action presumably due to incorporation with the alkaloid of a foreign chemical. Until the discovery that supposedly identical strychnines would vary decidedly in their killing efficiencies, a tremendous amount of work was done which cannot be compared critically with any later findings, because no reference standard for all tests had been deemed necessary. Within the past year, however, and since the report of our variation findings (5) we have adopted the policy of using a reference standard, so we have a reasonable relationship between various tests.

To emphasize the importance of this factor of variation further, we are presenting a brief summary of the detailed bioassays run at the laboratory during the past year as an introduction to the modification studies.

The system of testing used for all the tests reported in the body of this paper was a minor modification of that reported (5) last year. The rats used were of the same strain and were kept in the laboratory long enough to become acclimated and to have a uniform dietary history. The night before the test the animals were weighed and put into separate cages. They were given water, but no food. The next morning, the strychnine mixture to be tested was made into a suspension by means of 0.2% acacia in water. The animals were then stomach-tubed with the computed quantities in exactly the manner described before (5).

Readings were taken of the time of treatment, the time the animal went down in the first spasm (T/S), the duration of spasm period (T/T), and the time until

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death (T/D). Each series of animals used was given the same amounts by weight of strychnine alkaloid; namely, the doses used were 25.00, 22.50, 20.00, 17.50, 15.00, 12.50, 10.00 and 7.50 mg./Kg. Each test then required sixteen rats, as two rats were taken at each dosage. Appreciating the importance of the presence of survivals in a series of animals as far as the proper evaluating of an alkaloid was concerned, we adopted the system of dividing the averages in minutes obtained from the animals dying in the test—by the percentage of animals which succumb. In this way we represent the averages higher than they really are in proportion to the number of survivals—so that an indication is obtained of the effect of survivals on the average.

During the past year we have bioassayed eleven different lots of strychnine, received from five different manufacturers, in sufficient detail to demonstrate the variation of potency previously reported. A summary of these assays is of interest.

TABLE I.—VARIATION IN TOXICITY OF COMMERCIAL STRYCHNINE ALKALOIDS.

Manufacturer.	Number.	Averages. T/S.	T/T.	T/D.	Averages Interpolated for Survivals. T/S.	T/T.	T/D.
A	1	6.4	3.0	9.4	6.4	3.0	9.4
	2	8.0	3.2	11.2	8.0	3.2	11.2
B	1	15.5	5.0	20.5	16.5	5.3	21.8
C	1	15.5	3.0	18.5	16.5	3.2	19.7
	2	12.4	4.8	17.2	15.5	6.0	21.5
D	1	13.3	3.9	17.2	15.3	4.4	19.7
E	1	11.8	4.3	16.1	13.5	4.9	18.4
	2	10.0	4.5	14.5	11.5	5.2	16.7
	3	9.4	3.3	12.7	10.0	3.5	13.5
	4	7.6	4.7	12.3	7.6	4.7	12.3
	5	6.8	4.6	11.4	7.2	4.9	12.1

Also during the year we tested seven different samples which were sent to us under number only, for the purpose of checking the effectiveness of bioassay methods for detection of variation in strychnines of supposedly equal purity. The key to the point of origin of each of these samples is lacking, but the obvious differences are of value in this regard.

TABLE II.—VARIATION IN TOXICITY OF STRYCHNINE ALKALOIDS OF UNKNOWN ORIGIN.

Lot Number.	Averages. T/S.	T/T.	T/D.	Averages Interpolated for Survivals. T/S.	T/T.	T/D.
B 49106	6.85	3.4	10.2	6.8	3.4	10.2
B 52415	6.73	4.3	11.0	7.2	4.6	11.8
B 51528	11.2	3.4	14.6	13.8	4.2	18.0
B 48483	11.2	4.7	15.9	12.8	5.4	18.2
B 47914	12.0	3.6	15.6	14.8	4.4	19.2
B 46644	17.3	6.0	23.3	21.3	7.4	28.7
B 48127	18.9	5.2	24.1	23.3	6.4	29.7

From the viewpoint of economic poisoning it is apparent that there is little difference in the effectiveness of Lots 49106 and 52415; or of the next three samples; or of the last two, when compared with each other; but it is also evident that there is a great deal of difference between the first two and the last two. The first lot had no survivors, while the second had only one, with average T/D's of 10.2 and 11.8. The sixth lot had three survivors, as did the seventh, and the T/D's were 28.7 and 29.7, respectively. In deciding about the suitability of these samples for economic poisoning use, we would promptly pass the first two lots for any purpose; we would pass the next three for rodent poisons for the less discriminating animals; and we would prefer to refuse to use the last two lots.

Largely because we have been working toward some way to decrease this undesirable "spread," the tests reported below have been run with several substances combined with the poison to determine their effects on strychnine action. Our studies followed several lines of investigation: (A) The use of substances with strychnine which might potentiate the toxicity of the alkaloid; (B) the use of substances which might change the absorption of the poison by the modification of some physiological function in the animal; (C) the use of chemicals which might retard strychnine action in any way. It is not a simple matter to divide the materials used because the results obtained did not always follow the lines we would have expected from a knowledge of their pharmacological actions. Accordingly, the distribution is made largely for a matter of convenience in comparison of results. Averages based on "survival corrected" figures will be used.

A. SUBSTANCES WHICH SEEM TO POTENTIATE STRYCHNINE ACTION.

(*a*) Sodium Azide.

Sodium Azide.			Check Series.		
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
4.3′	1.8′	6.1′	10.5′	4.1′	14.6′

(*b*) Borax (1/2 as much borax as strychnine in the suspension).

T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
7.2′	3.1′	10.3′	10.5′	4.1′	14.6′
(2 times as much borax as strychnine.)					
6.6′	3.8′	10.4′	10.5′	4.1′	14.6′

(*c*) Sodium Thiosulfate. (3 times as much thiosulfate as strychnine.) Strychnine as the sulfate in both the series reported.

				Check Series.	
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
9.7′	8.7′	18.4′	14.1′	6.3′	20.4′

B. SUBSTANCES WHICH INCREASE THE SPEED OF STRYCHNINE ACTION DUE TO SOME MODIFICATION OF PHYSIOLOGICAL FUNCTION.

(*a*) Sodium Bicarbonate. (Equal quantities of soda and strychnine used.)

				Check Series.	
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
11.4′	3.9′	15.3′	13.8′	4.2′	18.0′

(*b*) Sodium Chloride (see under C-*e*).

C. SUBSTANCES WHICH DELAY STRYCHNINE ACTION.

(*a*) Tannic Acid. (Strychnine as the hydrochloride used.)

Dose Strychnine.	Dose Tannic Acid.	T/S.	T/T.	T/D.
20.00 mg./Kg.	20.00 mg./Kg.	11.0′	3.0′	14.0′
20.00 mg./Kg.	20.00 mg./Kg.	12.0′	11.0′	23.0′
20.00 mg./Kg.	20.00 mg./Kg.	27.0′	4.0	31.0
20.00 mg./Kg.	25.00 mg./Kg.	16.0	1.0	17.0
20.00 mg./Kg.	30.00 mg./Kg.	7.0	10.0	17.0
20.00 mg./Kg.	40.00 mg./Kg.	22.0	61.0	83.0
20.00 mg./Kg.	50.00 mg./Kg.	14.0	63.0	77.0
20.00 mg./Kg.	60.00 mg./Kg.	23.0	48.0	71.0

(*b*) Ethyl Alcohol. (Alcohol series was run with strychnine in 95% C_2H_5OH.)

	Test Series.			Check Series.		
Dose.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
400 mg./Kg.	4.0′	25.0′	29.0′	4.0′	3.0′	7.0′
300 mg./Kg.	6.0	18.0	24.0	3.0	3.0	6.0
200 mg./Kg.	6.0	12.0	18.0	5.0	7.0	12.0
100 mg./Kg.	8.0	12.0	20.0	10.0	2.0	12.0
50 mg./Kg.	17.0	5.0	22.0		Survived.	
25 mg./Kg.		Died over night.			Survived.	

(*c*) Activated Calcium. (Test series was run with suspension in 100% calcium water.) Standard doses and 16 rat series.

Test Series.			Check Series.		
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
23.8′	5.5′	29.3′	11.6′	6.5′	18.1′

(*d*) Magnesium Chloride. (Strychnine used was in the form of the hydrochloride.)

1. Magnesium chloride $^1/_{10}$ the concentration of strychnine.
2. Magnesium chloride $^1/_4$ the concentration of strychnine.
3. Magnesium chloride $^1/_2$ the concentration of strychnine.

1.			2.			3.		
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
10.5′	2.7′	13.2′	11.3′	5.2′	16.5′	13.2′	6.8′	20.0′

(*e*) Sodium Chloride.

1. Sodium chloride $^1/_2$ the concentration of strychnine.
2. Sodium chloride the same concentration as strychnine.
3. Sodium chloride 2 × the concentration of strychnine.
4. Sodium chloride 5 × the concentration of strychnine.

1.			2.			3.			4.			Check.		
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
18.0′	6.2′	24.2′	6.9′	4.6′	11.5′	9.8′	6.3′	16.1′	18.8′	7.1′	25.9′	15.3′	4.8′	19.7′

In addition to the differentiated studies given above, an additional series of tests was run to determine the effect of powdered yellow dextrin on strychnine toxicity, when this material was used in varying concentrations. The following tabulation is not included in the above tests because larger doses were employed than those reported in the other experiments, in that the dosage range was from 12.50 to 31.75 mg./Kg.

1. 2.5 × as much dextrin as strychnine.
2. 5.0 × as much dextrin as strychnine.
3. 7.5 × as much dextrin as strychnine.
4. 10.0 × as much dextrin as strychnine.

1.			2.			3.			4.		
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
8.6′	2.8′	11.4′	8.4′	3.4′	11.8′	11.0′	4.3′	15.3′	11.5′	2.5′	14.0′

Then, a test to confirm or contradict the belief that a mixture of strychnine and brucine was more toxic than strychnine alone. We first ran preliminary tests with the two alkaloids separately, followed by an experiment with a 50–50 mixture of the separate alkaloids. This latter test being checked by a commercial 50–50 mix supplied us for study by one commercial manufacturer.

The strychnine alkaloid was different from our standard reference poison because we wished to use a strychnine made by the same firm as our brucine.

Strychnine.			Brucine.		
T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
15.6′	3.7′	19.3′	No kills 50–150 mg./Kg.		

	Lab. 50–50 mix of the above alkaloids.			Commercial 50–50 mix.		
Dosage Range.	T/S.	T/T.	T/D.	T/S.	T/T.	T/D.
10–50 mg./Kg.	13.2′	4.5′	17.7′	10.9′	4.6′	15.5′
Total alkaloids	Survivors at 10–15 mg./Kg.			Survivors at 10 mg./Kg.		

In this test we failed to demonstrate any toxicity other than that carried in the strychnine. The obvious weakness of the brucine in the sample used was without doubt the reason for this.

In much of the laboratory work on rats, the tests were instituted to check observations previously made in the field by one of us, in work carried out with Zuni prairie dogs. Owing to the intensely practical phase of the outside work a fixed dose of 7 mg./Kg. was given to these small rodents by stomach tube, and the "reaction times" were recorded. From a list of approximately forty substances tested, we will select five to demonstrate the fact that the laboratory figures represent the way in which the results will show up under similar conditions in field testing.

Substance.	Ratio of Substance to Strychnine.	T/S.	T/D.
Strychnine alkaloid		31.5′	73.0′
Sodium azide	2 ×	8.4	20.3
Sodium nitrite	2 ×	19.5	39.5
Sodium thiosulfate	3 ×	30.0	59.5
Borax	1 ×	27.0	42.0 to ON

DISCUSSION.

In these tabulations it appears that we have shown a continued variation to be present in alkaloidal strychnine obtained from various manufacturers for use in economic poisoning.

It also appears that sodium azide exerts the most potent influence in speeding up the toxic action of strychnine, since the T/D dropped from 14.6 minutes to 6.1 minutes, when the azide was added to the suspension. Borax in quantities of from $^1/_2$ to 2 × slightly speeds strychnine action. Sodium thiosulfate resembles borax in action. Sodium bicarbonate in a quantity equal to that of strychnine gives a slight speeding of killing time. Tannic acid, ethyl alcohol and activated calcium seem to slow absorption, but do not detoxify the strychnine, so death eventually occurs. Magnesium chloride antagonizes strychnine in direct proportion to the concentration of the salt used, from the ratios $^1/_{10}$ to $^1/_2$ tested. Sodium chloride has an action dependent upon the concentration. There is apparently no consistency because $^1/_2$ and 5 × both slow the T/D, while 1 × and 2 × both speed the T/D. This is probably due to action on osmosis.

Powdered yellow dextrin had slight influence on strychnine action up to a ratio of 7.5 and 10.0 × the strychnine. At these figures there was a slight slowing of the strychnine speed of kill. In these tests brucine did not appear to exert a potentiating influence on strychnine, probably owing to a non-toxic brucine being involved.

CONCLUSIONS.

1. Strychnine alkaloids of apparently uniform chemical composition vary in their physiological activity.
2. Certain chemicals markedly influence the lethal rates of strychnine.
3. Sodium azide and sodium nitrite are most effective in speeding up the toxic action of strychnine.
4. Tannic acid, ethyl alcohol and activated calcium were the most effective in retarding the toxic action of strychnine.
5. Other substances tested were intermediate in their action, and did not greatly affect the toxicity of the poison.

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ASSAY OF FREE ACIDITY IN SHAVING CREAM.*

BY L. F. GABEL.[1]

The assay of free acidity or alkalinity in shaving cream is complicated by the fact that, in order to determine the reaction, it is necessary to use alcohol as a solvent.

Reaction in alcohol introduces a chemistry which differs from the reaction in water; this is particularly true of soap, due to the hydrolysis in water solution.

A series of assays was made in an attempt to note the reaction of soap by

(1) Colorimetric p_H
(2) Electrometric p_H
(3) Assay in 95% neutral alcohol
(4) Assay in small volume of water

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