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## STRYCHNINE IV: LETHAL DOSE STUDIES ON CATTLE AND SHEEP.\*

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The earlier papers in this series (1, 2, 3) have presented data on chemical and physiological tests for strychnine, and on masking of strychnine bitterness by certain chemicals. The present discussion deals with the toxicity of strychnine to cattle and sheep.

Literature on the feeding of measured doses of strychnine to domestic animals to determine the lethal doses is very deficient (4). It is because of this fact that the authors are presenting data accumulated under somewhat adverse conditions, since many more animals were involved in their tests than have been reported upon at any one time previously. In these studies 26 cattle and 65 sheep were used.

The obtaining of such a large number of test individuals was made possible through the cooperation of governmental agencies concerned with the administration of the cattle and sheep-buying programs in Idaho and Wyoming during the fall of 1934. The buying program was carried out as a drought relief measure to prevent starvation of a large number of animals on the depleted ranges during the winter. The animals purchased by the Government were graded down to a class that was called "condemned." These "condemned" animals, by the terms of purchase, were to be slaughtered on the owner's property and were to be destroyed. Many of these individuals were organically sound, but were emaciated to the extent that they would not be able to reach a shipping point in condition to be fed back to usable condition. By following the buying crews it was possible for the authors to obtain animals in fairly good condition for their experiments. The tests were always run with the full knowledge and consent of the owner of the "condemned" stock. Normal stock may differ in susceptibility.

Two major observations were made on each animal studied; *first*, the lethal dose; *second*, the rate and manner in which the animal accepted the ground squirrel poison which was the form in which the strychnine was fed. Because of the fact that both cattle and sheep refused the poisoned grain in many cases it was necessary to resort to forced feeding to obtain the lethal dose data needed. Tabulations will show where this forced feeding was used.

The results seem widely variable, so the correlation of dosage trials will prove of value.

Tables I and II indicate that four of eight "condemned" animals died at 15 mg./Kg.; two of three animals at 16 mg./Kg. and all died at doses of 18 mg./Kg. and above. Doses below 12.50 mg./Kg. were only occasionally dangerous, as in the case of a markedly susceptible animal, since only one of nine cows in this dosage range died.

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\* Scientific Section, A. P. H. A., Portland meeting, 1935.

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TABLE I.—STRYCHNINE EFFECTS IN "CONDEMNED" CATTLE.

Dose, Mg./Kg.	Bait.	Method of Application.	Symptoms.	Results.
20.00	Bran mash	Forced <sup>1</sup>	Spasms—25 min.	Death—40 min.
18.00	Grain	Fed	" --42 "	" --90 "
17.80	Bran mash	Forced	" --15 "	" --20 "
17.50	Grain	Fed	Completely refused the bait	-- Shot
17.50	"	"	" " " "	"
16.00	Bran mash	Forced	Spasms— 2 hours	Death— 4 hours
16.00	" "	"	" --33 min.	" --43 min.
16.00	" "	"	Stiff—no spasms	Survived 4 1/2 hours; shot
15.00	" "	"	Spasms—30 min.	Death—60 min.
15.00	Grain	Fed	None	Survived 4 hours; shot
15.00	"	"	Slightly nervous	" 2 " "
15.00	Bran mash	Forced	None	" 9 " "
15.00	" "	" <sup>2</sup>	Spasms—8 min.	Death—10 min.
15.00	" "	"	" --40 "	" --95 "
15.00	" "	"	" --35 "	" --80 "
15.00	" "	"	Slightly nervous	Survived
12.50	Grain	Fed	" "	Survived 4 hours; shot
12.50	"	"	None	Survived 2 1/2 hours; shot
12.00	Bran mash	Forced	"	Survived 6 1/2 hours; shot
12.00	" "	"	Slight spasm 2 1/2 hours	Survived 6 1/4 hours; shot
12.00	" "	"	None	
	After 18 hours animal normal Force fed 15 mg./Kg. more			Survived both doses
10.00	Grain	Fed	Spasms—32 min.	Death—60 min.
10.00	"	"	None	Survived 3 hours; shot
10.00	Bran mash	Forced	None	Survived 3 hours; shot
8.00	" "	"	"	Survived 2 1/2 hours; shot
5.00	" "	"	"	Survived 3 hours; shot

<sup>1</sup> These animals refused to eat the poisoned grain offered, so they were force fed the bran mash.

<sup>2</sup> This animal was highly nervous and excitable.

TABLE II.—CORRELATION OF STRYCHNINE EFFECTS IN "CONDEMNED" CATTLE.

Dose, Mg./Kg.	Results.
20.00	One animal fed this dose died in 40 min. 1/1 (40 min.)
18.00	" " " " " " " " 90 " 1/1 (90 ")
17.80	" " " " " " " " 20 " 1/1 (20 ")
17.50	Two animals " " " refused to eat 0/2 (--, --)
16.00	Two of three animals fed this dose died in 42 min. and 4 hours, respectively. One survived 2/3 (42 min., 4 hours)
15.00	Four of eight fed died in 60, 10, 95 and 80 min. 1/2 (60, 10, 96, 80 min.)
12.50	Two of two animals survived 0/2 (--, --)
12.00	" " " " " " " " 0/2 (--, --)
10.00	One of three died in 60 min. 1/3 (60, --, --)
8.00	One animal survived
5.00	" " " "

To determine what these figures would mean in terms of a ground squirrel poison containing 1 ounce of strychnine to each 10 quarts of steam-rolled oats—the strongest formula generally used in the areas where these animals were tested—the following computations are made. Assuming 15 mg./Kg. as a possible killing dose (LD<sub>50</sub>%), 1/10 of a grain of strychnine per pound of cow would be needed. This means 1.23 Gm. or 80 grains, for the average 800-pound "condemned" adult cow.

In the "1 to 10" ground squirrel poison, these 80 grains would be carried on 1 pound 15 ounces of the steam-rolled bait. Recalling how difficult it was to find even a "condemned" animal that would eat half this quantity of such bait, one can safely conclude that properly distributed ground squirrel poison carries no hazard to cattle. Furthermore, in heavy ground squirrel infestation this 1 pound 15 ounces of poison would be distributed over approximately 4 acres of cattle range. To obtain a possible killing dose, it would, therefore, be necessary for the "condemned" cow to eat every bait on the entire 4 acres, which is inconceivable.

Correlations of data following normal feeding of sheep (Table III) are somewhat badly spotted owing to very slow acceptance of the poison and to the necessity of killing the animals after a maximum of three hours in order that the skinning crew could maintain its schedule. This series does not indicate, however, that 12.50 mg./Kg. would be the dangerous dose for the average animal.

TABLE III.—STRYCHNINE EFFECTS IN "CONDEMNED" SHEEP.

(a) Normal feeding—salt baited—but not forced.

Dose, Mg./Kg.	Method of Application.	Symptoms.	Results.
25.00	Fed	Spasms—89 min.	Death 2 hours
24.20	"	Nervous—3 hours	Killed for crew 3 hours
24.30	"	Spasms—61 min.	Death 96 min.
18.40	"	Spasms—86 "	Death 96 "
17.10	"	Nervous—3 hours	Killed for crew 3 hours
14.70	"	Spasms—84 min. (up)	" " " 3 "
12.50	"	Spasms	Death 60 min.
12.50	"	None	Killed for crew 3 hours
10.10 and 10.00	"	"	" " " 3 "
6.80 and 6.80	"	"	" " " 3 "
6.40	"	Spasms—32 min.	Death 58 min.
5.00 and 4.10	"	None	Killed for crew 3 hours

(b) Forced feeding.

Dose.	No. Animals per Dose.	Symptoms.	Results.
40.00	2	Spasms 34, 13 min.	Death 52, 18 min.
35.00	1	" 25 "	" 44
30.00	2	" 17, 11 "	" 27, 19
28.00	1	" 17	" 25
27.50	2	" 25, 170	" 40, 200
25.00	3	" 15, 21, 54	" 26, 30, 74
22.50	2	" 33, 30	" 41, 35
20.00	3	" 49, 21, —	" 56, 53, over night
17.50	3	" 33, —, —	" 58, ON, killed
15.00	3	" 81, 105, —	" 109, 110, "
12.50	10	" 64, 36, 76, —, —, —, —, —, —, —,	" 74, 57, 129, 6 hours ON, ON, ON, ON, —, —.
10.00	10	" 2½ hours —, —, —, —, —, —, —, —, —,	ON, ON, ON, ON, ON, —, —, —, —, —.
7.50	2	None	Survived

Forced feeding data indicate that 10 mg./Kg. (1/14 grain per pound) would be a dangerous dose for "condemned" sheep. Approximately 6 grains (360 mg.) of

strychnine might kill an 80-pound sheep. From these figures it appears that slightly more than 3 ounces of a 1 to 10 formula might kill a sheep. These 3 ounces of poisoned grain would be exposed on approximately 1 acre of ground squirrel-infested sheep range. It is inconceivable that a sheep might get all the poisoned grain from an acre, even if feed on the pasturage was extremely short.

To test whether or not the amount of strychnine in the mixture would alter the animals' acceptance, a 1 to 16 bait prepared with a large amount of free salt in it was offered to six additional "condemned" sheep. They were allowed to eat all they would, then the balance was weighed back and the dose taken was computed.

#### ACCEPTANCE TEST 1 TO 16 POISONED GRAIN-FED SHEEP.

Dose Eaten, Mg./Kg.	1 to 16 Bait, Ounces.	Result.
4.85	2	No symptoms in 3 hours.
7.00	2 <sup>3</sup> / <sub>4</sub>	No symptoms in 3 hours
6.70	2 <sup>3</sup> / <sub>4</sub>	Very nervous. Killed after 1 <sup>1</sup> / <sub>4</sub> hours
10.95	4 <sup>1</sup> / <sub>2</sub>	Slightly nervous. Killed after 1 <sup>1</sup> / <sub>4</sub> hours
12.40	6 <sup>1</sup> / <sub>4</sub>	Stiff. Killed over night
18.50	7 <sup>3</sup> / <sub>4</sub>	Spasm 68 min. Dead 85 min.

From this record it would appear that where the grain is available in a formula carrying a high percentage of salt there is a higher palatability factor. For that reason care should be exercised with somewhat greater diligence when salty formulas are used on sheep ranges, particularly during these periods of the year when the grass is short and the animals are cropping very closely.

#### CONCLUSIONS.

1. Strychnine in the form of a 1 to 10 ground squirrel poison is not readily taken by either cattle or sheep.
2. There would be needed 1 pound 15 ounces of this 1 to 10 formula to carry a lethal dose for an 800-pound "condemned" cow, and slightly more than 3 ounces for an 80-pound "condemned" sheep.
3. The susceptibility of normal (healthy) animals might differ from that of "condemned" animals.
4. Because of the slowness with which these animals accepted ground squirrel poison voluntarily, many of the tests reported were based on force-feeding methods. This system of administration excited the animals and tended to lower the lethal dose.
5. Because of the small bait spots used in ground squirrel control operations, to get a possible killing dose, a cow would have to pick up *all* the scattered grain from about 4 acres, and a sheep from 1 acre of range.
6. Properly exposed ground squirrel poison offers no hazard to cattle and only a slight hazard to sheep.

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## EPHEDRINE SYNTHESIS I: THE PREPARATION OF PROPIOPHENONE DIETHYL ACETAL AND OF 1-PHENYL-1-ETHOXY-PROPENE-1.<sup>1</sup>

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Ephedrine, the chief alkaloid in the Chinese drug Ma Huang, was discovered by Nagai (1) in 1887. Its constitution was shown by Späth and Göhring (2) to be represented by the formula:  $C_6H_5 \cdot CHOH \cdot CH(NHCH_3) \cdot CH_3$ . Since this contains two asymmetric carbon atoms, four optically active forms may exist, together with racemic or other mixtures of these four. The two active forms in which the hydroxyl and methylamino groups are adjacent were believed to represent *l*- and *d*-ephedrine: the two in which these groups are on opposite sides of the axis, to represent *l*- and *d*-pseudoephedrine (3).

Both ephedrine and pseudoephedrine have been synthesized by various procedures, but the exact relationship existing between them seems still a matter of some doubt (4). The present investigation is an attempt to throw additional light upon this question.

### EXPERIMENTAL.

*Propiophenone Diethyl Acetal.*—The method employed follows that of Claisen (5) in which one mole of ketone in 3 moles of alcohol is treated with 1.1 mole of ethyl orthoformate in the presence of a catalyst (6, 7). Into an Erlenmeyer flask were put 72 Gm. of propiophenone, 88 Gm. of ethyl orthoformate, 84 Gm. of absolute alcohol and 0.3 cc. of 36% hydrochloric acid. The temperature increased about ten degrees, and after several hours there developed a beautiful cerise color which gradually faded into yellow. At the end of 24 hours the product was neutralized with alcoholic KOH and the ethyl formate, alcohol and excess orthoformic ester were removed by distillation. The acetal was fractionated under reduced pressure with yields, in successive operations, of 97.9%, 96.2%, 96.5% and 97.6% of the theoretical.

The product was a colorless, mobile liquid with a faint aromatic odor. A cryoscopic determination of the molecular weight in benzene showed 207.45. (Calculated for  $C_6H_5 \cdot C(OC_2H_5)_2 \cdot C_2H_5$  was 208.2.) Its boiling point under varying pressure was: (6 mm. = 93-96°), (8 mm. = 99-100°), (10 mm. = 100-101°), (14 mm. = 108-110°), (23 mm. = 119°) and (760 mm. = 226-228°). When boiled at atmospheric pressure, there was a slight decomposition into the monoethyl derivative. The refractive index was  $d_{25} = 1.4767$ . The specific gravity  $25^\circ/4^\circ$  was 0.94476.

*1-Phenyl-1-Ethoxy-Propene-1.*—When propiophenone diethyl acetal was boiled for some time under atmospheric pressure, it was partially decomposed into the monoethyl derivative and ethyl alcohol. In order to carry this decomposition to completion, some agent must be introduced as an alcohol acceptor (8). Acetyl chloride serves very well in this capacity, but since one by-product resulting is hydrochloric acid, which would effect complete hydrolysis of the acetal, this must be neutralized as formed. Pyridine functions admirably for this purpose.

<sup>1</sup> Reconstructed from a thesis by Ernest L. Beals presented in partial fulfillment of the requirements for the degree of Master of Science at the Oregon State College.

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