Factors Affecting the Toxicity of Red Squill*

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INTRODUCTION

The object of this work was to determine some of the factors affecting red squill toxicity for the purpose of making red squill more effective as a rat poison. Experiments were conducted to determine the effect of the sex of rats, heat, moisture, fat, pectin and diet on the toxicity of red squill.

Winton (1), Munch, Silver and Horn (2), LeBlanc and Lee (3), and Crabtree, Ward and Welch (4) discuss the effect of red squill on male and female rats. Winton (1), O'Connor, Buck and Fellers (5), LeBlanc and Lee (3), and Munch, Silver and Horn (6) report on the effect of heat application to squill. O'Connor (7) found that white rats prefer dry rather than moist baits containing red squill powder. The effect of fats and oils on the action of drugs and poisons is discussed by Underhill (8) and Sollman (9). It is interesting to note the observation of Manville, Bradway and McMinis (10) that galacturonic acid from pectin can be utilized by rabbits in the detoxification of menthol. Winton (1) states that abnormal diets have little influence on the susceptibility of the rat to squill poisoning. Smith (11) and Gortner (12) report on the effect of diet on selenium toxicity.

EXPERIMENTAL

Procedure of Assay.—The standard assay technique for red squill powders (5, 6) was employed with the exception that all rats were starved for 24 hours instead of 18 hours. The lethal dose was taken to be the smallest amount of red squill powder in mg. per Kg. of body weight that killed 90 to 100 per cent of the rats within 120 hours.

SOME FACTORS INFLUENCING THE TOXICITY OF RED SQUILL

(*1*) *Sex Differences.*—The differences in toxicity of 2 red squill powders due to sex were determined by feeding at various levels to male and female rats on the basis of body weight.

When a powder called Reference Standard Red Squill of 1934 was fed to females only, the results showed that the toxic level of this powder was 350 mg./Kg. toward female albinos. In feeding this squill to male rats, it was found to be 800 mg./Kg. in toxicity.

The toxicity of a commercial red squill powder was calculated to be between 300 mg./Kg. and 400 mg./Kg. for female rats and between 700 mg./Kg. and 800 mg./Kg. for male rats.

The above experiments show that Reference Standard Squill of 1934 was 2.27 times more toxic for female than for male rats, and that the squill obtained commercially was about twice as toxic for females as for males. This work shows the fallacy of using female rats or rats of both sexes in an assay for red squill because of the greater resistance of male rats. For this reason a standard assay of red squill should be based on male rats.

(*2*) *Application of Heat.*—Heat is destructive to many organic toxicologic substances. To determine the effect of heat treatment on the toxicity of red squill the following experiment was conducted.

Reference Standard Squill of 1934, testing 800 mg./Kg. for male rats, was used in this work. The squill was spread in thin layers on aluminum trays and heated in an electric oven. The temperature to which the squill was heated was measured by means of a thermocouple which was placed in contact with the squill. One hundred Gm. portions of red squill were heated for 30 minutes at 100°, 125°, 150°, 175°, 200° and 225° C. Powdered dextrose was added to make up for any loss in weight due to evaporation of moisture or charring.

The toxicity determinations were performed using male rats which were starved for 24 hours, and then fed on an 800 mg./Kg. level with the heat-treated samples of squill in baits containing about two Gm. of Fox Chow as carrier.

This experiment indicated that the higher the temperature applied to the squill powder, the less toxic it becomes. When heated at 100° C. for one-half hour in a hot air oven, the squill was only slightly changed in toxicity, while at 225° C. it was rendered non-toxic to male rats at the 800 mg./Kg. feeding level. These results are expressed in Table I.

Table I.—Effect of a 30-Minute Heating Period on the Toxicity of Standard Red Squill Fed at an 800 Mg./Kg. Level to Male Albino Rats

Temperature of Heat Treatment of Squill, ° C.	Result, Rats Killed/Rats Fed
100	7/10 (2 rats very ill)
125	6/10 (1 rat ill)
150	1/10
175	3/10
200	3/9
225	0/9

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(3) *Moisture in Squill Baits.*—In order to determine the effect of moisture on the toxicity of red squill baits, the following experiment was conducted.

Baits containing a lethal dose of Reference Standard Squill (5 per cent moisture) and Purina Fox Chow (8.46 per cent moisture) as a base were mixed with 0, 25, 50, 75 and 100 per cent of the rats' daily requirement of water. The average daily requirement was found to be about 190 cc. per Kg. for females and about 140 cc. per Kg. for male rats. These baits were fed to rats that had been starved and deprived of water for 12 hours. Female rats were fed on a 350 mg./Kg. level and male rats on an 800 mg./Kg. level of this squill.

It can be seen from the results shown in Table II that the amount of moisture in a bait bears no relationship to the toxicity of the bait.

Table II.—Influence of Water Content of Baits on Toxicity of Standard Red Squill

Amount of Water in Bait Per Cent of Daily Requirement	Rat Sex	Squill Level Mg./Kg.	No. of Rats Leaving Some Bait	Result, Rats Killed/ Rats Fed
0	M	800	1	4/5
0	F	350	0	5/5
25	M	800	0	5/5
25	F	350	0	5/5
50	M	800	1	5/5
50	F	350	3	4/5
75	M	800	2	4/5
75	F	350	5	2/5
100	M	800	5	3/5
100	F	350	5	1/5

(4) *Fat.*—Fats and oils have a retarding effect on the rate of absorption of certain poisons; they also decrease the toxic effect of these poisons on animals due to some purely physical action.

Hydrogenated fat (Crisco) and petrolatum were used in experiments to determine the effect of fat on the toxicity of red squill to rats. Each rat was fed a bait consisting of a known amount of powdered squill, a known percentage of fat and Fox Chow when necessary to make 1 per cent of the rat's body weight. These were fed the required percentage of fat and enough Fox Chow to equal 1 per cent of their body weights. The control rats evidenced no harmful effects from these baits which contained no squill.

It is seen from the results shown in Table III that fat lowers the toxicity of red squill. It is interesting to note that an increase in the dose of red squill from 350 mg./Kg. to 400 mg./Kg. overcame any protective action that the Crisco baits had on female rats.

(5) *Pectin.*—It is thought that pectin acts as a detoxifying agent because of its physical character and because on being broken down in the organism it gives rise to galacturonic acid. This acid is capable of forming conjugation products with toxic materials in the same manner as glucuronic acid (10). To determine the effect of pectin on red squill the following experiment was conducted.

Baits were prepared containing 10 and 50 per cent of dry citrus pectin. They were so prepared that when fed in the proportion of 1 per cent of a rat's body weight, they would contain an 800 mg./Kg. dose of Reference Standard Squill along with the desired percentage of pectin with Fox Chow as a carrier.

When male rats were fed an 800 mg./Kg. dose of the squill in a bait containing 10 per cent pectin, 7/10 died. When 50 per cent pectin was fed in a like bait, 3/8 died. Ten rats were used on the 50 per cent pectin bait, but as 2 rats refused to take the bait, they were not considered in the experiment (Table IV).

Table IV.—Effect of Pectin on the Toxicity of Reference Standard Red Squill Fed on an 800 Mg./Kg. Level to Male Rats

Pectin in Bait %	Result, Rats Killed/Rats Fed
10	7/10
50	3/8

This preliminary experiment indicates that pectin has a detoxifying action on red squill. It seems that the more pectin in a red squill bait, the less toxic will the bait be to rats.

Table III.—Effect of Fats on the Toxic Action of Reference Standard Red Squill of 1934 toward Albino Rats

Type of Fat	Added Fat in Bait, %	Red Squill Feeding Level Mg./Kg.	Rat Sex	Result, Rats Killed/Rats Fed
Control (no Crisco)	0[a]	350	F	10/10
Crisco	10	350	F	7/10 (2 rats ill)
Crisco	25	350	F	8/10 (2 rats ill)
Control (no Crisco)	0	400	F	10/10
Crisco	10	400	F	3/3
Crisco	75	400	F	4/4
Crisco	96	400	F	3/4
Control (no Crisco)	0	800	M	9/10
Crisco	10	800	M	7/10
Control (no petrolatum)	0	350	F	10/10
Petrolatum	10	350	F	7/10 (3 rats very ill)
Petrolatum	25	350	F	6/10 (2 rats ill)

[a] No added fat, fat content of ration 3.0%.

In addition to the control rats fed on the different levels of squill in baits without fat, control rats were used on all percentages of Crisco and petrolatum.

(6) *Diet.*—The action of poisons is sometimes affected by diet. The question was raised whether or not dietary factors might in some way affect the

toxicity of red squill. Preliminary experiments showed that considerable fat in red squill baits slightly lessens the toxicity of the baits. In feeding baits containing 92 per cent protein (casein) and 8 per cent of Reference Standard Red Squill of 1934 (800 mg./Kg. dose) to male rats in amounts equal to 1 per cent of their body weights, there was no change in red squill toxicity. Carbohydrate (cornstarch) was fed in like manner with identical results.

In an attempt to answer the question as to whether the type of diet has any effect on the killing power of red squill, diets were prepared as follows:

Diet I.—Low protein, high carbohydrate diet, consisting of 7 per cent protein, 73 per cent carbohydrate, 11 per cent fat.

Diet II.—High protein, low carbohydrate diet consisting of 45 per cent protein, 35 per cent carbohydrate, 11 per cent fat.

Diet III.—Low protein, high fat diet, consisting of 7 per cent protein, 37 per cent fat, 47 per cent carbohydrate.

Diet IV.—The control diet, Fox Chow, consisting of 20 per cent protein, 3 per cent fat, 46 per cent carbohydrate.

The composition of these experimental diets is shown in Table V. The rats used were normal male albino rats which had been maintained on a diet of Purina Fox Chow. Rat weights ranged from 100 to 320 Gm. Four groups of rats were fed the various diets over a period of 1 month. They then were left without food for 24 hours, after which they were fed an 800 mg./Kg. dose of Reference Standard Red Squill of 1934 in enough Fox Chow to equal 1 per cent of the rat's body weight. Fox Chow was used as the carrier rather than the experimental diets in order to minimize the physical interference which the diets might have on the toxicity of the squill.

Table V.—Percentage Composition of Experimenta Diets

	Diet Number			
Component	I, %	II, %	III, %	IV,[a]
Commercial casein	7	45	7	Purina Fox Chow
Cornstarch	73	35	47	
Crisco	9	9	35	
Cod liver oil	2	2	2	
Dried brewers yeast	5	5	5	
Osborne and Mendel salt mixture	4	4	4	

[a] Guaranteed analysis of Purina Fox Chow; crude protein 20%, nitrogen free extract 46%, crude fat 3% and crude fiber 6%. It also contains vitamins and minerals.

The results shown in Table VI are described in the following paragraphs.

Table VI.—Effect of Previous Diet on the Toxicity of Standard Red Squill Fed in an 800 Mg./Kg. Dose to Male Rats

Type of Diet Fed for 30 Days Previous to Squill Feeding	Result, Rats Killed/Rats Fed Squill
Low protein, high carbohydrate	8/9
High protein, low carbohydrate	2/10
Low protein, high fat	7/10
Control (Purina Fox Chow)	8/9

Diet I.—Ten rats were used in this experiment. One rat died at the end of 10 days on this diet, cause unknown, and, therefore, was not considered in the results. These rats appeared normal after 30 days on this diet. When fed squill 8/9 died, indicating that this diet has no influence on the toxicity of red squill.

Diet II.—Ten rats were used. These rats appeared normal after 30 days of this diet. They were then fed squill. Two out of 10 rats died, indicating that a high protein, low carbohydrate diet markedly decreases the toxicity of red squill.

Diet III.—In this group there were 10 rats. After 30 days these rats developed a greased appearance, but otherwise appeared normal. When fed the squill 7/10 rats died, indicating that this diet has a very slight effect on the toxicity of red squill. This slight effect is due perhaps to the amount of fat remaining in the alimentary tract of the rats because of the previous high fat diet. This resulted in spite of the fact that they were without food for 24 hours. In a previous experiment it was shown that large amounts of fat have a slight lessening effect on the toxicity of red squill.

Diet IV.—Ten rats were placed on this control diet. One rat died from causes unknown at the end of 29 days, and was not considered in this experiment. All nine rats appeared normal at the end of 30 days. On being fed the squill 8/9 died, indicating that this diet had no effect on the toxicity of red squill.

Experiments on rats indicate that the toxicity of red squill is affected by dietary factors. It is to be noted first that one-bait feedings of 92 per cent protein and 92 per cent carbohydrate with a lethal dose of squill have no effect on the toxicity of the squill; while one-bait feedings of various percentages of fat fed in like manner cause a slight decrease in the toxicity of squill. High carbohydrate, low protein; and high fat, low protein diets when fed to rats for 30 days seem to have no marked effect on the toxicity of red squill fed on the 31st day. However, a high protein (casein), low carbohydrate diet when fed in like manner has a marked effect in decreasing the toxicity of red squill.

The reason for the important role of protein (casein) in the diet on the toxicity of red squill is unknown. It may be due to raised tissue immunity toward red squill caused by the high protein diet, or to the combination of the poison with degradation products of protein present in the rat due to the diet.

SUMMARY AND CONCLUSIONS

1. Red squill is about half as toxic for males as it is for female rats. It is recommended that standard assays of red squill be based on male rats rather than female rats, or rats of mixed sexes.

2. The higher the dry air temperature

(above 100° C.) applied to red squill powder, the less toxic it becomes.

3. The amount of moisture in a red squill bait has little relationship to the toxicity of the bait.

4. Large percentages of protein (casein) or carbohydrate (cornstarch) in squill baits do not affect the toxicity of the baits. Considerable fat in red squill baits results in a slight decrease in the toxicity of the bait. The more pectin in a red squill bait, the less toxic will the bait be to rats.

5. Low protein diets containing (1) high fat and (2) high carbohydrate when fed to rats for 30 days, seem to have no marked effect on the toxicity of red squill. However, a high protein (casein), low carbohydrate (cornstarch) diet has a rather definite effect in decreasing the toxicity of red squill baits.

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Svante August Arrhenius (1859–1927) was awarded the Nobel Prize for chemistry in 1903 in view of the special value of his theory of electrolytic dissociation in the interest of the development of chemistry.

Pyrethrin Content of Pyrethrum Grown in India

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Pyrethrum flowers imported into India are from *Chrysanthemum cinerariæfolium*, which is the only species that is commercially important. The product imported is either the powdered flowers or their extracts or various preparations made from either of these. They are widely used as household insecticides, livestock sprays and horticultural dusts and sprays. Large quantities of pyrethrum or its preparations are now being used in India, where the control of disease due to insects is an acute problem, and in view of the vast economic possibilities for pyrethrum, attempts are being made to cultivate the plant in India. The experimental cultivation of pyrethrum flowers has been tried in Kashmir, Muree Hills, Kulu Valley, North Western Frontier Province, Kurrum Valley, United Provinces and other places, and it has been found that the plant grows well at altitudes of 5000–6000 ft. Very recently, however, pyrethrum has been grown successfully by Mr. S. N. Bal, Curator, Calcutta Museum, in the Mayurbhanj State (Orissa) at altitudes of 3400–3600 ft., and we are grateful to him for sending us two of these samples for our analyses. We are also thankful to Mr. R. L. Badhwar for securing samples of pyrethrum, grown in Kashmir and Murree Hills, from time to time for the present work.

The chemical assay of pyrethrum flowers depends upon the estimation of the insecticidal principles, pyrethrin I and pyrethrin II, which are the esters of the ketone-alcohol pyrethrolone with the two acids chrysanthemum monocarboxylic acid and chrysanthemum dicarboxylic acid methyl ester, respectively. The content of pyrethrin of the Dalmatian flowers ranges from 0.38% to 0.58%, the Japanese flowers from 0.58% to 1.21%, the Kenya flowers from 0.90% to 1.48%, the Californian flowers about 1.10%, the Spanish flowers about 0.57%,

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