A PHARMACOGNOSTIC STUDY OF CH'AN SU, THE DRIED VENOM OF THE CHINESE TOAD.*[1]

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I. HISTORICAL INTRODUCTION.

For centuries the toad has been known in the Western World to produce a poisonous secretion. Comprehensive historical accounts are given in the works of Faust (2), (3), (4), Abel and Macht (5) and Lewin (6), (7), upon which the following statements are based. The secretion of the toad or its internal organs was a favorite ingredient of poisoning mixtures used for criminal purposes in ancient times. Juvenal, the poet, for example, described the skill of Roman women in murdering their husbands with various agents, one of which was toads' lungs. In the beginning of the fourteenth century, Bishop Guichard of Troyes was accused of poisoning the wife of Philippe le Bel with a preparation of scorpions, toads and spiders. During the sixteenth century, processes of extracting the toads' poison with salt were devised by murderers, especially the Italian poisoners. It was stated that the victims who took the salt succumbed quickly. In the same period a subtle method of inflicting wounds on the enemy's body without injuring the part was to rub his skin with the toads' secretion. In warfare and in the pursuit of game the toad secretion has long been used as an arrow poison by some Indian tribes (cited by Abel and Macht). In the beginning of the eighteenth century, toad poison was added to explosive shells. It was probably assumed that the enemy's death would be doubly assured if explosives were reënforced with poisons. In dispensatories and medical treatises, toad or toad poison is mentioned and described as a therapeutic agent. Paré (1510–1590), for example, dedicated the thirty-first chapter of his famous book to toad poison (Faust). In this connection he described two cases of toad poisoning and cited a third.

Powdered toad is highly recommended for dropsy, epistaxis and other ailments in the "Thesaurus Pharmakologicus" of J. Schröder, published in Leyden in 1672, and in the "Pharmacologia" of S. Dale, published in London in 1672 (quoted by Abel and Macht). At one time it was believed that the toad derived its poison from the earth and poisonous leaves, especially the mushrooms—thus the origin of the English expression "Toad stool," and the Dutch term "Padden Stollen," for mushrooms.

The nineteenth century marked the beginning of actual scientific investigations of the toad poison. Thus the secretion has been definitely localized in the skin glands of the back and sides, and the "parotid glands" behind the ears (8), (9), (10), (11), (12), (13). Vulpian (14), (15) was the first to make accurate observations on the action of the poison on the heart together with the toxic syndrome, and to show that the toad is tolerant to its own poison. His results were confirmed by Fornara (16). Vulpian, Fornara and later workers (17), (18), (19) found that the toad is

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[1] A preliminary report of this work was made to the Society for Experimental Biology and Medicine (1).

comparatively resistant to digitalis bodies. Recent studies appear to indicate that certain ions such as Ca^{++} and K^{+} may play an important part in the establishment of the tolerance (20), (21), (22).

The chemical investigation of the toad poison was begun by Pelletier (23), and followed by Gratiolet and Cloëz (24), Fornara (16), Calmels (25), Phisalix and Bertrand (26), Bertrand (27) and Faust (2). The highly potent principles prepared by Phisalix and Bertrand, and Faust, were still amorphous in nature. Abel in association with Macht (5) was the first to isolate bufagin and epinephrine in crystalline form from the American tropical toad, *Bufa agua*. Wieland and Alles later succeeded in obtaining crystalline bufotalin and bufotoxin from the European common toad, *Bufo vulgaris* (28). Handovsky (29) separated from the same species an alkaloid which, he thinks, is a pyrrole derivative and distinctly different from epinephrine. Novaro (30) claimed to have found epinephrine in *Bufo marinus*, but did not obtain it in crystalline form.

In China, as in the Occident, the toad and its dried venom have been known for many years and are admitted to the materia medica. The dried venom is called Ch'an Su in Mandarin, Ch'an meaning the toad and Su the venom (31). According to Pentsao Kang Mu (32) the preparation can be employed by external application in the treatment of canker sores, sinusitis, and many local inflammatory conditions, in the relief of toothache, and in the arrest of hemorrhages from the gums. When administered internally in the form of a compound pill, it is said to be able to break colds. Special emphasis is placed on the toxic nature of the toad and its venom. It is stated that dogs biting toads have their mouths swollen. If the fresh venom comes into contact with man's conjunctiva, it produces congestion and blurring of vision.

Fig. 1.—The Chinese toad as illustrated in Pentsao Kang Mu.

Ch'an Su has been the subject of study under the name of "Senso" by several Japanese workers during the last few years. Hayashi (33) reported that it was 50 to 100 times more powerful than digitalis. Shimizu (34) separated cholesterol, and bufagin $C_{18}H_{24}O_4$, m. p. 209–210° C., which he claimed to be identical with Abel's bufagin. The same author separated an amorphous substance which he termed bufotoxin. Kodama (35), (36) gave the formula for bufagin as $C_{27}H_{34}O_7$, m. p. 222–223° C. and claims to have succeeded in separating bufotoxin in a crystalline form, $C_8H_{10}O_2$, m. p. 203–204° C.; while Kotake (37) on repeating his work found the formula for bufagin to be $C_{29}H_{38}O_7$, m. p. 220–221° C., and Kodama's bufotoxin to be a chlorine-containing compound, $C_{27}H_{35}O_5Cl$. Both Shimizu and Kodama maintained that bufotoxin had a picrotoxin action.

During 1923–25, one of us (Chen) made a preliminary study of several Chinese drugs in China. Ch'an Su was one of them. Owing to the extensive work on

ephedrine, a thorough investigation was delayed until in 1928. Jensen was then invited to undertake the separation and establish the nature of the active principles.

II. THE IDENTITY OF THE TOAD.

A picture of the toad as illustrated in Pentsao Kang Mu is reproduced in Fig. 1. At present we are not certain as to which species produces the poisonous venom, although attempts have been made to secure specimens of the toad for a detailed morphological study. Nieden (38) in his monograph on Amphibia included 145 species in the genus *Bufo* distributed in Europe, Asia, Africa and America. Of this number, 7 occur in China, as shown below:

Species.	Habitat.	Species.	Habitat.
Bufo vulgaris	North of Himalaya Mountains	*B. mammatus*	Szechuan Province
B. raddei	Tibet	*B. melanostictus*	Southern China
B. viridis	Mongolia	*B. himalayanus*	Himalaya regions
B. nouettei	Nanchang		

Recently, a new species was found in Mongolia (39).

The Pentsao Kang Mu (32), which was written over three centuries ago, gives a simple description of the toad. It attains a considerable size and lives in the marshes and other damp areas of the river and lake regions of China. It has a protuberant abdomen, wrinkles and warts on the back and poisonous secretions in the skin. It is slow in its movements, unable to jump and does not sing. These general statements are, of course, not sufficient for zoölogical diagnosis.

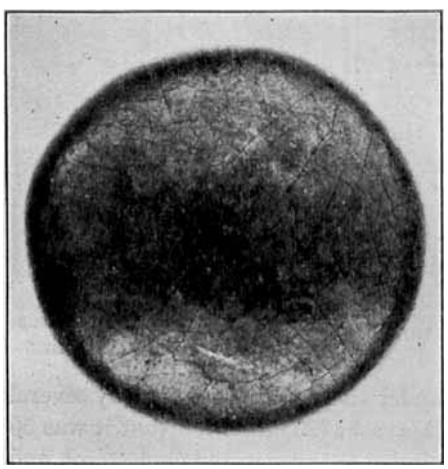

Fig. 2.—Illustrating a disc of Ch'an Su, the dried toad venom.

According to two modern Chinese biological dictionaries, the Chinese toad is identified as *Bufo vulgaris* in one (40), but *B. bufo japonicus* in the other (41). Either identification is open to question, from a chemical point of view. At present there has not been any report that all the toads yield the same active principles in their skin secretions. One is led to assume, from the published data, that each species produces its own characteristic secretion, although at least one constituent of each secretion has the typical action on the heart. It is therefore doubtful that the Chinese toad is identical with *B. vulgaris*, for epinephrine, which is present in Ch'an Su as will be seen below, has never been demonstrated in the European common toad. The toad giving rise to Ch'an Su is also less likely to be *B. bufo japonicus* because Kotake (42) isolated from the latter gamabufotalin, $C_{27}H_{38}O_6$, m. p. 261–263° C., which is not identical with the bufagin separated from Ch'an Su. Nieden (38) classified *B. bufo japonicus* as a variety of *B. vulgaris*. Further study is necessary to decide the correct species of this toad.

III. GENERAL CHARACTERISTICS OF CH'AN SU, THE DRIED VENOM.

The material for the present investigation, three catties in quantity, was purchased from Tung Jen T'ang, Peiping, one of the oldest drug stores in China (43). The dried poison comes in round, smooth and dark brown discs, as illustrated in Fig. 2. They are hard and thinner toward the margins. There is a hole in the center of each disc through which a string is passed. Obviously, they are hung up and air-dried. They vary in size, thickness and weight, as shown in Table I. Their diameter ranges from 6.95 to 8.80 cm., their thickness from 1.45 to 2.15 cm., and their individual weight from 47.03 to 109.34 Gm. The total number of discs in our supply was 21, and total weight 1729.30 Gm.

TABLE I.—SHOWING THE DIAMETER, THICKNESS, AND WEIGHT OF THE DISCS OF CH'AN SU.

Disc no.	Greatest diameter in cm.	Greatest thickness in cm.	Weight in Gm.	Remarks.
1	6.95	1.45	47.03	Broken
2	7.90	2.10	101.25	
3	7.90	1.65	75.21	
4	8.80	1.90	106.52	
5	7.70	2.05	83.49	
6	7.40	1.85	74.63	
7	8.30	1.80	85.71	
8	7.80	1.70	79.80	
9	7.80	1.95	83.12	
10	7.90	1.50	72.81	
11	7.85	2.00	86.75	
12	8.55	2.05	109.34	
13	8.10	1.75	89.42	
14	7.50	1.70	65.25	
15	7.25	1.45	61.68	
16	8.15	2.15	98.33	
17	8.40	2.05	106.39	
18	8.35	1.80	90.06	
19	7.85	2.00	83.22	
20	7.00	1.60	47.96	Broken and incomplete
21	8.10	1.65	81.33	Broken and incomplete
		Total	1729.30	

At present no knowledge has as yet been obtained as to exactly how these discs are made by the druggists. According to Pentsao Kang Mu (32), the secretion, white in color, may be collected on oil paper by squeezing the head of the toad between and behind the eyes (probably from the parotid glands). Another method is to administer garlic and paper into the toad's mouth, and later scrape off the secretion from the body with bamboo sticks (probably from the whole skin glands). Inquiry is being made as to whether or not these methods are still employed to-day.

The discs of Ch'an Su are very difficult to break. The broken surfaces are smooth and the color may be lighter in the center. When ground in a mortar or in a machine, one notices, if unprotected, a tickling of the nose, followed by continuous sneezing. The tip of the tongue becomes anæsthetized, and the throat is first dry for a very brief period, succeeded by an increase in secretion. There is no irritation of the eyes. These effects may last for over half an hour depending on the amount

inhaled. Sneezing and numbness of the tongue were previously experienced by Shimizu (34).

A microscopic examination of a No. 60 powder, which is brown to the naked eye, shows that it consists of particles, angular and irregular in shape, greenish black in color, somewhat translucent, with clearly defined edges. When a drop of water is added to the powder, the particles at once swell up and assume a granular appearance with indistinct margins. Disintegration does not take place unless the mass is disturbed mechanically. No definite cellular elements can be found when stained with methylene blue, or Wilson's reagent. The original contours of the particles are preserved if ether or chloroform is dropped on the powder under the microscope.

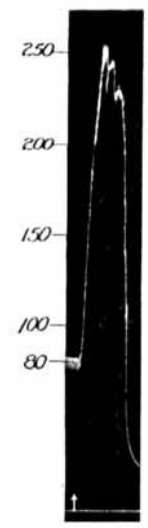

Fig. 3.—Showing the physiological effects of an alcoholic extract of Ch'an Su.

Cat, male, dark brown, 2.7 Kg., decerebrated and pithed, artificial respiration at the rate of 24 per minute. At signal, 0.5 cc. of an alcoholic extract (100 cc. of 80 per cent alcohol to each gram of Ch'an Su on water for an hour), was injected intravenously. The blood pressure rose from 80 to 258 mm. Hg, soon followed by a permanent fall and death of the animal. The ventricles were found fibrillating when the chest was opened.

In a test-tube one can see the particles swell if water is brought into contact with the powder, which now assumes a whitish appearance. When the powder is ground with a quantity of water (100 cc. for each Gm. of the powder), a fine emulsion is obtained. Filtration is practically impossible, and the coarse particles can be removed only by means of a strainer. The emulsion thus obtained is slightly acid in reaction, gives a positive biuret test, and a red color with Lugol's solution. It has a marked pressor action in anæsthetized dogs or pithed cats, when injected intravenously in small quantities (0.5 cc.). A cold acetic acid extract also raises the blood pressure in experimental animals. An alcoholic extract, made on a water-bath with 100 cc. of 80 per cent alcohol to each Gm. of powder, yields a clear solution, brown in color. It gives a positive catechol reaction with ferric chloride, and is very toxic to animals. A quantity of 0.5 cc. caused a marked rise of blood pressure but was soon followed by the death of the animal as shown in Fig. 3. The net rise of blood pressure in that experiment was 178 mm. Hg. After the blood pressure fell to the fatal level, the ventricles were found in fibrillation when the chest was opened. This powerful response of the animal is due to the component action of epinephrine, bufagin and a *N*-containing compound as will be seen below.

IV. ACTIVE CONSTITUENTS OF CH'AN SU.

So far four principles have been successfully isolated in crystalline form. They are as follows:

1. *Cholesterol*, m. p. 146° C., shows an ergosterol content of two parts per thousand, when examined spectroscopically.

2. *Bufagin*, m. p. 217° C., is toxic to frogs' and cats' hearts.

3. *A N-Containing Compound*, m. p. 200° C., is more toxic than bufagin.
4. *Epinephrine*, m. p. 212° C.

We are the first to have succeeded in obtaining epinephrine and the N-containing compound from Ch'an Su in crystalline form. Detailed accounts of the chemical and pharmacological studies will appear elsewhere.

V. DETERMINATION OF MOISTURE, VOLATILE SUBSTANCES, AND ASH CONTENTS OF CH'AN SU.

Samples of 0.5 Gm. of the powdered material were taken and studied in the same manner as in the investigation of Ma Huang published previously by one of us in THIS JOURNAL (44). The following results were obtained:

		Average.
Moisture in p. c.	5.10, 4.98, 5.04	5.04
Volatile substances in p. c.	1.08, 1.06, 1.12	1.09
Ash in p. c.	2.94, 3.16, 3.20	3.10

The ashing of the powder deserves comment. During ignition, a peculiar empyreumatic odor, somewhat sweet, somewhat pungent, is evolved. The powder becomes dark brown and then black, melts and rises. If care is not taken, it may easily run over the crucible. Because of this fact, each sample is best ignited in the air, and later incinerated in an electric muffle furnace. During the early stage, the inner walls of the crucible are covered with carbon particles. As combustion proceeds the charred material gradually sinks to the bottom until finally the ash assumes a grayish white color.

VI. ANALYSIS OF ASH.

The water soluble, acid soluble (in 10 per cent HCl), and insoluble portions of each ash were determined. The common elements were tested for with a larger incinerated sample. Iron and phosphorus are present in a considerable quantity. The results are shown below:

Analysis for		Average.		
H_2O soluble in p. c.	10.20, 11.39, 12.41	11.33	Chlorine	Trace
			Sulphur	Trace
			Phosphorus	Present
HCl soluble in p. c.	38.10, 37.34, 36.25	37.23	Iron	Present
			Potassium	Present
			Sodium	Present
Insoluble in p. c.	51.70, 51.27, 51.35	51.44	Manganese	Present
			Calcium	Absent

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PYORRHEA.

Bacteriologic study of pyrorrhea alveolaris. T. J. Cook and E. C. Stafne.—*Dental Cosmos*, 61 (1929), 115.—Through *Squibb Abstract Bulletin*, Feb. 13, 1929.

The present study was undertaken for the purpose of throwing further light on the relationship of pyorrhea alveolaris to metastatic infection. Streptococci were isolated from pyorrhea pockets and inoculated into animals to determine their virulence and selective affinity. Pathologic lesions resembling those of arthritis were found in 30% of the rabbits that were injected intravenously with the streptococci from the pyorrhea pockets of patients suffering from arthritis; in those injected with cultures obtained from patients suffering from ulcerative colitis; similar results were obtained in the case of ulcers of the stomach and duodenum, nephritis, cholecystitis, etc., in each case streptococci from the pyorrhea pockets of such patients producing similar pathologic conditions in a certain percentage of the animals so injected. The mortality rate of the infected rabbits was 44%; the controls received injections of cultures from pyorrhea pockets of subjects showing no sumptoms of any of the above diseases and displayed the various lesions mentioned above only to a very limited degree. This contrast of the percentages in the two groups is evidence, the authors contend, of the elective localizing power of streptococci obtained from pyorrhea pockets. The specific powers of elective localization of *streptococcus viridans* was not evident with other organisms present in *pyorrhea alveolaris*. Bacteria and their toxins which are produced in deep pyorrhea pockets can find access to distant parts of the body by way of its abundant vascular supply, this study proves.—J. P.