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Title INVESTIGATIONS ON THE TOXIN OF *TARICHA GRANULOSA*

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There are essentially no publications dealing with the skin toxin of *Taricha granulosa.* This study has undertaken investigations with this toxin in an attempt to determine its potency and its effect on the newt's potential predators.

Test solutions were made by macerating a known volume of newt skin in a known volume of saline solution. A known volume of this mixture was injected into a test animal to check the animal's susceptibility to the toxin. The skin solution was found to be toxic when used in oral, subcutaneous, and intraperitoneal injections. Tests were also made by feeding, both voluntary and by force, portions or entire newts to potential predators.

It was found that one mouse unit (dose, injected intraperitoneally needed to kill a 21 gr. white mouse in ten minutes) equaled 0.0002 cc. of back skin from an adult terrestrial *Taricha granulosa.*

Forty-two vertebrate taxa (3 fish, 6 amphibians, 14 reptiles, 5 birds, and 14 mammals) were tested either with injections or by

feeding; all except *Thamnophis sp.* were conclusively proven to be susceptible to the newt skin toxin. *Thamnophis sp.* were killed by very large injections but survived after eating adult newts.

A toxin was also found to be present in ovarian eggs, oviducal eggs, and the blood of adult males (females were not tested). Very little or no toxin is present in larvae.

Terrestrial newts appear to be more toxic than aquatic newts and back skin appears to be more toxic than ventral skin.

INVESTIGATIONS ON THE TOXIN OF _TARICHA GRANULOSA_

by

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INVESTIGATIONS ON THE TOXIN OF *TARICHA GRANULOSA*

I. INTRODUCTION

The mechanisms by which salamanders are protected from predation have long been of interest to scientists. The primary defense mechanism studied has been that involving integumental toxins. Apparently the first investigations of salamander skin toxins were done in France by Gratiolet and Cloez in 1851 and 1852 (Hubbard, 1903), who studied the properties of the secretions of the skin glands of *Salamandra maculosa*. Research was also done during this period in Germany, by Albini in 1854 and 1858 (Hubbard, 1903) on *Salamandra maculata*. These and other studies during this period by Bochefontaine in 1879, Capparelli in 1883, Calmels in 1884 (Harmon and Pollard, 1948), Drasch in 1892, Vulpian in 1856 and 1864 (Hubbard, 1903) and Dutartre (1889) were primarily interested in the physiological effect of the skin toxins on laboratory animals. During a period extending from 1889 to 1922, C. Phisalix and M. Phisalix did extensive research on animal venoms, including salamander integumental toxins. Among other things, they described the physiological action of the toxin on predators, the occurrence of the skin toxin throughout the life cycle, and the natural immunity of the European viper to the toxin of the European salamanders.

Three alkaloid poisons, collectively discussed in Noble (1931), were isolated from European salamanders and chemically analyzed during this period. Two of these poisons were isolated from Salamandra salamandra, (discussed in Phisalix-Picot, 1900): (1) samandarine ($C_{26}H_{40}N_{2}0$) described by Zalesky in 1866, affecting the respiratory centers in the central nervous system (this same poison was named salamandrine by C. Phisalix in 1889), and (2) samandaridine, described by Faust in 1899, a less soluble, less toxic, but more abundant toxin.

Samandatrin, the third alkaloid poison, was isolated from the granular gland of Salamandra atra. All three of these isolated alkaloids and several unisolated ones have the same general physiological effects as described for samandarine.

Hubbard (1903) was the first person to study salamander defense mechanisms in the United States; although she was concerned primarily with tail autotomy, she made observations on the noxiousness of salamanders in regard to predation by snakes. Among the salamanders she studied was Taricha torosa, the California counterpart of Taricha granulosa, in Oregon. Storer (1925) commented on the protection of Taricha torosa (Triturus=Taricha): "I do not believe that this restriction of daytime hunting is due to fear of enemies as Triturus is abundantly supplied with dermal poison glands and it has never been reported as being fed upon by any of the local land dwelling vertebrates whose food habits are known. In this connection see the experiments of Hubbard (1903)".

This and a superficial paper by Stuhr (1936) who observed the effect of *Taricha granulosa* integumental toxin on the heart rate and respiration of experimental animals, especially frogs, appear to be the extent or research involving integumental toxins of salamanders of the genus *Taricha*.

The embryos of *Taricha torosa* have, however, been extensively studied. The toxic properties of *Taricha torosa* embryos were first discovered by Twitty and Johnson (1934) when transplants of *T. torosa* paralyzed embryos of *Ambystoma tigrinum.* Further work by Twitty (1935, 1937), Twitty and Elliott (1934), Horsburgh, *et al.* (1940), van Wagtendonk (1942), Fuhrman and Field (1941), Davenport and Smith (1942), and Turner and Fuhrman (1947) established the physiological effects of embryonic *Taricha torosa* toxin and also that it occurs in mature ovaries and the blood of some adult females.

The physiological actions of the embryonic toxin of *Taricha torosa* are, in order: (1) weakness of the limbs, (2) progressive loss of coordination, (3) period of inactivity with respiratory increase, (4) convulsions, (5) complete paralysis of limbs and spasmodic contractions of thorasic muscles, (6) cyanosis may or may not appear, and (7) continued heart beat after cessation of respiration (Horsburgh *et al.*, 1940).

Later work by Brown and Mosher (1963), Kao and Fuhrman (1963), and Fuhrman *et al.*, (1963) further isolated, purified, and re-established the physiological effects of the embryonic toxin of *Taricha torosa.* Brown and Mosher (1963) named this toxic extraction "Tarichatoxin" and established it as a non-proteinaceous neurotoxin. The chemical formula of tarichatoxin was established as $C_{11}H_{17}N_3O_8$ (Buchwald, *et al.*, 1964) and found to be identical to tetrodotoxin isolated from the ovaries of *Sphoeroides rubripes,* the Japanese Fuga or puffer fish.

Mosher *et al.*, (1964) have synthesized all available knowledge concerning tarichatoxin and have determined the toxin of the embryo of *Taricha rivularis* to be identical with that of *Taricha torosa.* They have also determined that the eggs of *Taricha granulosa* are toxic but have not isolated or identified the toxin. Mosher *et al.* imply that the integumental toxin of *Taricha torosa* and both the embryonic and integumental toxin of *Taricha granulosa* are identical to tarichatoxin (from *Taricha torosa* embryos); this has not been conclusively shown, but is in fact, at variance with Twitty (1937) who feels the embryonic and integumental toxins of *Taricha torosa* are distinct.

The aim of this study was primarily to determine the presence of toxin in the skin of adult *Taricha granulosa.* Furthermore, an attempt was made to sample as many potential predators as possible to determine if Taricha granulosa enjoys partial or complete protection from vertebrate predators. A mouse unit (mu.) was designated for use in comparison of levels of susceptibility among possible predators. Brief studies were made on ova, larvae, and blood of adult newts to determine presence of toxin in them, and tests were also made to determine relative abundance in aquatic vs. terrestrial adult newts and back skin vs. belly skin in adult newts. Another brief test was made to check the activity of the ingested toxin.

II. METHODS AND MATERIALS

To establish susceptibility of vertebrates to the integumental toxin of *Taricha granulosa*, it is desirable to test as many potential predators as possible. The objective of this study was primarily to study the toxicity of adult integumental toxin, but since others have determined the presence of toxin in the other stages of the life cycle in closely related forms, possible predators on the eggs and larvae have been tested as well as possible predators of the adult.

Several fish are possible predators on eggs, larvae, and adults of *Taricha granulosa*; bluegills, largemouth bass, and catfish all live in the ponds where newts were collected for use in these investigations.

Few amphibians could be considered as potential predators on *Taricha granulosa* adults, but *Rana catesbeiana* and *T. granulosa* adults would very possibly prey on the egg and larval stages.

The most likely predators on *Taricha granulosa* adults are reptiles, particularly *Thamnophis sirtalis concinnus*, which is very abundant around ponds. Two other reptiles, *Thamnophis ordinoides* and *Gerrhonotus multicarinatus*, are also common around ponds and, due to their food habits, are potential predators on recently transformed or young individuals.

Birds which depend upon their fishing ability for food, such as the green heron, the great blue heron, the belted kingfisher, and possibly some ducks, may be considered as possible predators as they could easily substitute Taricha granulosa for fish in their diet.

Predatory birds, hawks and owls, may also be predators, especially owls, since they are nocturnal and would be hunting when the newts are most active.

Since the habitat of Taricha granulosa ranges from ponds and ditches to wooded areas, any mammal which occasionally eats meat could be considered as a possible predator, especially those frequenting the pond or ditch habitats. Among these mammals are moles, raccoons, mink, weasels, and feral cats. Since these animals are difficult to obtain and to maintain in captivity, smaller mammals were used in most of the actual tests. It is assumed that susceptibility of possible predators would be similar to that of the smaller animals tested (a greater volume is allowed for larger animals).

Susceptibility of animals to toxin is tested by two general methods, feeding and injection. In this study, most of the sub-mammalian forms were tested by feeding, either voluntary or force-feeding; it was usually necessary to force-feed these animals. This was done by placing a newt or a section of a newt's tail into the mouth or throat of the test animal. If the mouth was then held closed, the animal would usually swallow the newt.

Injection was used in testing mammals so as to get more quantitative data. In the early experiments, three methods of injection were used, as follows: (1) oral, (2) subcutaneous, and (3) intraperitoneal. In later experiments, after these methods were compared for consistency of results, only intraperitoneal injections were used.

In all susceptibility tests, the time, amount, and method of administration were recorded. Any visible change in the test animal was recorded and in case the injection or feeding was lethal, the time to death; this was recorded to the nearest 30 seconds, due to difficulty in determining the point of death in some animals. In most cases an animal was assumed to be dead when its heart beat could not longer be detected.

For use in the injections, a suspension of Taricha granulosa skin was prepared. In the early experiments, a known area of skin was macerated in a mortar and pestle with a known volume of Ringer's solution. When mammals were tested, mammalian Ringer's was used; when amphibians or reptiles were tested, amphibian Ringer's was used. In later quantitative tests, a known volume of skin was blended with a known volume of Holtfreter's solution. A Waring blender was used and the mixture was blended until no color was apparent in the skin remnants left in the bottom. Unless otherwise indicated, all tests were made with skin from the back of adult male Taricha granulosa in the terrestrial stage. All newts used for preparation of suspension were collected from Benton and Polk counties in Oregon.

Other methods have been used for collecting poison from the skin of salamanders. Boulenger (1892) indicates three of these methods which have been widely used: if a salamander has enlarged parotoid glands or other concentrations of poison glands, subjecting the animal to an electric current or pressing the glandular area with a pair of forceps will cause the secretion to be "squirted out with great strength and sometimes a considerable distance." The third method, described by Bourlenger, was to inject subcutaneously a dose of barium chloride. This treatment should cause the salamander to exude a secretion over its entire body.

All three of the methods for collecting the skin secretions of amphibians indicated by Boulenger are connected with weighing the secretions and mixing and quantifying the data by weight. Since equipment was not available for the exact weighing of minute amounts, it was decided a measure of skin area or skin volume must be used for preparation of skin toxin for injection.

As previously mentioned, a stock solution was first prepared by macerating a known area or volume of Taricha granulosa skin in a known volume of Ringer's or Holtfreter's solution. Dilutions were made of stock solutions by mixing it with Ringer's or Holtfreter's solution, whichever had been used as the solvent of the stock. Dilutions were designated as to percentage of the stock solution. From the percentage and the volume of solution injected, one can compute the actual amount of skin used in any injection.

Using skin area, a standard concentration was assigned and termed 100%. As defined, 100% equals one sq. cm. of skin macerated in one cc. of Ringer's solution. All dilutions were made by starting from the 100% stock. Table 1 indicates how the actual area of skin injected is determined for each dilution and dosage.

Table 1. Relationship of volume and concentration to skin area

Concentration	skin area per 1.0 cc	skin area per 0.5 cc	skin area per 0.2 cc
*100%	1.0 cm^2	0.5 cm^2	0.2 cm^2
50%	0.5 cm^2	0.25 cm^2	0.1 cm^2
25%	0.25 cm^2	0.125 cm^2	0.05 cm^2
12.5%	0.125 cm^2	0.0625 cm^2	0.025 cm^2

*100% = 1 sq. cm. of skin/1 cc. of Ringer's solution

In those tests involving skin volume each mixture was different and each was designated as 100%; these were later correlated through the determination of skin volume in each injection. Table 2 indicates how skin volume per injection was determined for mixture number 25, one of the most often used mixtures in this study. One hundred percent in mixture 25 equals 1.4 cc. of back skin from adult terrestrial Taricha granulosa to 138.6 cc. of Holtfrater's solution making 140 cc. of stock solution. As with solutions of skin area, all dilutions were made from the 100% stock.

Table 2. *Relationship of volume and concentration to skin volume.

Concentration	skin volume per 1.0 cc	skin volume per 0.4 cc	skin volume per 0.2 cc
100%	.01 cc	.004 cc	.002 cc
75%	.0075 cc	.0003 cc	.0015 cc
50%	.005 cc	.002 cc	.001 cc
25%	.0025 cc	.001 cc	.0005 cc
10%	.001 cc	.004 cc	.0002 cc
5%	.0005 cc	.0002 cc	.0001 cc

*Regarding mixture #25

In all tests where several test animals of one species were available, controls were run. Usually controls were injections of the solution used in the dilutions, i.e., Ringer's or Holtfreter's solution. In some cases however, macerated portions of subintegumental skeletal muscle were used on the controls. The latter controls were made to assure that any toxin was from the integument and also to check the possibility of a protein reaction.

To better compare degrees of susceptibility, a standard mouse unit (mu.) was established and used in designating the amount of toxin used in each injection. One mu. is defined as the amount of toxin, injected intraperitioneally, needed to kill a 21 gram white mouse in ten minutes. One mu. was found to equal, in back skin preparations of terrestrial adult Taricha granulosa, 0.0002 cc. of actual skin.

For comparison, twenty adult male terrestrial Taricha granulosa used in these experiments averaged 1 cc. of skin on the back between the fore and hind legs.

Table 3 indicates the various volumes of skin equaling different numbers of mouse units.

Table 3. Relationship of volume of skin to mouse units

Mouse Units	Volume of skin in cc.
2000	0.2
1000	0.1
750	0.075
500	0.05
100	0.02
50	0.01
20	0.004
10	0.002
5	0.001
2	0.0004
1	0.0002

III. RESULTS

All vertebrates tested with the integumental toxin of Taricha granulosa display the same symptoms. Generally these symptoms are, in order: (1) muscular weakness, especially prevalent in the hind limbs, causing a splayed gait, (2) loss of righting reflex, (3) convultions, (4) gasping, gaping and regurgitation, (5) flaccid paralysis, (6) fall in blood pressure, due to vasodilation as seen in the blood capillaries of the web of frogs and the ear of white mice, and (7) continuous heart beat after the cessation of respiration.

These symptoms were mainly observed in white mice and white rats, but most held true for the other mammals, birds, reptiles, amphibians and fish tested.

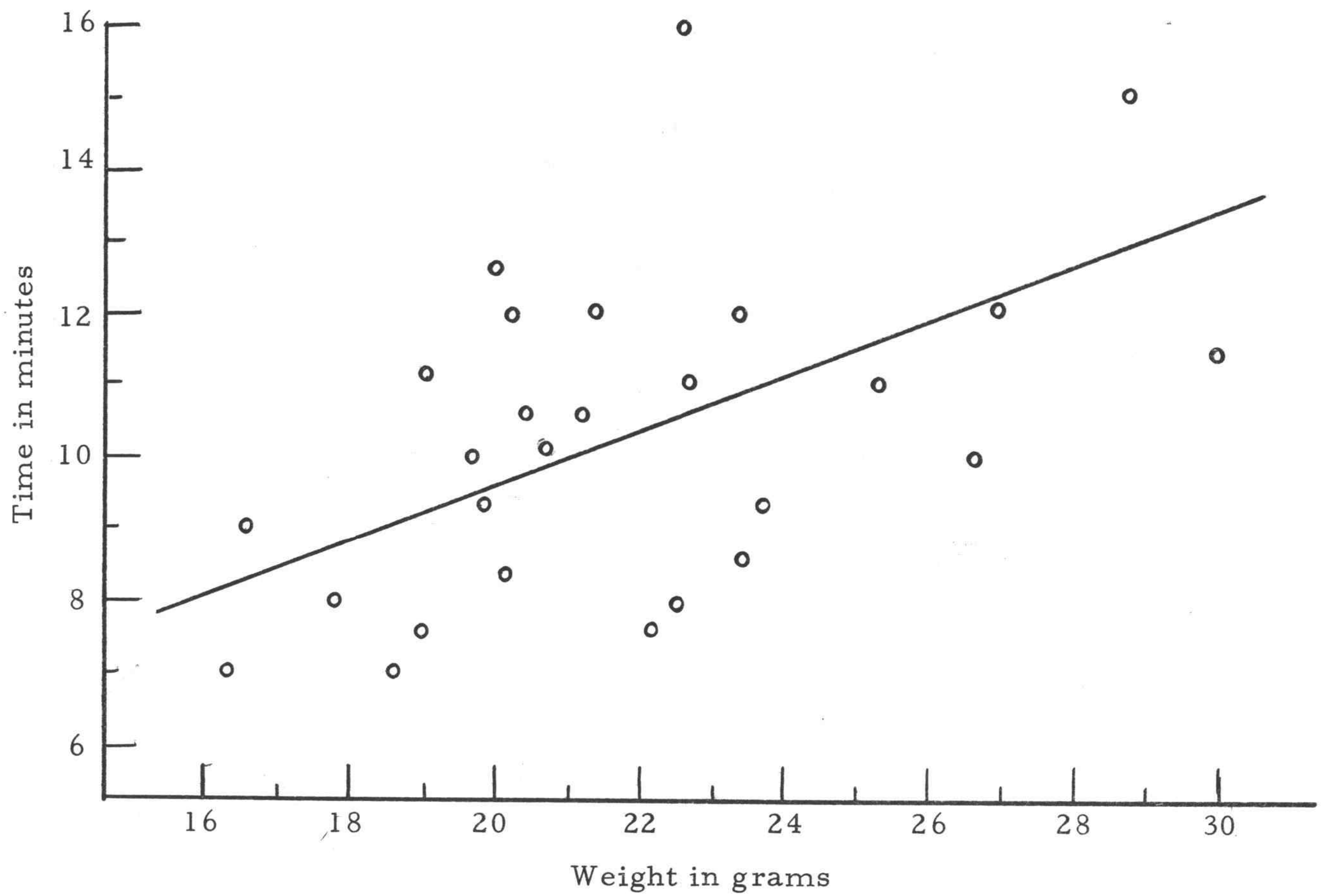
Susceptibility of Mammals to Intraperitoneal Injections of the Integumental Toxin of Taricha granulosa

All mammals tested were susceptible to newt toxin, but most quantitative data was gathered from tests on white mice, due to the ease with which they were obtained and kept. White mice were used in determination of levels of susceptibility, as well as establishment of the mouse unit.

Table 4 indicates for white mice the body weight and amount of toxin injected, in mu., related to the time of death. Graph I shows

Table 4. White mice susceptibility to intraperitonial injections of *Taricha granulosa* integumental toxins

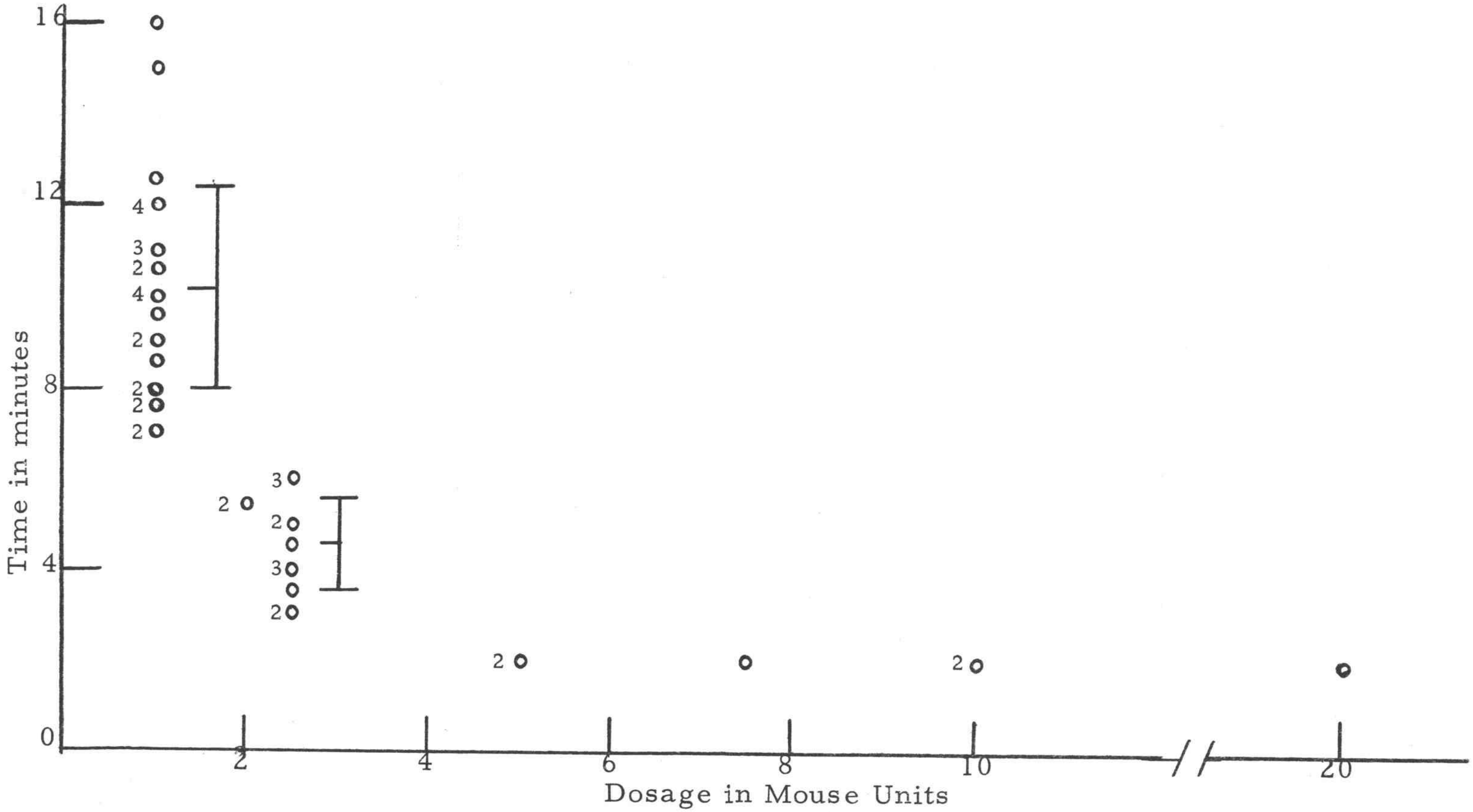
Wt. in gm	Time to death in min.	Wt. in gm	Time to death in min.
1 mu.		2 mu.	
16.4	7	21.1	5.5
16.5	9	24.5	5.5
17.8	8		
18.7	7	2.5 mu.	
18.9	11		
19.0	7.5	16.3	4
19.6	10	17.0	4
19.7	9.5	17.1	4.5
20.0	12.5	17.3	3
20.1	8.5	18.6	4
20.3	12	21.9	3.5
20.6	10.5	21.9	6
20.7	10	22.2	5
21.3	10.5	24.2	6
21.5	12	24.4	3
22.2	7.5	26.8	5
22.5	8	30.3	6
22.5	16	5 mu.	
22.7	11		
23.3	9	19.3	2
23.5	12	20.5	2
23.7	10	7.5 mu.	
25.5	11	23.9	2
26.7	10		
26.9	12	10 mu.	
28.7	15		
		21.3	2
		20 mu.	
		25.3	2



Graph I. Susceptibility of white mice to intraperitoneal injections of one mouse unit of the integumental toxin of Taricha granulosa how the level of a mouse unit was determined, as it plots body weight against time to death for all mice injected with a certain amount of toxin (one mouse unit). A line was fitted to the graph statistically by the method of least squares (Holman, 1962) and intersected the 21 gram axis at the ten minute axis. A mouse unit is then defined as the amount of toxin injected intraperitoneally needed to kill a 21 gram white mouse in ten minutes.

Graph II was used to indicate the relationship between the dosage, in mouse units, and the time to death in minutes for all data in Table 4. The mean and standard deviation are shown for one mouse unit ($\bar{X}$=10.25, s=2.26) and two and one-half mouse units ($\bar{X}$=4.5, S=1.11). It is apparent from this graph that 5, 7.5, 10 and 20 mu. all cause death in white mice within two minutes.

White rats were also used to some extent to indicate levels of susceptibility and these data are indicated in Table 5. Several other species of mammals, usually possible predators, were also tested by injections; these data are presented in Table 6. All of these mammals were adult except the feral cats, Felis catus, which were eight weeks old and had just been weaned.



Graph II. Susceptibility of white mice to intraperitoneal injections of varying doses of the integumental toxin of <u>Taricha granulosa</u>

The bar symbols indicate the mean and two standard deviations

Table 5. White rat susceptibility to intraperitoneal injections of *Taricha granulosa* integumental toxins

Wt. in grams	Dose in mu	Time to death in min.
152	5	*NL. (Affected hind leg coordination)
135	5	11
135	5	19
167.9	5	15.5
180	5	NL
182	5	NL
129	10	4.5
132	10	5
137	10	5
137.4	10	6
165.3	10	4
180	10	8
183.3	10	13
155.7	17.5	5
184.2	25	2.5
180	100	1.5

*NL=non-lethal

Table 6. Mammalian susceptibility to intraperitoneal injections of *Taricha granulosa* integumental toxins

Species	Wt. in grams	Dose in mu.	Time to death in min.
Zapus sp.	21	1	17
Mustela erminea	57.5	10	2.5
Scapanus townsendi	--	25	2
Neotoma lepida	81.8	10	8.5
Felis catus	290	5	NL
	350	10	NL
	400	25	23.5
	414	25	17
	425	25	11.5
	440	50	6
Ondatra zibethica	670	10	32
Citellus beecheyi	669	25	NL
Citellus beecheyi	727	100	19
Myocastor coypus	3085	500	12
Lynx rufus	8000	750	13

Susceptibility of Lower Vertebrates to Intraperitoneal Injections of the Integumental Toxin of *Taricha granulosa*

Due to the ease with which fish, amphibians and birds may be force fed, injections were not used extensively in testing susceptibility of these groups. Fish and amphibians were not injected at all (with the exception of *Taricha granulosa* itself, which will be covered in a later section) and only a few tests were made on birds. Table 7 shows the tests made with birds, indicating weight of birds, dosage in mouse units, and time to death in minutes.

The birds tested exhibited similar symptoms to those seen in white mice.

Table 7. Susceptibility of birds to intraperitoneal injections of the integumental toxin of adult terrestrial *Taricha granulosa*

Species	Wt. in gms.	Dosage in mu.	Time to death in min.
Starling	--	1 mu.	N. L.
	--	1 mu.	N. L.
	72	2.5 mu.	4.5
	85	2.5 mu.	7.5
	74	2.5 mu.	10
Robin	73.2	2 mu.	N. L.
	76.2	4 mu.	5
	80.7	4 mu.	28

Reptiles, particularly snakes, are possible predators on newts, and therefore, several species of snakes were tested for susceptibility by injections. Tables 8 and 9 indicate the data from

Table 8. Susceptibility of garter snakes to intraperitoneal injections of the integumental toxin of adult terrestrial *Taricha granulosa*

Species	Wt. in gms.	Dosage in mu.	Lethality	Remarks
Thamnophis elegans vagrans	14.6	1000	59 min.	
	58.5	2000	2.5 days	
	70.8	2000	6 hrs, 20 min.	
Thamnophis ordinoides	32.5	5	NL	Full recovery after 12 hours
	11.1	10	NL	Full recovery after 12 hours
	11.7	250	8 hrs.	
	25.9	500	50 min.	
	12.3	1000	29 min.	
	18.	1000	38 min.	
	25.5	1000	68 min.	
	27.5	1000	36 hrs.	
	32.2	2000	44 min.	
T. sirtalis fitchi	79.3	1000	NL	Full recovery after 4 days
	46.0	2000	80 min.	
	55.7	2000	36 hrs.	
	129.0	2000	68 hrs.	
	27.4	2500	100 min.	
	47.4	2500	120 min.	
T. s. concinnus	6.7	1000	86 min.	
	37.9	2000	36 hrs.	
	38.8	2000	25 hrs.	
	105.0	2500	2.5 days	
T. s. c. X *T. s. f.* (intergrade)	64.0	2500	2.5 days	
T. marciana	65.5	2000	62 min.	

Table 9. Susceptibility of snakes to intraperitoneal injections of the integumental toxin of adult terrestrial *Taricha granulosa*

Species	Wt. in gms.	Dosage in mu.	Lethality	Remarks
Coluber constrictor mormon	42.6	10	10 min.	
	52	10	NL	affected for several hours
	54.6	20	NL	not affected
	48.3	150	NL	complete recovery after 6 days
	49.7	500	45 min.	
	93.9	1000	24 min.	
	45.9	2000	65 min.	
Pituophis catenifer	90.1	10	NL	complete recovery after 5 days
	86.8	100	3 days	
	26.5	1000	30 hrs.	
	28.5	2000	30 hrs.	
Crotalus viridus	110.0	10	NL	complete recovery after 5 days
Masticophis taeniatus	110.4	1000	17 min.	
Charina bottae	48.8	500	42 min.	
Contia tenuis	4.4	100	15 min.	

these tests; Table 8 applies to garter snakes and Table 9 to other snakes. As is evident from these tables, snakes were tested with much larger relative doses than were mammals; all tests were made at room temperature. Snakes represented in these tables that were affected by the toxin were effected within a few hours; the symptoms were much like those in mammals. Loss of muscle coordination was evident in the snakes' inability to crawl, elevate their heads, or extend their tongues. These symptoms were usually followed by loss of righting reflex and cessation of breathing. Some snakes lived several days; these snakes usually were considered to be alive only due to heart beat or slight response to mechanical stimuli. Four snakes recovered completely after being effected by the toxin. These snakes were considered to be completely recovered when they: (1) were able to right themselves quickly, (2) could elevate their head and (3) were active and alert.

Measurements of Susceptibility of Vertebrates to Newt Integumental Toxin by Feeding

Mammals were not tested by force feeding and refused all attempts made at voluntary feeding. The writer has, accidently, while collecting newts, touched his fingers to his tongue and mouth and experienced a severe burning sensation. Mammals, i. e. cats, react as if the newt is extremely distasteful after biting into one.

Birds are easily force-fed and this was, therefore, the primary method used in testing them. Feeding is not a good method to obtain quantitative data but is, however, the most valid method of establishing susceptibility. A male robin, 65.9 grams, was force-fed a 20 mm. piece of the tip of the tail of an adult terrestrial Taricha granulosa. This bird was affected after one hour and 30 minutes as shown by lack of coordination and inability to stand erect. After 24 hours, the bird still showed these symptoms along with the following: (1) does not close eyes when touched, (2) does not attempt to fly or to break fall when dropped, (3) exhibits righting reflex and stands unsteadily but if not harassed lies down with its head on the table surface, and (4) reacts, by jumping, to vibrations or touch but not to light, sound, or movement around it. The robin makes no attempt to defend itself or escape and cannot perch or grip with its feet. The bird was observed constantly and at 24 hours and 55 min. it suddenly stiffened, flapped its wings, and died. The robin was autopsied and the digestive tract dissected; all remains of the newts tail had been digested and were in the lower tract. The remains were evident by a slight orange color.

Other birds tested showed these same symptoms. An adult red-shafted flicker was tested with a 40 mm. tip of an aquatic newt's tail and died between 10 and 20 minutes after the feeding.

The two other birds tested by force-feeding, a kingfisher and a great blue heron, are the most likely avian predators on Taricha granulosa. The kingfisher, an adult male, 120 gms., was force fed a 30 mm. section from the middle of an adult terrestrial newt's tail. Two minutes after the tail was placed in the bird's mouth, the bird was dead. The kingfisher was autopsied to ascertain if its digestive tract had been injured by the force feeding. The esophagus was found not to have been injured in any way and the section of newt's tail was found in the proventriculus.

A great blue heron having a wing span of 4'9" and weighing 1260 gms., was fed 50 mm. of the tip of the tail of a terrestrial Taricha granulosa. The section of newt's tail was placed in the great blue heron's mouth; the bird promptly spit it out, but the tail was again placed in the bird's mouth, this time in the rear of the mouth, and the bill was tied loosely shut. In one minute, the bird was tethered to a chair; by one minute thirty seconds the bird was unable to stand upright, and by two minutes the great blue heron was dead. This bird also was autopsied and found not to have been injured by the force=feeding; in this case, the newt's tail was found in the lower esophagus, just in front of the proventriculus.

Susceptibility of reptiles, particularly lizards but to some extent snakes, was also tested by feeding; voluntary feeding was usually used with reptiles due to difficulty in force feeding. The only possible predator among lizards is Gerrhonotus multicarinatus. Six of these were tested by picking up the lizard, who then attempted to bite, whereupon the tail of an adult terrestrial Taricha granulosa was placed in the lizard's mouth; the lizard then would bite down on the newt's tail, sometimes chewing to the point of drawing blood. Usually the lizard would rapidly release the newt and show apparent distress by rubbing the sides of its mouth in the gravel of its terrarium. The first two lizards, when their mouths were held closed and the newt's tail was pulled from their mouth, died in five minutes and 15 minutes. One lizard bit and chewed the newt's tail and was dead in 30 minutes. Another lizard bit solidly on the newt's tail, but did not chew; it died in one hour. The symptoms shown by this lizard were also typical of the other lizards tested: one minute--regurgitated and frothed at the mouth; three minutes--gaping; four and one-half minutes--voids cloaca; six to ten minutes--lies motionless with eyes closed, yet rights itself rapidly; 11 to 13 minutes--same, but rights more slowly; 14 minutes--writhes and rolls, no righting reflex; 19 minutes--violent convulsions (two of the lizards shed their tails in convulsions, but this one did not); 20 to 57 minutes--heart beating but no other sign of life; 60 minutes--heart has ceased to beat, and the animal is dead.

Two *Gerrhonotus multicarinatus* survived after biting the tail of an adult terrestrial *Taricha granulosa*. One lizard bit down on but immediately released the newt's tail; this individual exhibited much the same symptoms as the previous individual and by 35 minutes had lost its righting reflex, and after 90 minutes the animal still showed a faint heart beat. The lizard showed no signs of life except the heart beat for 24 hours after which it exhibited a slow righting reflex, recovering completely 40 hours later. One lizard bit only very lightly on the newt's tail and, although it showed distaste, none of the other symptoms was evident.

Another species of lizard, *Crotaphytus wislizeni*, not a possible predator on newts, was also tested. This lizard bit the newt's tail and chewed it for 30 seconds; after releasing the newt, it exhibited the same symptoms as the alligator lizard, lost its righting reflex in three minutes, and was dead four and one-half minutes after the newt had been released.

One crocodilian, *Caiman* sp., obviously not a possible predator, was tested by force feeding, similarly to that done in birds. The *Caiman* was fed a 42 mm. section of the tail (tip) from an adult terrestrial *Taricha granulosa* and its mouth was tied shut. The Caiman lost its righting reflex after six minutes and was dead in ten minutes.

Snakes were tested only in as much as it was recorded when one ate a *Taricha granulosa*. The only snake the writer personally has observed eating a newt voluntarily was *Thamnophis sirtalis concinnus*. Several of these garter snakes have eaten adult terrestrial *Taricha granulosa* in the laboratory. The star performer is an individual 96.5 mm. in total length which eats newts with no apparent difficulty. Even though the snake may froth around the mouth while eating a newt, it (the snake) shows no apparent discomfort. This snake has eaten eight adult terrestrial *Taricha granulosa* in two weeks, occasionally two a day.

One amphibian was tested for susceptibility by feeding; a bullfrog (*Rana catesbeiana*) was force-fed an adult terrestrial female *Taricha granulosa*, 58 mm. snout-vent length. The frog's mouth was opened and the newt was inserted head first until the body was completely in the frog's mouth. The bullfrog bit down and within five minutes had gulped the newt down; the bullfrog showed no apparent distaste while eating the newt. After eight minutes (three minutes after the newt had been completely swallowed) the frog could not right itself or control its hind legs in any way. After 15 minutes (ten minutes after the newt had been completely swallowed) the bullfrot was dead. Five minutes after the frog's demise, the newt emerged from the frog's mouth apparently unscathed, but covered with a heavy coating of what was apparently skin secretions. The newt lived until preserved several days later.

Fish susceptibility was tested entirely by force-feeding; these tests are summarized in Table 10. Both aquatic and terrestrial adult newts were used; the terrestrial newt toxin killed catfish in 19 and 20 min., the aquatic newt killed a catfish in three hours and 40 minutes. It is interesting to point out that three of the four whole newts fed to fish were alive after the fish died. In these three cases, the newt remained in the fish's mouth until the fish was dead and was then removed from the fish's mouth and found to be alive and seemingly unhurt. The fourth newt was swallowed and not recovered. Death in fish was not ascertained by heart beat, but rather a fish was considered to be dead when it would no long pump water through its gills and would not react to physical stimulus, i.e., pinching with a large forceps.

Re-injection With a Non-lethal Dose

Three white mice were injected with non-lethal doses in an attempt to observe either a build up of immunity or an accumulative effect of newt skin toxin. Two of these mice (one weighed 29.2 gm. and the other seemed to be the same size, but was not weighed)

Table 10. Susceptibility of fish to adult Taricha granulosa by force feeding.

Fish	Wt. in gms.	Stage of newt	Amount of newt	Time to death in min.
Bluegill	108	Terrestrial	Entire newt* 105 mm.	13
Bluegill	75	Aquatic	Tip of Tail 20 mm.	14
Large mouth Bass	145	Aquatic	Base of Tail 20 mm.	20
Large mouth Bass	180	Aquatic	Base of Tail 20 mm.	30
Catfish	414	Terrestrial	Entire Newt* 122 mm.	19
Catfish	750	Terrestrial	Entire Newt* 148 mm.	20
Catfish	957	Aquatic	Entire Newt* 160 mm.	220

* newt lived after being removed from the dead fish's mouth

were injected with 0.5 mouse unit once each day for nine consecutive days; neither mouse showed any reaction to these injections. On the tenth consecutive day, the mice were again injected, one with 0.5 mu., and the other with 1 mu.; neither mouse was affected by the injection.

Seven days later (no injections were made during this period) these same two mice were again injected with 0.5 and 1 mu. respectively. The mouse injected with 0.5 mu. was unaffected, but the mouse injected with 1.0 mu. was killed (no exact time was recorded) in less than one hour.

One other white mouse, a very heavy one weighing 38 g., was injected on three consecutive days with 1 mu. The mouse was affected (as indicated, by loss of coordination), but recovered after each injection. No further tests were made on this individual since it was, by mistake, fed to the snakes.

Self-susceptibility of *Taricha granulosa* to Intraperitoneal Injections of Their Own Integumental Poison

To give an indication of self-susceptibility, three adult aquatic male *Taricha granulosa* were injected intraperitoneally with large doses of their own skin toxin. Previously newts had been injected with dosages from 1 to 100 mu. and had exhibited no symptoms of susceptibility nor were seemingly in any way ill affected.

One newt, 16.5 gm., was injected with 1000 mu. and two newts, 15.6 and 17 gm., were each injected with 2000 mu.; all three were killed by the injection, in 17.5 hrs., 18 hrs., and 22 hrs., respectively.

The three newts exhibited similar reactions to the poison as those seen in white mice. The newts first lost muscle coordination, as early as six min., and this was followed by loss of righting response, cessation of respiration, and finally cessation of heart beat. In these tests, the newts were able to swim well after they had lost ability to coordinate their movements on land.

Some typical symptoms were flaccid paralysis of skeletal muscles and relaxed cloaca resulting in the passing of what was evidently a spermatophore (these males were in breeding condition). One of the animals tested (15.6 gm., 2000 mu.) had stopped breathing after four hours, 50 min., but its heart continued to beat and its eyes closed when stimulated mechanically for 17 hours; this animal when tested after 18 hours indicated neither heart beat nor eye contraction when touched.

Adult newts have, in the laboratory, been observed to eat both eggs and larval forms, both those with and without skin granules; in none of these voluntary feedings was the adult newt in any way affected.

Stability of Toxin

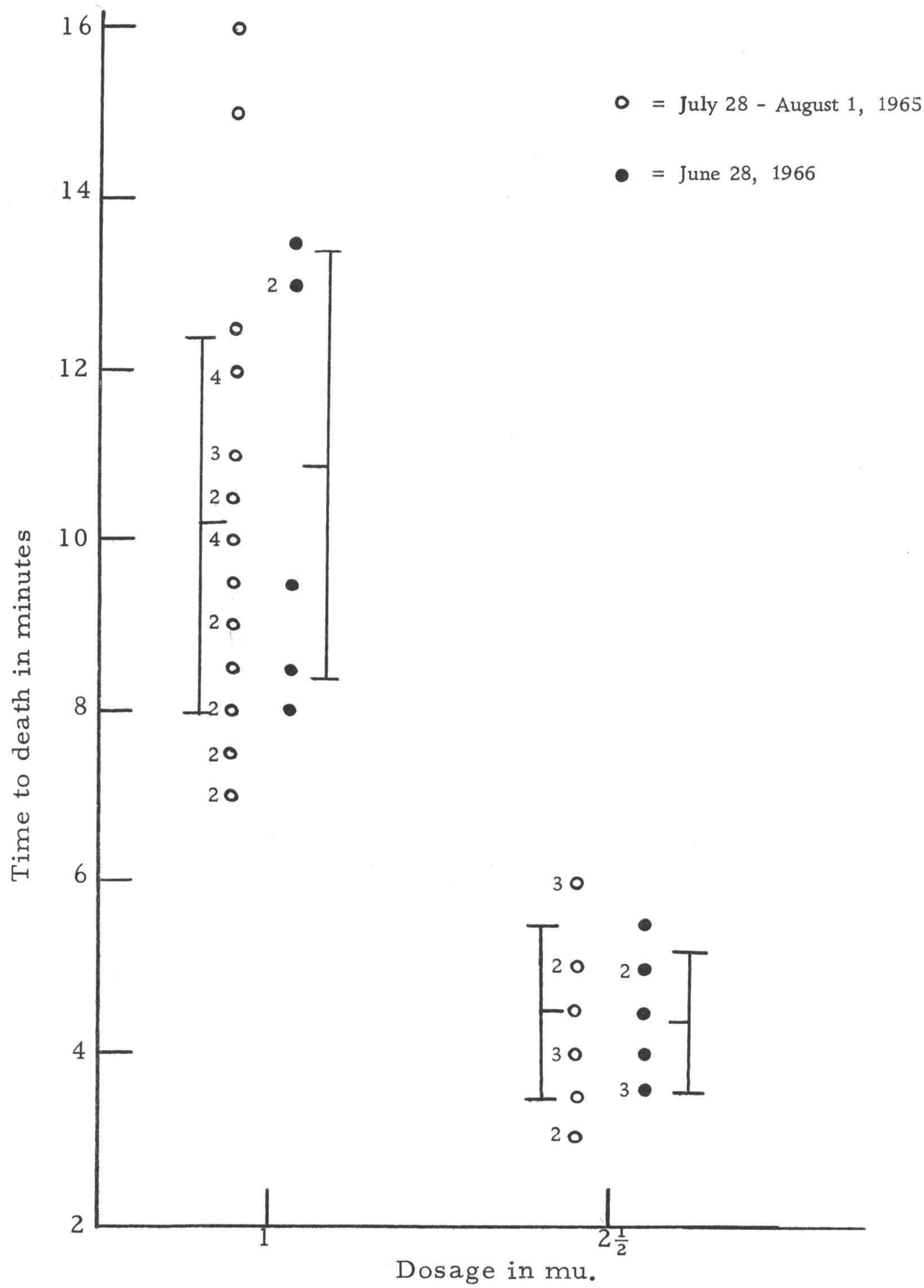
Several mixtures of integumental toxin of adult *Taricha granulosa* were used in this study; one of these mixtures, designated as mixture #25, was used over a period of 11 months, from July 28, 1965 to June 28, 1966. Graph III indicates for one and two and one-half mu. the relative potency of this mixture from the first five days to the last day. This mixture was kept at 4°C throughout this period, but was allowed to reach room temperature, 22°C, for each test.

The means and standard deviations plotted on Graph III give an indication as to relative toxicity of the mixture over an 11 month period, thereby indicating degree of stability.

It should be pointed out that the trials with the fresh mixture used mice averaging 21.5 gm., while the 11 month trial used mice averaging 23.0 gm.

Presence of Toxin in Blood and Ova of *Taricha granulosa*

In an attempt to determine presence of toxin in the blood of *Taricha granulosa*, three frogs (*Rana pipiens*) were injected with blood from three different adult aquatic male newts. Each injection involved 0.1 cc. of blood and 0.1 cc. of Holtfreter's solution, and was made intraperitoneally. All three frogs exhibited the same



Graph III. Stability of toxin.
The bar symbols indicate the mean and two standard deviations.

general symptoms as shown by test animals injected with newt integumental toxin. The frogs stopped breathing in 2, 17, and 73 minutes; all three frogs exhibited heart beat for several hours after the cessation of breathing, but none recovered.

To determine the presence of toxin in ova, extracts of ovarian and oviducal eggs were made. Oviducal eggs were obtained from female newts which were depositing eggs when collected. A solution of oviducal eggs and Holtfreter's solution was injected intraperitoneally into frogs and white mice. Only one frog, (Rana pipiens, 28.6 gm.) was tested; an injection equaling 0.1cc. of oviducal eggs was made intraperitoneally, and the frog was found to be susceptible. After one minute, the frog exhibited no righting response; after four minutes there was a cessation of respiration, but the heart continued to beat for 35 minutes.

Table 11 indicates the susceptibility of white mice to this same mixture of toxin and shows relationship of amount of toxin to time to death. These white mice exhibited similar symptoms to those shown by mice injected with the integumental toxin of Taricha granulosa.

Ovarian eggs were tested by a solution of developing ova and ovaries from adult female Taricha granulosa as they were leaving ponds after completion of depositing eggs; these were developing eggs

Table 11. Susceptibility of white mice to intraperitoneal injections of oviducal eggs from *Taricha granulosa*

Species	Wt. in gm.	Volume of ova in cc.	Time to death in min.
White Mice	21.43	.1	1
	24.9	.025	2
	25.2	.01	2
	20.0	.005	3
	20.5	.001	5
	19.9	.0005	NL*
	16.4	.000125	NL (not affected)

*was affected by loss of coordination, but recovered within 12 hrs.

for the next laying period and were in the early stages of development. Two white rats were injected with the equivalent of 0.004 and 0.003 cc. of ovarian eggs respectively and were not affected. A frog (*Hyla regilla*, 5.1 gm.) was injected with the equivalent of 0.004 cc. of ovarian eggs and was also not affected. As a further test with this mixture, a white mouse, 20.6 g., was injected with .2 cc. of precipitate, apparently yolk, and died in four minutes.

Presence of Toxin in Larval and Recently Metamorphosed *Taricha granulosa*

Since it is difficult to collect the large numbers of larvae and young individuals necessary to prepare a skin solution for use in injections, all tests involving these stages were made by feeding.

Blue gills were the only fish used in testing these forms. Three inch bluegills were observed to eat *Taricha granulosa* larvae lacking roughened skin in the laboratory.

In one trial, three *Taricha granulosa*, one without roughened skin and two recently transformed individuals, were introduced into the aquarium with several three inch bluegills; the one without skin granules was eaten immediately; the two with skin granules were nosed but were not eaten or even mouthed.

Four amphibia, three of them potential predators, were fed larval or newly transformed *Taricha granulosa*. An adult bullfrog, *Rana catesbeiana*, was force-fed four larvae without skin granules; the frog showed no distress signs and was apparently not affected. One week later this same frog was force-fed two larval *Taricha granulosa*, 20 and 25 mm. snout-vent length, with developed skin granules (the skin appeared to be identical to that of adult newts); again the frog was not affected. This was the same frog later killed by a feeding of adult newt.

One toad, Bufo boreas, 258 gm., was force-fed a transformed Taricha granulosa, 38 mm. total length. The toad was apparently affected; 15 minutes after the feeding the toad gaped, opening its mouth. No other symptoms were evident and perhaps this gaping was due to the force feeding.

One test was made with the African clawed frog, Xenopus sp. A larval Taricha granulosa, just prior to metamorphosis with apparently adult skin, was introduced into the frog's aquarium and was immediately siezed and swallowed by the frog. The newt was too long and the tail could not be swallowed, but the frog chewed and attempted to swallow the newt for two minutes. After two minutes, the newt was spit out; the frog retched for three minutes and was still cleaning its mouth (fore feet were repeatedly placed in the frog's open mouth) ten minutes later. No other susceptibility symptoms were evident.

One salamander, Dicamptodon ensatus, was tested by placing larval Taricha granulosa into a container with larval Dicamptodon. The Dicamptodon voluntarily ate larval newts without evident skin granules and no ill effects were noted. No attempted feedings were made with newt larvae having developed skin granules.

Two garter snakes, Thamnophis sirtalis concinnus and T. ordinoides, were tested by introducing larval and newly metamorphased newts into a water pan in their terrium. Both snakes ate many of each stage newt and there was no apparent distaste or ill effect.

Two birds, red-shafted flicker and kingfisher, were tested with transformed Taricha granulosa. The kingfisher was force-fed a transformed newt, 40 mm. in total length; the bird showed no ill effects. The red-shafted flicker was force-fed a sub-adult newt, 20 mm. snout-vent length and 60 mm. total length; this bird also exhibited no ill effects. Each of these birds was later tested with comparable respective portions of adult newts and both were found to be susceptible to this toxin. These birds were then autopsied and each had digested the newt.

Relative Toxicity of Different Skin Areas and of Aquatic and Terrestrial Adult Newts

In some early tests, prior to standardization of the toxin into mouse units, several tests were made examining various stages and areas of skin to determine their relative toxicity. The amount of skin used in each injection (all injections were intraperitoneal) was recorded as to skin area; the test animals were Peromyscus maniculatus and Microtus townsendi. Table 12 indicates the relative toxicity of two skin areas of aquatic Taricha granulosa, these being the back or dark pigmented area, and belly or light pigmented region.

Table 12. Susceptibility of Microtus townsendi to intraperitoneal injections of dorsal and ventral skin of Taricha granulosa

skin area injections in cm^2	0.2	0.1	0.05	0.025
dorsal skin		2 min.	4 min.	NL
ventral skin	4 min.	NL		

Adult Taricha granulosa may be categorized into two phases, the terrestrial, non-breeding phase and the aquatic, or breeding stage. The male newts undergo a remarkable change at the onset of the breeding season. "Their skin becomes smooth and puffy and the tail broadens into an effectiv swimming organ" (Stebbins 1954, p. 42). Table 13 indicates the relative concentration of toxin in the tail skin of aquatic male and terrestrial male newts as determined with Peromyscus maniculatus. In another tests, 0.3 cm^2 of terrestrial newt skin was needed to kill a white rate in two minutes; 0.6 cm^2 of aquatic newt skin was needed to kill a white rat in 27 minutes (both injections were intraperitoneal).

Table 13. Susceptibility of Peromyscus maniculatus to intraperitoneal injections of tail skin of adult male aquatic and terrestrial Taricha granulosa

skin area injected in cm^2	0.2	0.1	0.05	0.025
aquatic	1 min.	2 min.	35 min.	NL
terrestrial				20 min.

Action of Taricha granulosa Skin Toxin in Relationship to Method of Injection

Table 14 indicates for Peromyscus maniculatus the effects of injections of the skin toxin of Taricha granulosa. Oral, subcutaneous and intraperitoneal injections were made.

Table 14. Action of Taricha granulosa skin toxin in relationship to method of injection

Skin area in cm^2	1.0	0.8	0.2	0.1	0.05	0.025
Peromyscus maniculatus intraperitoneal injection			1 min.	2 min.	35 min.	NL
Peromyscus maniculatus subcutaneous injection			10	37	NL	NL
Peromyscus maniculatus oral injection	84 min.	180 min.	NL			

IV. DISCUSSION

There are, in the literature and from reliable sources, many reports concerning various predators which have eaten *Taricha*. Both *T. granulosa* and *T. torosa* will be discussed since the symptoms caused by tarichatoxin (from the embryos of *T. torosa*) seem to be non-distinguishable from those observed with the skin toxins of *Taricha granulosa*. These reports will be compared with data previously presented.

Presence of Toxin in Ova and Embryos of *Taricha*

One test was made with ovarian eggs of *Taricha granulosa* and it appeared they were non-toxic; however, when the precipitate (apparently yolk) was tested, it was found to be toxic. This agrees with the findings of Twitty and Elliott (1934) who suggested that the most highly toxic portion of *Taricha torosa* embryos is the yolk.

Fish are conceivable predators on developing embryos but no tests were made and there are no reports involving fish predation on embryos. One frog, (*Rana pipiens*, 28.6 gm.) was injected with .1 cc. of oviducal eggs and found to be susceptible. Other amphibia (*Ambystoma*, *Dicamptodon*, *Aneides*, and *Bufo*) have also been found to be susceptible to the toxin in newt embryos (Twitty, 1937).

Adult _Taricha granulosa_ were observed in the laboratory to eat their own eggs. Others have found that _Taricha granulosa_ prey on their own eggs in nature (Chandler, 1918, and Evenden, 1948). In stomach counts from one locality, Evenden (1948) found that their own eggs made up 36% of all stomach contents of adult _Taricha granulosa_.

No tests were made examining susceptibility of reptiles and birds to ova or embryos because these groups are not considered to be potential predators on the eggs of _Taricha_.

Although mammals are not possible predators on eggs of _Taricha_, white mice have been used extensively in testing the presence of toxin in their ova and embryos. As seen in Table 11, the minimum lethal dose for white mice is approximately 0.001 cc. of oviducal eggs; half this amount effected a mouse, but was not fatal. Tests with the embryos of _Taricha torosa_ have been largely on white mice (Buchwald _et al._, 1964; Horsburgh _et al._, 1940; Mosher _et al._, 1964; and Twitty, 1937). These workers, especially Horsburgh _et al._ (1940) have described the action of this toxin; their description is indistinguishable from this writer's observations on the skin toxin from _Taricha granulosa_.

Presence of Toxin in Larval and Newly Metamorphosed Taricha

Pond-dwelling fish would be potential predators on newt larvae. To examine this possibility, several bluegills were brought into the laboratory and fed on a diet of *Taricha granulosa* larvae. The bluegills ate numerous newt larvae, without developed skin granules, without apparent distaste. When larvae with skin granules were introduced into the aquarium, they were ignored completely. This appears to indicate that *Taricha granulosa* larvae do not have a toxin and that the development of skin granules is accompanied by the development of some noxious substance (not necessarily toxic).

Several amphibians may be considered to be possible predators on larval and newly metamorphosed newts; *Rana catesbeiana*, *Bufo boreas*, and *Dicamptodon ensatus* were tested. *Dicamptodon* voluntarily ate newt larvae without skin granules (none with skin granules were used in the tests), *Bufo* was force-fed a newly metamorphosed newt; and *Rana* was force-fed larvae both with and without skin granules; none of these animals showed ill effects although the toad gaped slightly after the feeding. One other amphibian, *Xenopus* sp., was tested; it was offered a larval newt with developed skin granules. The frog siezed the newt but after two minutes the newt was spit out, followed by retching. There was obviously a noxious substance involved as this frog was a voracious feeder and had eaten larger objects. Twitty (1937) has found that extracts of Taricha torosa paralyze Ambystoma larvae but that as larvae increase in size they decrease in toxicity.

Adult Taricha granulosa have been observed, in the laboratory, to eat larvae and newly metamorphased individuals of their own species; this phenomenon has also been observed by Chandler (1918) and Pimentel (1952).

Amphibians appear to show the same susceptibility as fish; they eat larvae without skin granules. It is apparent from the Rana and Bufo evidence above that some amphibia are able to eat newly-metamophosed newts; yet these two were force fed and it is not known if, in nature, they would actually prey upon this stage. Adult newts are able to eat their own larvae and appear to eat them as readily as other food items offered (Chandler, 1918).

Vying with adult newts as the top predator on larvae and newly metamorphosed newts, are garter snakes. Thamnophis sirtalis concinnus and T. ordinoides have been fed numerous newt larvae in the laboratory and at no time has distaste on the part of the snake been observed. Storm (1948) observed large specimens of T. s. concinnus feeding on newt larvae in a shallow pool (Fitch 1940 and 1941) has stated that larval Taricha are eaten by T. sirtalis sp. and T. elegans hydrophila. From these accounts, it is evident that if toxin is possessed by larval or newly metamorphosed Taricha, it is ineffectual as a defense against predation by garter snakes.

Two birds were tested with newly metamorphased Taricha granulosa. Neither bird, kingfisher nor red-shafted flicker, showed ill effects by the force-feeding of these newts. Each bird was later dissected and in each case the newt had been digested.

It is apparent from these tests on potential vertebrate predators, that larval Taricha granulosa are poorly protected by toxins. This is not surprising since French workers have long noted that the larvae of some European salamandrids are less toxic than their respective adults (Dutarte, 1889 and Phisalix-Picot, 1900).

Presence of Toxin in Adult Taricha granulosa

Susceptibility of Fish

Large fish would be potential predators on adult newts and there are, in fact many reports of fish predation on newts. Primarily aquatic adults would be preyed upon, although a terrestrial adult could be taken shortly after entering the water. Terrestrial adult newts or parts thereof were force fed to bluegills and catfish; all fish succumbed quickly to the toxin.

Aquatic newts were fed to bluegills, large mouth bass, and catfish; again all fish tested succumbed to the toxin. The tests on catfish, two with terrestrial adults and one with an aquatic adult, appear to indicate that aquatic adult newts are less toxic than terrestrial adult newts. That catfish are susceptible is confirmed by a report from a graduate student in Fisheries and Wildlife at OSU, who observed a dead catfish with the tail of Taricha granulosa protruding from its mouth (pers. comm., Lynn Goodwin). Mosher et al. (1964), refering to the collection site of Taricha torosa embryos, states, "during the spawning season, numerous dead catfish were observed floating in this pond." Mosher does not make it clear that he believes adult newts or their embryos are the cause of the demise of these fish and evidently he made no stomach samples.

There are several reports of Taricha granulosa being found in the stomachs of fish; unfortunately, the persons reporting these are not explicit as to whether or not the fish were dead. These fish are assumed to have been alive when taken. A black bass (Chandler, 1918), a white perch (pers. comm., Dr. Bond), and several trout have been found with Taricha in their stomachs. Dr. Bond (pers. comm.) reports a brook trout with two Taricha in its stomach and Dr. Metter (pers. comm.) reports that big cutthroat trout are found regularly in western Washington with Taricha in their bellies.

Taricha granulosa mazamae have been found in the stomachs of two rainbow trout taken from Crater Lake. One of these is reported by Vincent (1947) and the other, a four-pound fish (Pimentel, 1952), is known to have been alive when collected (R. Storm per. comm.). Pimentel (1952) feels the occurance of newts in the stomachs of fish are a very rare happening. As supporting evidence, he sites R. E. Dimick, former Head of the Department of Fish and Game, OSU, who analyzed a few thousand fish stomachs from localities where Taricha granulosa were common; no newts were found in the stomachs examined.

Susceptibility of Amphibians

Predation upon adult newts by amphibians would be very unlikely due to size. One Rana catesbiana, however, was tested by feeding it an adult newt. The bullfrog quickly succumbed and the newt crawled from the frogs mouth, apparently unharmed.

Of primary interest is self-susceptibility of Taricha granulosa. It was found that adult Taricha granulosa are susceptible to injections of their own toxin; this is true only of massive doses. When adult newts were tested with 200 mu. or less, no signs of susceptibility or ill effects were evident; when injected with 1000 or 2000 mu., the newts were killed by the toxin. The difference in time to death using 1000 mu. (17.5 hrs.) and those using 2000 mu. (18 hrs., 22 hrs.) are not significantly different but do show the great resistance newts have to their own toxin. The resistance of

Taricha torosa and also *T. granulosa* has been noted in regard to tarichatoxin; they are approximately 3,000 to 30,000 times more resistant than frogs (Kao and Fuhrman, 1963; Buchwald *et al.*, 1964; and Mosher *et al.*, 1964). Taylor (1934) states that "these animals" (*Taricha granulosa*)" are highly sensitive to even small quantities of their own poison. I found that often the inserting of the needle through the skin in the course of suturing the wounds made by the operation was sufficient to bring about the death of the animal." Taylor was thyroidectomizing the newts in question and the "extreme nervous sensibility" he describes as a symptom is not typical of animals effected by the skin toxin of *Taricha granulosa*. This writer feels the cause of death of these newts was due to other than their own skin toxin.

Susceptibility of Reptiles

Since reptiles are the most likely predators on adult newts many tests were made involving these animals. All lizards tested were found to be highly susceptible to skin toxins of adult *Taricha granulosa*. All lizards which bit down solidly on a newt tail died from the skin toxin. No injections were made to attain quantitative data on lizard susceptibility, but it is apparent that they are very likely the most susceptible of all animals tested.

One crocodilian was tested by feeding it a small piece of newt tail; the newt was not swallowed by the Caiman, but was kept in the mouth. Like the lizards, the Caiman is very susceptible. Snakes, particularly garter snakes, are potential predators on adult newts. Many tests were made involving snakes found within the range of Taricha granulosa. Susceptibility of snakes to injections of mixtures of the skin of adult Taricha granulosa may be sharply divided into two groups, garter snakes and other snakes. Six genera of snakes other than garter snakes were tested (Table 8). These snakes appear to be approximately 200 times more resistant to the toxin of newts than white mice. It should be pointed out, however, that the toxin needed to kill a 110.4 gm. Masticophis, a 265 gm. Pituophis, and a 93.9 gm. Coluber is equal to only one-half cubic centimeter of back skin from an adult newt.

Many garter snakes, Thamnophis sp., were injected with the integumental toxin of Taricha granulosa (Table 8).

From these data it is apparent that garter snakes are susceptible only to large amounts of newt integumental toxin. Too few tests were made to determine if the resistance of garter snakes is confined to garter snakes sympatric with Taricha or if this resistance is found in all garter snakes.

Further evidence that Thamnophis are able to resist the newt toxin is the feeding of *T. sirtalis concinnus* on adult *Taricha granulosa* in the laboratory. This evidence is further substantiated by reports from R. A. Hendon (pers. comm.) who has observed a *T. s. fitchi* from Moscow, Idaho to eat adult *Taricha granulosa* both from that locality and from Corvallis, Oregon.

Farner and Kezer (1954) observed, on Wizard Island, Crater Lake, Oregon, a *T. s. fitchi* in the process of eating an adult *Taricha granulosa mazamae*; unfortunately they were collected before the newt could be eaten. There are also numerous unsubstaniated reports by students who apparently have observed garter snakes preying on newts.

It must be mentioned that most persons who have commented on garter snake predation feel that the integumental toxin of *Taricha* is an effective protection against predation (Hubbard, 1903; Fitch, 1941; Pimentel, 1952; and Mosher *et al.*, 1964). No evidence explaining this difference of opinion was evident from tests made in this research.

Susceptibility of Birds

Two species of birds, not likely predators, were tested by injections (Table 6); there were no apparent differences between the susceptibility of these birds and those of white mice. Other tests, involving the feeding of adult newts to potential bird predators made it apparent that these birds could not eat adult newts and survive. All birds tested were found to be susceptible. Others, have commented on the effect of newt toxin on birds.

> "During the winter of 1947-8, a female Mallard duck was brought into the Fish and Game laboratory at Oregon State College, whose crop contained an adult male Triturus. This duck had been found dead, and it can probably be assumed that she either choked to death on this rather large morsel (an unlikely occurrence) or that poison from the newt brought about her demise" (Storm, 1948, p. 47).

Pimentel (1952) discusses information given to him by Dr. E. M. Dickinson, Professor of Veterinary Medicine, relating reports of turkeys and chickens dying after eating Taricha granulosa. These were birds brought in by poultrymen for autopsy, usually after many of their flock had died from an unknown cause.

There is the report of ducks near Trout Lake, Klickitat Co., Washington apparently preying upon newts with immunity.

> "In 1958 I talked to a farmer who described 'water dogs' that could only be Taricha. He recognized them when I showed him several types of salamanders. He said that a couple of ponds on the back of his farm had been full of them until he moved some domestic ducks to the ponds. He states there were soon no 'water dogs' left and that he had seen the ducks actively hunting and eating them." (D. E. Metter, pers. comm.)

This writer feels the farmer has mistaken some other salamander for Taricha, although there is a possibility of regional variation in degree of toxicity. This possibly has not been examined except for specimens from Moscow, Idaho; these newts were found to be toxic (Gerrhonotus multicarinatus died after biting a newt's tail).

Susceptibility of Mammals

All mammals tested were similarly susceptible to injections of newt skin toxin. To better correlate and study levels of susceptibility, a mouse unit was designated and used to describe all dosages.

Table 4 and Graph I indicate the relationship between weight and time of death for one mu. for white mice. A line plotted by the method of least squares indicates the correlation between weight and time of death. As would be expected, the heavier animals live longer than the lighter animals. There is a great degree of individual variation of white mice of any weight and this writer feels the range of weights is too short to indicate any great difference in susceptibility by weight.

Graph II plots time to death against dosage in mu. for white mice (no allowance is made for weight). The mean and standard deviation as well as the range of time are plotted. It is significant that the ranges of one mu. and two and one half mu. do not overlap but that all successively larger (5-20mu.) dosages killed the mice in two minutes. Apparently two minutes is the shortest time needed for the action of newt skin toxin in white mice.

The white rats tested reacted like the white mice (a larger dose is of course required for a larger animal).

Ten other species of mammals were injected with varying amounts of the toxin (Table 6). Some of these animals (Scapanus townsendi, Ondatra zibethica, Myocastor coypus, Mustela erminea, Felis catus, and Lynx rufus) are potential predators on adult Taricha granulosa. No apparent resistance to the toxin was noted for any of these possible predators.

Only one report of a mammal preying on adult Taricha is known. G. Clothier (pers. comm.) observed a four month old kitten playing with a dead Taricha granulosa. (The newt had been dead for less than 24 hours). The kitten later regurgitated Taricha skin and was dead one hour after it had been playing with the newt.

Re-injection With a Non-lethal Dose

It is evident from data presented that the skin toxin does not build up so as to make successive doses accumulative.

It appears that after nine consecutive days of injections with a sublethal dose, one mouse gained resistance; a dose of one mu. (normally a lethal dose) failed to kill it. Seven days later when this mouse was again injected with one mu., it was killed.

The possibility remains that a resistance to the integumental toxin may be acquired by animals after successive non-lethal injections. This possibility needs to be further explored.

Stability of Toxin

In an attempt to determine if mixtures of integumental toxin of *Taricha granulosa* are stable, a quantity was mixed and stored in a 4°C refrigerator. Graph III indicates relative potency of this toxin from the first week the mixture was prepared to eleven months later. From this graph, it is obvious that the toxin has not lost potency.

Relative Toxicity of Different Skin Areas and of Aquatic and Terrestrial Adult *Taricha*

The possibility exists that the concentration of integumental toxin differs in various regions of the skin. To check this possibility, two regions were tested. It was found that back skin is approximately four times as toxic as belly skin. The belly skin is still very toxic, as only 0.2 cm^2 is needed to kill a mouse in four minutes.

The relative toxicity of terrestrial and aquatic adult newts is apparent from Table 11. Data from tests on white rats also point out the greater toxicity of terrestrial individuals. All measurements relating skin areas to toxicity were made by area; if they had been made by volume, the terrestrial newt would have been even more toxic in relationship to the aquatic newt due to the thicker skin of the aquatic newt. This difference in toxicity explains the much longer time needed to kill a catfish when an aquatic instead of a terrestrial newt was used.

Presence of Toxin in the Blood of Adult *Taricha*

In order to test presence of toxin in the blood of *Taricha granulosa*, three adult males were used as donars. The blood of all three of these newts was found to be toxic in small amounts when injected into frogs. This is in variance with findings by Twitty (1937) who found all males tested (22) lacked toxin in their blood; of 40 females tested, one-half had toxin in their blood and one-half had no toxin. "There is some indication that the presence or absence of the paralyzing toxin in adult female blood may be correlated with the stage of ovarian development," (Twitty, 1937, p. 78). The difference in results may be accounted for by difference in techniques; 0.1 cc. of actual blood was used in these tests. It is not known how much blood Twitty used in his study.

Action of Toxin in Relationship to Method of Injection

It is evident from Table 14 that the method of injection has a great deal of effect on the time to lethality for mammals. It is evident that a larger dose is needed when administered orally. Oral injections were not carried out on other mammals; but the tests on *Peromyscus maniculatus* show the toxin is lethal when introduced into the esophagus. There are two possible explanations for this lethality: (1) The toxin is not neutralized in the digestive system, or (2) absorption of the toxin from the oral and esophageal surfaces is sufficient to kill the mice. Regardless of which explanation is correct, ingestion is lethal and therefore intraperitoneal injections are considered as valid tests for susceptibility.

V. CONCLUSIONS

From the preceeding data, one may draw the following conclusions:

(1) There is indeed a potent toxin in the skin of adult *Taricha granulosa*.

(2) Very small amounts of this toxin kill most would-be predators.

(3) The mixture of newt skin is toxic whether injected or injested.

(4) A toxin (not necessarily the same as in the skin) occurs in the ovary, eggs, and blood of adult newts.

(5) The dorsal skin is more toxic than the ventral skin and terrestrial newts are more toxic than aquatic newts.

(6) Larval newts either lack completely or have small amounts of toxin.

(7) Garter snakes, at least *Thamnophis sirtalis concinnus*, are able to eat adult *Taricha granulosa* and are apparently immune to their toxin when eaten; further work is needed to determine the nature of this immunity.

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