

The unenlightened reader may wonder how even a nominal defense can be made of such practices. It is to be found in the fact that among the other U. S. P. requirements is one that the fluidextract must darken the cock's comb when tested as prescribed. Since the Pharmacopœia says "Preparations shall be made only from drugs meeting the physical descriptions and tests," it is clear that there should not be any fluidextract made from such ergot as I have described, but the fact is that the practice has grown of disregarding every other requirement except this one. Things have even gone so far that when the fluidextract does not show the required strength, the official formula is changed so as to use a larger amount of drug to produce the liter of fluidextract! And the defense for this is that it is not yet proved that a fluidextract that darkens the comb is not necessarily active therapeutically!

That physicians should take little interest in this matter is not surprising. Not one in a thousand of them knows the difference between grains of ergot and licorice drops, and too few of them are observant of the effects of the medicine when administered. But with the pharmacist, the case is very different. He is directly responsible for the merits of a fluidextract used in a prescription, regardless of whether it is of his own manufacture or not. With the knowledge that *ergotamine* cannot be extracted in quantity from Russian ergot, and that the British Pharmacopœial Laboratories have found it "uniformly deficient in the specific alkaloid," and that the U. S. Department of Agriculture have found that 10 out of 14 samples of Russian ergot were without appreciable activity, American pharmacists should recognize that their own interests, quite aside from those of their customers, are endangered by a continuance of the determination of certain American manufacturers to "prevent a market for Spanish ergot," and compel the use of the Russian article, in plain violation of the Pharmacopœia and the statutes.

### CARE OF ANIMALS FOR BIOLOGIC ASSAYS.

(Continued from p. 257, March JOUR. A. PH. A.)

BY PAUL S. PITTINGER.

#### FROGS.<sup>1</sup>

*Distinction of Batrachians from Fishes and Reptiles.*—"The Batrachians represent a Class of Vertebrate animals occupying a position between Fishes and Reptiles. There is considerable variation in general appearance among the different living members of the Class, so that a Batrachian is not as easily defined and identified as is a fish, a bird or a mammal. There is no one characteristic by which it may be known, as there is in each of these other Classes."

*Two Orders of Living North American Batrachians—Urodela and Salientia.*—"The living North American Batrachians differ enough to allow classification into two distinct Orders, the Urodela and the Salientia. The Urodela are the Tailed Batrachians, or Salamanders, with various popular names, such as Mud Puppies or Water Dogs, Tritons, Newts and Efts. The Salientia are the Tailless Batrachians, *i. e.*, the Toads, Tree Frogs, Frogs and all Batrachians that have the frog-like form."

<sup>1</sup> Quotations from "The Frog Book" by Mary C. Dickerson.

*Development and Metamorphosis.*—"The North American representatives of the Salientia deposit the eggs in water, usually in shallow, stagnant water. The eggs may be laid singly or in small cluster, as in the case of some of the tree frogs; in large masses, as is the habit among the frogs; or in long unbroken strings, as in the case of toads. The eggs are sometimes free in the water, but more often are attached to water-weeds or other objects. If free, the egg mass has such buoyancy that it floats at the surface of the water (*Rana sylvatica*)."

"The egg is spherical, and is provided with a large amount of light-colored yolk. When the eggs are first laid, part of this yolk can be seen occupying the lower portion of the egg under the more or less pigmented upper portion. The dark pigmented portion of the egg at the top (later the whole surface becomes black or brown in color) allows a greater absorption of the sun's heat than would a lighter color."

The time required for the development of the frog's egg varies with the species and with the temperature in case of a given species. The eggs of the *Rana pipiens* hatch in two to three weeks.

"The length of life of the tadpole before its change into the frog may vary from two or three weeks to as many years. Some of the interesting points possible to observe during the metamorphosis are the following: The arms and legs develop simultaneously, but the arms are concealed under the opercular membrane. The horny parts of the mouth are dropped and the mouth cavity increases in size. The tail becomes smaller by absorption from within. The tadpole takes on habits of rushing to the surface or of resting wholly out of the water, showing that the lungs, which for some time before had been functional in company with the gills, now take on all of the respiratory work (except that performed by the skin)."

*The Families of the Salientia or Tailless Batrachia.*—"The Tailless Batrachia of North America, as far as known, are represented by seven families, containing together twelve genera and fifty-six species."

Frogs belong to the family *Ranidae*.

"The family contains a large number of genera, and it is very widely distributed. Australia, New Zealand, and southern South America are the only parts of the world not possessing representatives of the true frogs. Some two hundred species (by far the largest number) are found in the tropical portion of the Eastern Hemisphere. North America has only one genus, *Rana*; there are seventeen known species, the one form of the rank of a sub-species (*Rana c. latiremis*). They are most numerous in the eastern part of the continent, though several species are found west of the Sierra Nevada Mountains."

The official species, used for the assay of the cardiac stimulants, is *Rana pipiens* Shreber commonly known as the Leopard Frog, Spring Frog and Grass Frog.

The color of the official leopard frog is green, grey or brown, with somewhat rapid change from one color to the other.

"The Leopard Frog is one of the most beautiful in coloring of all our common frogs. It is better known than others not only because of its wider distribution and greater numbers, but because it has the habit of going considerable distances from its pond, or marsh."

Frogs of about 2½ inches in length are the proper size for assay purposes.

*Age of Leopard Frogs.*—The age of a leopard frog may be approximately

estimated from its body-length. The average body-length of the official leopard frog is as follows: One year old, one and one-half inches; two years old, two inches; three years old, two and one-third inches and four years old, two and three-quarters inches.

*Sex.*—The sex of the frog may be distinguished as follows: The male leopard frog has the thumb of the forefoot much enlarged on the inner edge and has a vocal sac between each ear and shoulder. These vocal sacs can be demonstrated by seizing the frog around the waist just in front of the hind limbs and alternately squeezing and relaxing the pressure. In this way the male will inflate the sacs.

The ripe *females* are very gravid and swollen and have no vocal sacs and no enlarged thumbs.

When disturbed frogs jump for safety. The jump is oftentimes accompanied by the squirting of water. This habit of squirting water—as though in the face of the enemy—may sometimes result in protection to the frog, since the water is of disagreeable odor.

When the frogs are removed from the tank for assay purposes each individual animal should be held until it has finished squirting water before being weighed.

The slipping skin, the slender body and the strong hind legs make it difficult to hold a frog. He gives most unexpected and vigorous jerks of the body to free himself. They are most easily held by firmly gripping about the posterior part of the body and thighs.

*Temperature and Hibernation.*—“All Batrachians, like Fishes and Reptiles, are cold-blooded, *i. e.*, they have a variable body heat, depending on the temperature of the environment. In this they are distinguished from Birds and Mammals, which have an unvarying body temperature.

“Because of this variation of the body temperature with that of the surroundings, Batrachians can endure extremes of heat and cold, but are greatly influenced by them. With decreasing temperature the processes of respiration and circulation gradually slacken speed, and the animals become more and more lethargic until they sleep. With rise of temperature to a limit varying from 20° to 30° Centigrade in the different species, they gain increased activity.”

*Shipping.*—There are several methods of shipping frogs. Some dealers ship in wet boxes without moss or hay. Others ship in wet moss. The author has found, however, that a large percentage of the frogs die during shipment if not properly crated. The method of packing should be governed by the temperature at time of shipment. *In the very hot summer months* they should be placed in boxes with wet moss. The one side of the box should be covered with fly screening. Care should be taken not to use too much moss as this mats and kills the frogs. The greatest loss of life occurs when too many frogs are placed in a single box or compartment. In such cases the frogs and moss mat in one corner of the box which kills a large number of the animals. Not more than 30 frogs should be shipped in one box or compartment. The most satisfactory method is to divide the box into compartments holding 30 to 35 frogs each, *loosely packed* with wet moss. *During the winter months* frogs may be packed as described above for shipping short distances. For shipping long distances they should be packed in loose moist (not wet) soil covered with about four inches of hay. *The hay must not be wet.* Do not ship more than 40 or 50 frogs in one box or compartment.

"Members of the *Salientia* can endure an astonishing amount of cold; even freezing in the water of a pond or in the mud at its bottom will not of necessity cause death. The circulation and all life processes may stop, but if the blood and protoplasm of the heart do not fall much below freezing point, the frozen parts will recover. Since sleep is induced in specimens at varying temperatures below 10° C., it is easy to understand why Batrachians are not found in the extreme north."

"This hibernation or sleep induced by cold continues until a return of high temperature."

Frogs that become frozen during shipment may, in many cases, be revived and used for assay purposes. Frozen frogs, however, should not be placed in a tank of water to "thaw out" as this rapid thawing usually kills most of them. Frozen frogs should be placed in a cold place where they will thaw out gradually. If this procedure is followed very few of the frogs will die unless they have been frozen for too long a period of time.

*Food.*—"The frog's method of eating is attended with much nervous alertness, and sometimes with unsatisfactory results to the frog. The food consists, in general, of living worms and insects, which are seized and swallowed alive. The frog uses his hands to help put the food into his mouth. The mouth has the sense of touch highly developed, but the sense of taste is present in only small degree. *In all cases, movement of a small living object gives the visual stimulus, and, psychologically speaking, brings to the frog the suggestion of something to eat.* Long experience of the race has taught that only immediate and swift motor response will result in capture of the food—the miller or grasshopper may take wing, the slug disappear under a board, or the caterpillar roll into a ball and "play dead." Usually, the result of the immediate seizure of the moving object is satisfactory, since *almost all small insects and worms are part of a toad's or frog's menu.* But sometimes lack of examination of the object brings dire results. Such is the case when a large stag beetle is swallowed. Its huge pinching mandibles produce terrible effect at once in the frog's stomach. Fortunately, the frog has a wide, short oesophagus, so that any disagreeable object may be disgorged immediately.

If frogs pursued their prey in the water as do fishes there would be no particular need for a tongue, and some of the most aquatic frogs of the world have little or no tongue as a consequence. Most frogs, however, possess a thick, adhesive protrusible tongue, which is fastened at its forward end. The posterior end of the tongue can be shot forward and then quickly retracted with the prey affixed or held.

It is not necessary, however, to feed frogs which are being stored for testing purposes. Feeding is very difficult and unsatisfactory, so it is better to keep them under conditions where very little energy is used. When stored at the comparatively low temperature of 15° C. or below, as directed by the U. S. P., frogs are quite inactive and require practically no food. It is necessary, however, to supply running water from which they presumably obtain sufficient nourishment.

*Storage.*—Frogs should be stored in tanks supplied with running water maintained at a temperature of 15° C. The tank should be so constructed that the depth of the water is about one-half inch deep over one half of the bottom and about four inches deep over the other half.

The tank should be divided into several compartments so different lots of frogs may be kept separated. This is essential owing to the variation between different lots in their susceptibility to Ouabain.

The sides and bottom of the tank should be thoroughly scrubbed with a small brush at least twice a week.

*Diseases.*—The most common and deadly disease of frogs is known as red-leg. This was originally thought to be an infectious disease. It is no longer considered as such but is explained as being a capillary condition produced by too much activity under unnatural conditions or to other unfavorable conditions under which the frogs are kept. There is apparently no cure for the disease and all animals in which red-leg is noted should be immediately removed from the tanks and discarded.

#### GOLD FISH.

*Carassius Auratus.*—The Golden Carp more commonly known by the name of Gold Fish owing to its golden hue, is a native of some of the ponds and sluggish streams of China. In their native waters, however, they are more of a silver color and do not have the bright golden coloring which is common to our usual aquarium varieties.

The Chinese breeders, who have for many centuries devoted all of their skill to breeding fine specimens, are to a great extent responsible for the much admired, bright golden red color.

Gold fish are classified according to shape as Common Gold Fish, Japanese Fan Tails, Japanese Fringe Tails, Comets and Telescopes. Fine colors are found in all the classes—golden red, silver, white, black and amber. These colors are often beautifully variegated.

#### HOUSING.

The aquarium may consist of any water-tight vessel. Its shape is of little importance. The size depends upon the number of fish to be kept and whether or not the aquarium is supplied with aquaria plants or running water.

In aquaria without plants or running water a half gallon of water should be allowed for every fish of medium size. If the aquarium is supplied with growing plants one gallon of water is sufficient for four fish. If running water is supplied the number of fish per gallon of water may be greatly increased.

Aquaria without running water should not be placed in the sun as the water becomes warm and unsuitable for fish. Another objection to too much sunlight is that it accelerates the growth of algae.

*Plants for the Aquaria.*—If running water is not supplied, the aquarium should contain an abundance of aquaria plants. These plants in the presence of light, exhale oxygen which is required by the fish to maintain life. If the number of fish is not in excess of four to the gallon of water and there are plenty of aquaria plants present the water need not be changed except when it becomes necessary to clean the aquarium which should not exceed two or three times a year.

Various plants are suitable for growing in aquaria. The most common of these are the Umbrella Plant or *Syperus*, *Colomba* or *Aquaria Moss*, *Ludwigia*, *Lemma* or *Duckweed* and *Sagitaria*.

If plants are to be placed in the aquarium the bottom should be covered to a depth of about  $\frac{1}{2}$  inch with well-washed sand. This should be covered with

a layer of clean pebbles and a few shells. It is not advisable to use too many shells as the lime hardens the water.

After adding water to a depth of about 2 inches the plants should be inserted into the sand and weighted down until roots grow sufficiently to hold the plants in position.

*Scavengers.*—The properly stocked aquarium should contain some scavengers to consume decaying vegetable matter, and keep down as much as possible the growth of confervae. Snails, tadpoles and newts are used for this purpose. Snails and tadpoles subsist largely on the growing plants in the water but newts should be fed scraped raw beef, earth worms or insects.

*Water.*—Ordinary "tap" water, spring or well water is best. Cistern or distilled water should not be used. The temperature of the water should be kept at about 60° to 75° F.

*Feeding.*—The best food for gold fish is the rice flour wafer or the mixed food containing dried meat sold under the name of Prepared Fish Food and Prepared Mixed Fish Food, respectively.

Fish kept in running water may be fed raw beef or chopped fish worms. All food containing yeast such as bread, crackers, etc., must be strictly avoided. Care should be taken not to overfeed. A piece of the prepared food  $\frac{3}{4}$  inch square per day is sufficient for a medium-sized fish.

*Diseases.*—Very little is known about the diseases of fish but salt water has been proven to be a specific for many fish diseases. Salt water apparently acts as a tonic which by invigorating the fish enables it to throw off the disease.

The usual treatment for a sick fish is to place it in a shallow dish containing a solution of one teaspoonful of salt to a quart of water. When the fish floats at the surface from exhaustion (usually after about two minutes) it should be removed to a small aquarium or other vessel containing a solution of one teaspoonful of salt to one gallon of water. Repeat the above treatment with the stronger salt solution daily until the fish has apparently recovered.

Among the common diseases of fish are the so-called fungus, asphyxia, twitters, dropsy and bladder complaint.

As only the common cheap variety of fish are used for laboratory purposes it is more economical to discard fish afflicted with any of these diseases than to spend time trying to treat them.

#### GUINEA-PIGS.

The Cavy, more commonly known as the Guinea-Pig is a native of South America and belongs to the rabbit family.

The first white explorers of South America found that the Indians had domesticated the Cavy. Besides being used for food purposes they were kept as household pets.

The wild Cavies of South America are still hunted as game and considered a delicacy.

Although guinea-pigs are used as food and as pets, they are in demand in far greater numbers for laboratory purposes.

The most satisfactory size for testing purposes is from 250 to 500 Gm. which size they attain in from six weeks to six months. Full-grown guinea-pigs, however, weigh from 675 to 1400 Gm. Their growth is comparatively rapid as they attain approximately their maximum size in about eighteen months.

The origin of the name guinea-pig is apparently unknown. Some think it is perhaps due to the fact that their shape suggests a small pig and that the name Guinea is a corruption of Guiana, a country in South America.

There are three principal varieties of Cavies distinguished mainly by their fur—the English, Abyssinian and Peruvian.

The Abyssinian and Peruvian are long ruffed-haired fancy breeds. The English or short smooth-haired cavies are the ones generally used for laboratory purposes. They come in a variety of colors, such as black, brown, cream, white, silver, gray, brindle, fawn or a mixture of these colors. The whites are usually albinos and have pink eyes.

*Housing.*—The size and style of the guinea-pig cage or cages depends primarily upon the number of animals to be housed. In our laboratory we keep these animals in multiple cages of the type shown in Fig. 3. Each unit of this cage accommodates about 25 pigs. While the animals are under test we use cages of the type shown in Fig. 1. Two to four pigs, of different color for identification purposes, are placed in each cage. The small cages are very serviceable as they may easily be carried into the laboratory where the animals are weighed and injected with the preparations to be tested.

The cages must be kept *dry* and clean. Guinea-pigs can stand low temperatures if the cages are dry and the animals are not in a draft. On the other hand they die rapidly if kept in a draft or wet cages.

The trays of guinea-pig cages should be covered with sawdust to a depth of about  $\frac{1}{2}$  inch to absorb moisture. On top of this a bedding of about three or four inches of hay should be placed. If the animal house is not heated during the winter months the hay helps protect the animals from the cold. At all seasons of the year the pigs will eat a considerable portion of the hay and the coarser parts make good litter.

The trays should be emptied at least twice a week and washed with an anti-septic solution before filling with fresh sawdust and hay.

In unheated houses the cages should be covered, during extremely cold weather, with burlap. This keeps out all drafts and at the same time allows sufficient air for breathing purposes.

*Feeding.*—Hay, grain and green foods form the principal diet for guinea-pigs. Hay should be before them all the time. Grain should be fed daily during the winter but sparingly in summer. Green food should be fed the year round and varied as much as possible. Of the grains, oats is the best although bran, wheat or rye serve the same purpose.

Guinea-pigs will accept almost any kind of greens such as grass, young wheat, alfalfa, clover, green corn stalks, melon rinds, apple peelings, cabbage, celery, kale, etc. Carrots and beets may also be classed as green food.

Green food should be fed sparingly during the winter and plentifully during the summer. *All green food must be in good condition.* Spoiled, sour, musty or moldy food will kill a large number of pigs. Decayed portions of green foods should be carefully removed before feeding.

Lettuce, celery and beet tops are among the doubtful foods. Too much of these greens seems to kill the animals. Also never feed grass or young wheat after it has been frosted or when it is wet with dew.

Do not overfeed as this is apt to cause bowel trouble. Do not feed at one time more green food than the pigs can eat in an hour. They are not apt to overeat hay but will eat too much green food if it is kept before them all the time. Best results are obtained by feeding twice a day—morning and evening.

*Water.*—Water should never be given while feeding green foods. When feeding only hay and grain, water is necessary. Change water daily.

*Breeding.*—Most laboratories find it more convenient and cheaper to buy their supply of guinea-pigs than to breed them.

For those who prefer to breed their own stock the following information is of value.

Guinea-pigs have from three to five litters per year with two to five to a litter. Young females should not be bred until three months old.

The period of gestation is from 65 to 70 days.

The young are fully developed at birth and are usually able to run around within a few hours.

The young begin to eat green food within two to three days after birth.

The young may be weaned when two weeks old.

About five females and one male should be kept in each breeding cage.

For best results each female should not have over four litters per year.

Several females with their young should be removed from the males and placed in a cage together. The females nurse each other's young as the young apparently cannot distinguish between the nursing mothers.

*Female Guinea-Pigs for Pituitary Assays* should be segregated at time of weaning and kept out of sight and smell of males.

*Diseases.*—Practically all diseases of guinea-pigs can be traced to improper care or food. As before stated damp cages, drafts and decayed food will cause the death of many pigs.

*Bowel Trouble.*—Too much green food especially in winter produces bowel trouble. In unheated houses this condition is also caused by sudden changes of the weather or insufficient bedding. When suffering from this complaint the coat gets rough and stands on end. The animal appears humped and many times the disease kills within 24 hours.

Separate all sick animals, administer castor oil, supply thick bedding and disinfect cages.

*Colds and Pneumonia.*—These diseases are rare in properly heated and ventilated houses. In unheated and drafty houses, however, it is not uncommon to have the entire stock wiped out with epidemics of these diseases.

As these diseases are contagious all affected animals should be removed and the cages disinfected. The sick pigs should be placed in warm cages and treated with a few drops of spirit of ethyl nitrite twice a day.

*Lice.*—Treatment is the same as for dogs.

*Other Diseases.*—Guinea-pigs are also subject to such diseases as Paralysis, Worms and Tuberculosis. As only the common cheap variety of guinea-pigs are used for laboratory purposes it is usually cheaper to kill animals suffering with these diseases than to spend the time necessary in trying to cure them.

(To be continued)

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#### ISOPROPYL ALCOHOL WARNING.

Concerning the use of isopropyl alcohol, the Anhalt Department of the Interior (Germany), draws attention to the fact that since isopropyl alcohol is employed in industry in place of ethyl alcohol in the manufacture of medicaments, liniments, cosmetics and perfumes, it is deemed necessary to issue a warning against the indiscriminate use of this substitute, and to draw attention to the possibility of injury to health which might be occasioned by the use of isopropyl alcohol in medicines in place of alcohol.