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Review Article

# Pharmacology and Toxicology of Toad Venom

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## HISTORY

FOR CENTURIES the toad has been known to produce a poisonous secretion. The Roman women used the toad, although unsuccessfully, as a murdering agent for their unfaithful husbands (1). William Shakespeare, born about 400 years ago, pictured the toad as wearing a precious jewel in its head. In Macbeth, Shakespeare made the witch throw a toad into the hell-broth. It has been long believed that handling of toads causes warts. In a small area of the state of Illinois, folklore has 300 references to warts of toad origin, particularly their cures (2). These superstitions can of course be disproved by laboratory experience. Houssay (3) operated on 15,000 toads for endocrine studies and we handled more than 10,000 toads for the collection of their poisons—with no ill effects. In form of a votive animal, the toad is associated with the uterus and various gynecological diseases (4) in Central Europe. Bronze toads of the Perm culture of Northeastern Russia were among the archaeological findings dating from the middle of the 1st century A.D.

Toad medicine has been advocated all over the world. For many years the Chinese have used a preparation of toad venom, ch'an su, for the treatment of canker sores, toothache, sinusitis, and local inflammations (5). During the 15th century a European physician wrote a book, "De Venenis," in which he mentioned that the toad's blood was of value in the treatment of difficult breathing (6). Pharmacopeias and dispensatories of early dates gave the dried toad a prominent place among healing agents (7, 8). The South American Indians have made use of the skin secretion of the toad as arrow poison. These arrows are shot into game or enemies from blowtubes.

The English word "toad" is applied to several tailless amphibians, but the various species under

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discussion belong to the genus *Bufo* of the *Bufonidae* family. The members of this genus all have a pair of well-defined skin glands behind the eyes, which secrete venom and are called "parotid" glands. The latter, however, have no relation to salivary glands. The longitudinal axis of the toad's parotid glands is parallel with the body axis, although that of exceptional species may be perpendicular to it—*B. peltocephalus* and *B. asper* (9, 10). Additional glands may appear on arms and legs, *e.g.*, *B. alvarius* (11). The venom can be expressed from the parotid gland between the thumb and index finger, and into a glass evaporating dish held over the toad's head. The toad has warts over the dorsal part of the body, and their secretion is difficult to obtain and may or may not be identical with the parotid secretion. Vulpian (12, 13) discovered that the toad venom had a digitalis-like action and that the toad's heart was relatively immune to its own poison. The histology of parotid glands and warts of European, South and North American toads has been documented by several workers (14–16).

Ugly and venomous, one species of toads, *B. marinus*, has saved sugar crops and other cultivated fields in the Hawaiian Islands and Puerto Rico by devouring the insects which do damage to the plantations. Although the toad is reputed to enjoy longevity, it lives in the laboratory from 8.5 to almost 16 years (17).

Attempts to isolate pharmacologically active substances from the European common toad were initiated over a half century ago (18). Abel and Macht (8) succeeded in isolating "bufagin" and epinephrine from the parotid venom of *B. marinus*. This was followed by the eventual identification of bufotalin and bufotoxin from *B. bufo bufo* by Wieland and Weil (19) and cinobufagin and cinobufotoxin from ch'an su (20). Marked advances were made at the University of Basel (21, 22), for numerous cardioactive substances were isolated from ch'an su, and later from the venoms of other species by elegant modern methods. It is now clear that the chemical composition of the venom of each species of *Bufo* differs from one another, although there are many similarities. Most species elaborate in addition, noncardiotonic sterols, indolealkylamines, and several, catecholamines.

## CHEMICAL INGREDIENTS OF TOAD VENOM

### Pharmacology of Bufadienolides

The cardioactive sterols are naturally the center of interest. In all instances the lactone ring is 6-membered of $\alpha$-pyrone type, attached to $C_{17}$. They have a free secondary hydroxy group at $C_3$, and are known as bufagins corresponding to aglycones of plants. The structural formulas shown in Fig. 1 represent the better known substances which are chemically bufadienolides (23) and synonymous with bufagins. Because different species of toads do not synthesize identical bufadienolides, prefixes have been employed for distinction. The Japanese toad apparently can produce all of them (24). In our laboratory gamabufagin and gamabufotalin are used interchangeably.

None of the bufadienolides in the toad venom conjugates with a carbohydrate to form a glycoside, but a few couple with suberylarginine to become bufotoxins. The first bufotoxin was isolated from the European common toad (25). To distinguish it from other bufotoxins, Wieland's compound has been renamed vulgarobufotoxin (26), and a structure of 14-suberylarginine bufotalin has been proposed (22–27). Among the amphibians, *Bufo* is the only genus elaborating bufagins and bufotoxins (28). Recently it has been reported that a North African grasshopper, *Poekilocerus bufonius*, feeding on milkweeds, is capable of storing glycosides of cardenolide type (29).

**Bufagins**—The action of bufagins upon the heart follows almost the same pattern as that of digitalis glycosides. Perfusion by various methods with appropriate concentrations in the frog results in systolic standstill. The absorption is generally poor. The positive inotropic action is easily demonstrated in mammals, such as the cat, by slow infusion of a dilute solution of regularobufagin. Electrocardiographic changes are most instructive. Figure 2 serves as an example. The prolongation of P-R interval, bradycardia, the diphasic T-wave, the nodal rhythm, the A–V dissociation, and the idioventricular rhythm are obvious. The slowing of heart rate can be abolished by atropinization but not by vagotomy. The effect of bufagins on the heart is less cumulative than that of digitoxin, being more like that of ouabain. There is a low order of absorption through the gastrointestinal tract in mammals. Thus, no more than 15% of cinobufagin is absorbed by oral administration in anesthetized cats, and 50% of bufalin (36) by intrajejunal injection in nonanesthetized dogs.

Because the chemical structures of bufagins differ from one another, their cardiac activity varies. Table I shows the potencies of 27 compounds. Their mean (geometric) lethal doses (LD's) are determined in groups of 10 or more etherized cats. It should be noted that bufota-

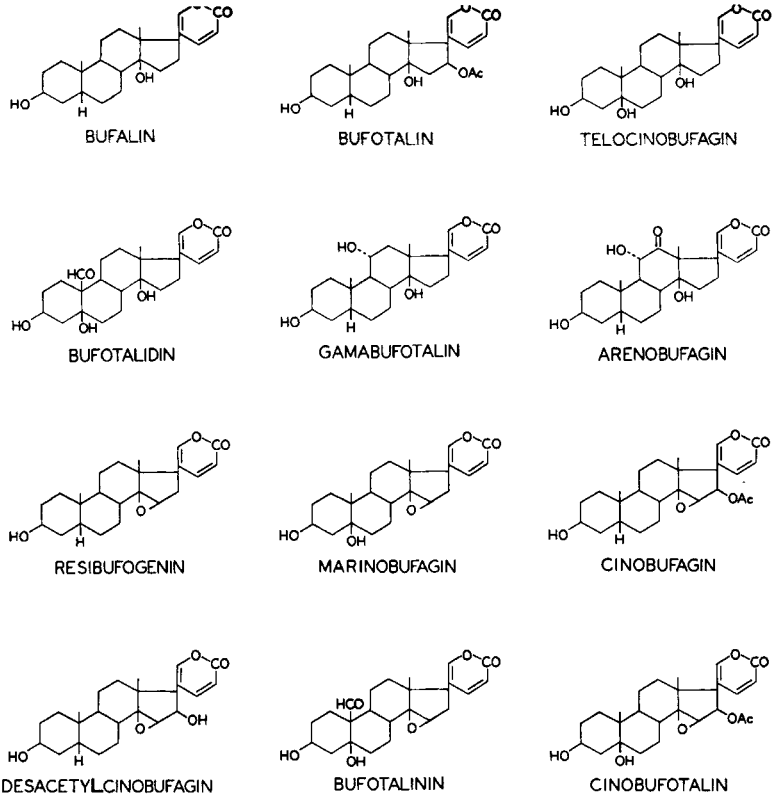


*Fig. 1—Structural formulas for better known substances which are chemically bufadenolides (23) and synonymous with bufagins.*

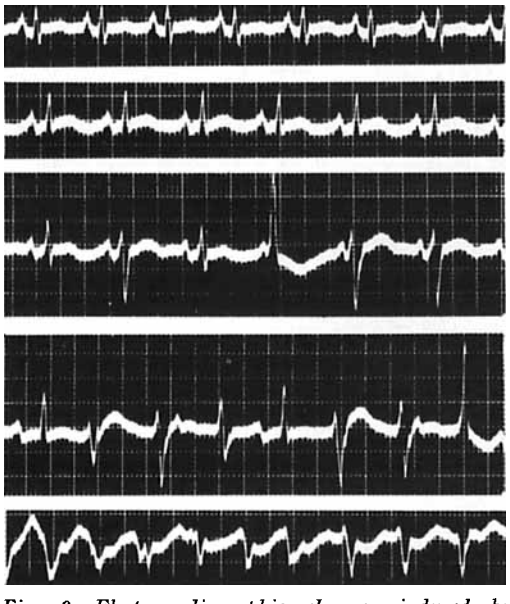

*Fig. 2—Electrocardiographic changes induced by regularobufagin in a cat. Regularobufagin in percent of fatal dose (top to bottom): 0, 57, 60, 67, 99.*

lidin is identical with hellebrigenin, an aglycone of the root of *Helleborus niger* (34). That toads can synthesize hellebrigenin and hellebrigenol of plant origin is exemplified by several species (37–39). When the means are converted to their reciprocals, they become the number of LD's per mg. as given in Table I, expressing the direct relationship of activity. The very high potency (toxicity) of some of these substances can be appreciated if one realizes that 1 Gm. of acetyl bufotalidin may have 15,600 cat LD's.

The contributions of the Basel scholars make it possible to arrive at certain trends of structure-activity relationship (30). If we consider bufalin the simplest bufadienolide, substitution of H with 5$\beta$-OH in telocinobufagin, or with 11$\alpha$-OH in gamabufagin increases the activity (Table I). A 12-keto group in arenobufagin further enhances the activity. The presence of a free 16-OH in desacetyl bufotalin reduces the toxicity, but 16-acetyl in bufotalin brings back the potency of bufalin. In other cases, 16-desacetylation abolishes the cardiac effect of cinobufagin and cinobufotalin. 3-Acetylation of cinobufotalin, marinobufagin, and bufotalidin (hellebrigenin) has a favorable influence on the potency to various degrees, but the same reaction with cinobufagin produces the opposite effect. A surprising feature is that 14$\beta$:15$\beta$-epoxides preserve a substantial amount of cardiac activity

TABLE I—ACTIVITY OF BUFAGINS AS MEASURED BY LD'S IN CATS

| Name | Mean (Geo.) LD, mg./Kg. | No. of LD's/mg. | Ref. |
|---|---|---|---|
| Arenobufagin | 0.08 ± 0.01 | 13.0 ± 1.14 | (30) |
| Bufotalin | 0.13 ± 0.01 | 7.6 ± 0.40 | (31) |
| Desacetylbufotalin | 0.26 ± 0.02 | 3.8 ± 0.28 | (32) |
| Cinobufagin | 0.20 ± 0.02 | 5.0 ± 0.44 | (33) |
| Acetylcinobufagin | 0.59 ± 0.04 | 1.7 ± 0.11 | (30) |
| Desacetylcinobufagin | inactive | ... | (30) |
| Cinobufotalin | 0.20 ± 0.02 | 5.0 ± 0.62 | (33) |
| Acetylcinobufotalin | 0.18 ± 0.01 | 5.6 ± 0.40 | (30) |
| Desacetylcinobufotalin | inactive | ... | (30) |
| Marinobufagin | 1.49 ± 0.09 | 0.7 ± 0.03 | (32) |
| Acetylmarinobufagin | 0.95 ± 0.05 | 1.1 ± 0.05 | (30) |
| 12β-Hydroxymarinobufagin | 3.00 | 0.3 | unpublished |
| Bufotalidin (hellebrigenin) | 0.08 ± 0.01 | 13.0 ± 0.93 | (34) |
| Acetylbufotalidin | 0.06 ± 0.00 | 15.6 ± 0.70 | (30) |
| Resibufogenin | inactive | ... | (30) |
| Acetylresibufogenin | inactive | ... | (30) |
| 12β-Hydroxyresibufogenin | 4.16 | 0.2 | unpublished |
| Bufalin | 0.14 ± 0.01 | 7.3 ± 0.55 | (33) |
| Telocinobufagin | 0.10 ± 0.02 | 9.8 ± 0.64 | (33) |
| Bufotalinin | 0.62 ± 0.12 | 1.6 ± 0.31 | (30) |
| Artebufogenin | inactive | | (35) |
| Gamabufotalin | 0.10 ± 0.01 | 9.9 ± 0.50 | (31) |
| Vallicepobufagin | 0.20 ± 0.02 | 5.0 ± 0.43 | (31) |
| Quercicobufagin | 0.10 ± 0.04 | 10.3 ± 0.39 | (31) |
| Viridobufagin | 0.11 ± 0.01 | 9.0 ± 0.66 | (31) |
| Regularobufagin | 0.15 ± 0.01 | 6.5 ± 0.25 | (31) |
| Fowlerobufagin | 0.22 ± 0.01 | 4.6 ± 0.24 | (31) |

as compared with their free 14β-OH analogs—marinobufagin *versus* telocinobufagin, cinobufagin *versus* bufotalin, and bufotalinin *versus* bufotalidin. It is difficult to explain the absence of activity of resibufogenin and artebufogenin (35). Telocinobufagin with a methyl group at $C_{10}$ is less potent than the aldehyde derivative of the same structure, bufotalidin. The cardenolides of plants—digitoxigenin, periplogenin, oleandrigenin, sarmentogenin, and strophanthidin, corresponding to bufalin, telocinobufagin, bufotalin, gamabufotalin, and bufotalidin—have lower toxicities. The same order of difference has been demonstrated on the contractility of guinea pigs' auricles (40). The superiority of α-pyrone at $C_{17}$ to the cyclobutenolide ring at the same position is unequivocal.

The nature of cardiotonic action is not yet clearly elucidated. An enormous amount of data with cardiac glycosides on tissue respiration, carbohydrate metabolism, high-energy phosphate bonds, ion equilibrium, and contractile proteins is probably also applicable to bufagins (41, 42).

Like ouabain, any of the bufagins causes vomiting in cats or pigeons following intravenous injection of one-half LD. The mechanism of action is presumably also due to the excitement of the chemoreceptor trigger zone where the impulse is mediated to the vomiting center (43).

Restlessness and convulsions occur in frogs with cinobufagin. The action appears to be on the spinal cord. Convulsant action has also been observed with acetyl cinobufagin, marinobufagin and its acetyl derivative, and bufotalinin, not only in frogs but also in cats (30).

As a class bufagins constrict arterial blood vessels and stimulate isolated rabbit's intestines and uterus (44–46). The action must be directly on the smooth muscles.

Some of the bufagins, if not all, have a local anesthetic action. Cinobufagin in 0.1–0.5% solutions produces numbness of the tongue when applied locally. With the stronger solution, sweet and bitter tastes may be lost. There is disappearance of tactile sensation. Local anesthesia can also be demonstrated on the rabbit's cornea after instillation and the guinea pig's skin following intracutaneous injections. Its action is not on nerve fibers, but is confined to sensory nerve endings. So far tested, bufalin, cinobufotalin, and gamabufagin also have a local anesthetic effect.

**Bufotoxins**—The principal action of a bufotoxin is also upon the heart. The electrocardiographic changes during the slow infusion of cinobufotoxin in a cat are illustrated in Fig. 3. They are similar to those with bufagins or cardiac glycosides. Their potencies are lower than those of corresponding bufagins. Thus vulgarobufotoxin is weaker than bufotalin. The data on

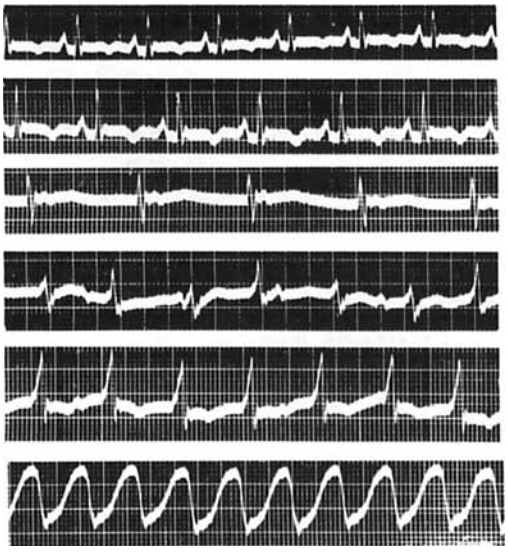

*Fig. 3—Electrocardiographic changes caused by cinobufotoxin in a cat. Cinobufotoxin in percent of fatal dose (top to bottom): 0, 19, 37, 75, 77, 100.*

nine bufotoxins are given in Table II. All of them are easily dissipated in the animal organism.

Extracardiac effects are repetitious of those of cardiotonic substances—emesis, stimulation of smooth muscle organs, and bitter taste when applied locally.

**Clinical Trials**—Because of the wide interest in the therapeutic value of toad venom in folklore, a clinical study of one of the bufadienolides was carried out. Cinobufagin can be used as a model, since it is easily available (47). Cases of auricular fibrillation are most suitable for this purpose. Indeed intravenous injection of 1 mg. slows the ventricular rate in some patients, and that of 2 mg. causes a marked fall of pulse rate. The effect lasts for about 5 hr. Nausea and vomiting occur in the majority of instances with the larger dose. It is obvious that cinobufagin offers no advantage over ouabain due to its brevity of action and frequency of side effects. In spite of such evidence, "bufomarine" has been recommended for the treatment of heart ailments of the aged (48). Aside from the curiosity, none of the toad cardiotonic principles can be justifiably employed in cardiology.

TABLE II—ACTIVITY OF BUFOTOXINS AS MEASURED BY LD'S IN CATS (31)

| Name | Mean (Geo.) LD, mg./Kg. | No. of LD's/mg. |
|---|---|---|
| Viridobufotoxin | 0.27 ± 0.01 | 3.70 ± 0.16 |
| Vulgarobufotoxin | 0.29 ± 0.02 | 3.43 ± 0.21 |
| Cinobufotoxin | 0.36 ± 0.02 | 2.79 ± 0.19 |
| Gamabufotoxin | 0.37 ± 0.03 | 2.67 ± 0.19 |
| Arenobufotoxin | 0.41 ± 0.01 | 2.46 ± 0.07 |
| Marinobufotoxin | 0.42 ± 0.02 | 2.40 ± 0.12 |
| Regularobufotoxin | 0.48 ± 0.03 | 2.10 ± 0.12 |
| Alvarobufotoxin | 0.76 ± 0.08 | 1.32 ± 0.13 |
| Fowlerobufotoxin | 0.79 ± 0.05 | 1.26 ± 0.09 |

### Catecholamines

**Epinephrine**—The discovery of epinephrine in the venom of *B. marinus* marks the first extramedullary source of a very potent sympathomimetic amine (8). What is more remarkable is the large quantity present in a pair of parotid glands. By a pressor method in pithed cats the dried secretion contains more than 5% of this agent. Not all species of toads produce epinephrine in the venom. To date *B. marinus*, *B. bufo gargarizans*, *B. regularis*, *B. arenarum*, *B. formosus*, *B. blombergi*, and *B. peltocephalus* are known to elaborate epinephrine in the parotid glands (9, 47, 49), while *B. bufo bufo*, *B. viridis viridis*, *B. alvarius*, *B. americanus*, *B. valliceps*, *B. fowleri*, *B. quercicus*, *B. melanostictus*, and *B. asper* are devoid of it (10). The pharmacology of epinephrine is so frequently reviewed (50, 51) that it will be superfluous to give an account here.

**Norepinephrine**—The identification can be made on the cat's blood pressure and nictitating membrane as well as by paper chromatography. Norepinephrine occurs in the epinephrine of *B. marinus* (52, 53) in amounts of 2–5%. The *l*-isomer of this catecholamine has also been found as an impurity from the sample of epinephrine, isolated from ch'an su (54). The detection of such a small quantity cannot be achieved by using the toad venom without isolating the bulk of epinephrine (55, 56). A monograph on the pharmacology of norepinephrine is available for reference (57).

### Indolealkylamines

The first indole base was isolated from the venom of *B. bufo bufo* (58). Its structure is 5-hydroxy-dimethylaminotryptamine (59). Indolethylamine derivatives occur in other species of toads (9, 10, 60). As shown in Fig. 4, cinobufotenine is the methylbetaine of bufotenine. 5-Hydroxytryptamine or serotonin and dehydrobufotenine are present in the parotid secretion of *B. marinus* (61, 62). Bufothionine occurs in the skin secretions of *B. viridis* (63).

The pressor activity in pithed cats can serve to distinguish indoleamines of different species (26). Cinobufotenine is the most potent. In fowls intravenous injection of these substances is followed by a fall of blood pressure (64). Application of the toad indoleamines stimulates

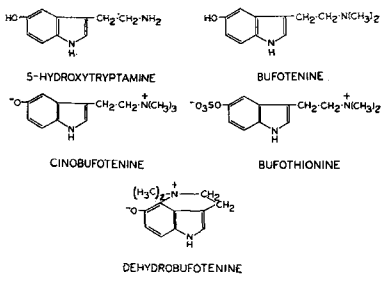


*Fig. 4—Substances appearing in the parotid secretions of the toad.*

the isolated uterus of the rabbit, the guinea pig, and the rat, and contracts the isolated ileum of the rabbit and the guinea pig. An extensive review on 5-hydroxytryptamine and its related indolealkylamines is available (65, 66).

### Noncardiotonic Sterols

The petroleum ether fraction of the ethanol extract contains a mixture of noncardiotonic sterols.

**Cholesterol**—The bulk can be identified as cholesterol by melting point, spectrogram, and color reactions in addition to digitonin precipitation.

**Provitamin D**—An admixture of the cholesterol sample of different species of toads is a phytosterol which varies from zero to 14 parts per thousand. The spectrogram is characteristic of ergosterol and after irradiation the material causes bone healing in rachitic rats.

**γ-Sitosterol**—Sterols of the plant kingdom occur in the venom of toads. γ-Sitosterol has been isolated from *B. bufo bufo*, *B. formosus*, and *B. arenarum* (67).

**Miscellaneous Substances**—The fresh venom of the different species of toads contains an average of 51–61% of water (11). During squeezing a typical scent is emitted. After drying and extraction for active principles, the residue swells in water, suggestive of mucoproteins. Free suberic acid and arginine are identifiable. The significance of these inert products have not been explored.

## USE OF THE VENOM TO THE TOAD

**Protection from Enemies**—The venoms of most animals are *a priori* intended for self-protection. If the toad finds it possible to introduce its poison into the enemy's bloodstream, it would be fatal to the latter. But the toad does not have teeth to bite, nor does it realize a danger by accurately squirting the venom. It blows itself up when approached by a human hand; when a dog or a cat bites a toad, prolonged salivation takes place but vomiting rarely follows (68). In our experience, one dog was induced to swallow a toad (*B. valliceps*) with complete recovery (47). Some snakes and rats feed on toads (69). The death of a little terrier from biting a toad (*B. alvarius*) was recorded (70). The bitter taste and local irritation of the nose and the eye when one comes in contact with the toad venom is well known (71, 72).

The process of regeneration of the venom is slow. If the pair of parotid glands (*B. marinus*) are manually expressed, it takes about 11 weeks for the toad to restore two-thirds of the original amount. It would be impossible for the animal to furnish large quantities of the poison repeatedly for defensive purposes.

It appears that the toad (*B. marinus*, *B. valliceps*) can dispense with the parotid glands. Surgical removal of these skin organs is compatible with the normal life of the animal. Observations of such an experiment were extended over a year (47).

**Significance to Behavior**—Recent work on psychopharmacology in higher mammals is centered around the amines of the brain. There is no information about the significance of 5-hydroxytryptamine and catecholamines to the toad. If a large toad, *B. marinus*, is dissected under local anesthesia for the blood supply and nerve innervation of a parotid gland, no epinephrine is demonstrable in the vein following electric stimulation of the glandular nerve which is a branch of the jugular ganglion. The result is not comparable to the splanchnic stimulation in mammals, liberating medullary epinephrine of adrenal glands. The toad's serum cholesterol averages 153 mg.%, lower than that of human adults. Is it possible that γ-sitosterol regulates it to this depressed level?

In short, toads of various species are capable of synthesizing in the parotid glands sterols of either *trans-* or *cis*-configuration, catecholamines, and indolealkylamines. All are pharmacologically active, but apparently serve little purpose to the toads.

## NATURAL TOLERANCE TO CARDIAC GLYCOSIDES AND AGLYCONES

For more than a century it has been known that the toad's heart is resistant to its own venom and to digitalis. This is a distinct example of pharmacogenetics. By comparison, the frog, *Rana pipiens*, is many times more susceptible to scillaren, ouabain, cymarin, and coumingine

HCl than the toad, *B. valliceps* (73, 74). Similar differences can be demonstrated by perfusions with gamabufagin, viridobufagin, vulgarobufotoxin, or regularobufotoxin. The exact mechanism of the toad's cardiac resistance is not known, but it offers a great challenge for elucidation. A suggestion has been made that the toad resistance is not due to the fate of cardiotonic substances, but determined by the reactivity of "digitalis receptors" (75). Aside from the classical studies, certain methods employed in electrophysiology may throw light upon this subject (76, 77).

## RESPONSE OF OTHER TISSUES TO DRUGS

Epinephrine on one hand and cinobufotenine and fowlerobufotenine on the other increase the tone and contraction of the frog's and the toad's heart to the same degree when perfused through the inferior vena cava (73, 78).

The central nervous system of the toad is almost equally susceptible as that of the frog to strychnine, picrotoxin, cocaine, caffeine, pentobarbital, and curare. The toad shows similar responses to gonadotropins as *Xenopus laevis* and *Rana esculenta* (79). The exhaustive investigations of Houssay (3) on the Argentine toad, *B. arenarum*, reveal more resemblance than exceptions to higher vertebrates. The natural tolerance of the toad's heart to cardiotonic steroids is unique and is only shared by rodents such as the rat. More studies to search for the reason will contribute to our understanding of this biological anomaly.

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