

2. A method for the extraction and titration of ephedrine in its salts has been developed, by refining well-known procedures and showing that ammonia if carried over in the solvent has no effect on the titrations by this method.

3. The limits of accuracy of the method are within one per cent, which is considered acceptable for alkaloidal extraction assays.

4. It can be extracted from its salts and titrated by the method submitted herewith in a period of one hour.

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## CARE OF ANIMALS FOR BIOLOGIC ASSAYS.

(Continued from p. 359, April JOUR. A. PH. A.)

### RABBITS.

The rabbit is indigenous to virtually every part of Europe and America. Australia has also a species of rabbit somewhat akin in type and habits to the American "Jack."

The rabbit is a different species from that of the hare. The rabbit is of the species *lepus cuniculus* while the hare is of the species *Lepus timidus*. Both belong to the family *rodentiae*—a creature with long rat-like front gnawing teeth.

In its gregarious habits the rabbit also differs widely from those of the hare, and in the wild state is said to be monogamous. This latter trait, however, ceases with domestication, and either sex becomes altogether polygamous. It also differs from the hare in that its young are born immature, with eyes closed and the body nude of hair, in a nest lined with fur pulled from the mother, burrowed in the ground whenever possible; while those of the former (hare) are born with eyes open, and body nicely covered, in a "form" on top of the ground.<sup>1</sup>

*Varieties.*—There are many varieties of domestic rabbits the more common of which are the American, Dutch, Lop, New Zealand, Polish, English, Angora, Spotted, Flemish Giant, Belgian Hare, the Tan in Black, the Silver in Brown, Fawn, Grey and Blue, the Havana, Imperial and Sells.

*Housing.*—The cages shown in Figs. 3, 5 and 7 are very satisfactory for housing rabbits. The bottom of the trays should be covered with sawdust on top of which is placed three or four inches of hay. If the rabbits are to be kept outdoors wooden hutches are preferable as they are warmer in winter. The character and size of the hutches depends upon the number of rabbits to be housed. Any style or shape box is apparently satisfactory. Five sides of the hutch should be constructed of wood. The sixth side should contain the door made of a wooden or metal frame covered with wire screen. For breeding purposes one end of the hutch should contain a nesting compartment, separated from the rest of the hutch by a wooden partition containing a hole sufficiently large to permit the rabbits to pass from one compartment to the other. Each compartment should contain a removable metal tray to facilitate cleaning. It is not necessary to heat outdoor hutches as rabbits can stand cold weather. The hutches should, however, be protected from drafts and storms in winter and excessive heat and

<sup>1</sup> Deardorff, Rabbit Culture and Standard.

direct sunlight during the summer. During extremely cold weather burlap- or muslin-covered frames should be provided to close opening in the hutches. These frames, by reducing the rapid circulation of air, tend to retain the warmth generated by the animal bodies, and at the same time permit the escape of impure air through the fabric.

During cold weather the bedding of hay should be increased to a depth of five or six inches. This helps to protect the animals from the cold.

All cages or hutches should be regularly cleaned and disinfected as described under "General Principles."

*Exercise.*—The rabbits should be placed in the cages or hutches in such numbers as to permit sufficient room for proper exercise. If too crowded the animals do not receive sufficient exercise to maintain a healthy condition.

*Feeding and Watering.*—Rabbits should be fed and watered practically the same as guinea-pigs. Dandelion seems to be injurious and plantain should be fed only sparingly. Turnips and carrots are a good relish now and then but hay-clover, alfalfa or timothy and oats should form the principle diet. Water should not be given when greens are fed. *Never give wet green food.*

Some prefer a mixed food containing cracked corn 10%, hominy 5%, whole wheat 10%, ground oats 20%, whole oats 25%, bran 30%. To this should be added one-half ounce of salt to each four quarts of mixed feed.

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

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

Rabbits have from six to eight litters per year with four to eleven to the litter.

The period of gestation is 31 days and if it varies more than one or two days longer something is wrong.

The doe will begin building her nest, and pull fur with which to line it any time from 10 days to an hour before due to kindle. It is necessary, therefore, to have short hay or straw present at all times for her use in building the nest.

A few days before a doe is due to kindle have before her continually a dish of clean, fresh water. Many does become feverish at this time, and will not infrequently destroy their young if the desire for water is not satisfied.

After the doe has kindled remove any dead from the nest. Best results are secured by reducing the litter to six. Above this number calls for extra attention, food and care.

During the time a doe is suckling her young she should have constantly before her a dish of bread and fresh milk. Do not allow the milk to sour in the dish. The acidity of sour milk will cause what in rabbits is known as "slobbers" which is a very annoying trouble.

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

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

Bucks become virile at about four months of age. It is best, however, to use bucks that are at least eight months old.

Does may be kept together in one compartment until bred but each adult buck must be kept in a separate cage or hutch as they are liable to kill each other by fighting. When breeding it is advisable to take the doe to the buck's hutch.

*Handling.*—When handling rabbits *do not pick them up by the ears*. The proper method of handling is to grasp the loose skin over the middle of the back with one hand and allow the rump to rest on the other. When carrying grasp as above and allow weight to rest on forearm.

*Diseases.*—If proper attention is given to the housing and feeding of rabbits, they are generally hardy and free from disease.

The diseases which are most common to rabbits are snuffles, slobbers and vent disease.

Animals suffering with *snuffles* should be isolated immediately and the cage or hutch thoroughly washed with a disinfectant solution before using for other animals. The treatment for snuffles is to place the animal in a warm cage well bedded with hay and administer two or three drops of tincture of aconite three times a day. If the animals are part of an experiment and thus of sufficient value to warrant the time and attention necessary, it is advisable to daily pipette into the nostrils a solution of equal parts of Solution S-T-37 (Hexylresorcinol 1-1000) and water or some equally efficient non-toxic germicide.

*Slobbers.*—This trouble is apparently due to indigestion caused in most cases by the too liberal feeding of greens, wet or decayed greens or sour milk. This trouble should therefore be *prevented* rather than treated. It may be treated by administering the proper doses of any good digestive stomachic.

*Vent Disease.*—Animals infected with this disease, sometimes called vent gleet, should be immediately isolated and the cages disinfected. If the animals are of sufficient value inject daily with Solution S-T-37 or a 5% solution of colloidal silver.

*Other diseases* to which the rabbit is susceptible include ear canker, diarrhea, paralysis, skin eruptions, constipation, etc. Very little can be done for these diseases and it is usually cheaper to discard afflicted animals than to try to cure them.

*Fleas and Lice.*—Treatment is the same as for cats.

#### RATS.

The albino rat (*Mus norvegicus albinus*) is the most satisfactory animal for vitamin assays and other nutritional investigations, since the effects of an exclusively vegetable diet or those of a strictly animal diet may be observed. The rat is to be preferred, therefore, for metabolism experiments such as studies in dietary deficiencies and the biologic assays for Vitamins A, B, D, E and the P-P Factor.

The rat is of especial value in many kinds of experimental work owing to the large number in each litter. This makes it possible to select both the control and experimental animals from the same litter and thus avoid variations which may exist between animals of the same age but from different litters.

*Housing.*—The albino rat is one of the cleanest of all laboratory animals if properly housed. Rats so housed are free from odor and there is no necessity for the nauseating odor so often prevalent where rats are stored. If the cages are properly constructed, cleaned semi-weekly, provided with fresh water daily and have an absorptive bedding, hundreds of rats may be housed in one room without any objectionable odor.

Cages constructed of metal are more durable and easier to clean and disinfect than wooden cages. The climate and the conditions of the experiment should govern to a marked degree the materials from which the cages should be constructed. Metal cages are to be preferred, but should be placed in a well-heated room away from drafts. The temperature of the room should range between 65° and 75° F. and *must not be damp*. Small metal cages such as used for vitamin

assays should be placed on wooden shelves with hinged wooden fronts to help retain the animal heat and avoid drafts. The wooden fronts, however, should only be three quarters of the height of the space between shelves.

*Breeding cages should always be constructed of wood* as the nests are frequently located so that the outer wall of the cage forms one side of the nest. All cages, whether constructed of wood or metal, should have bottoms made of wire screen with a metal tray underneath for holding sawdust. The front of the tray should be about  $\frac{1}{2}$ " longer and wider than the opening into which the tray slides so that when in position the front prevents drafts by closing the opening to the space beneath the wire-screen floor.

Light and proper ventilation are essential. The animals generate a lot of heat and give off quantities of moisture which must be dissipated by proper circulation of air.

The cage shown in Fig. 2 is suitable for housing two to three full-grown or six to eight small *stock rats*. The water is placed in the drinking fountain shown attached on the side of the cage. The bottom of the cage is of wire screen which allows the droppings to pass through into the tray beneath, which is one-half filled with sawdust.

Each unit of the cage shown in Fig. 3 is suitable for housing twenty-five stock rats.

For best results the *breeding cages* should be of special construction with one compartment for nesting and another for exercising.

Very satisfactory cages for this purpose were designed at the Wistar Institute of Anatomy and Biology by Greenman and Duhring<sup>1</sup> which they describe as follows:

"For the production of the best animals two types of cages are desirable. First, a dormer cage and, second, an exercising cage. A revolving cage or a turntable is used for the latter purpose."

"The two forms of cages which we have adopted after considerable experimental work with several types of cages are here described."

#### DORMER CAGE.

"To distinguish them from other types of cages, like the exercising cage, the special cages used for experiments in nutrition, etc., we have designated as 'dormer cages' (*Dormire*, sleep) those cages in which stock animals are bred, reared, and housed."

"The dormer cage is constructed of New England white pine  $\frac{1}{2}$ " thick, and is 35" long,  $12\frac{1}{2}$ " high, and 17" deep, all outside measurements. It is divided in the middle by a partition into two compartments, each compartment measuring 16" x 16" inside, with a height of  $8\frac{1}{2}$ " from the removable screen floor to the ceiling. The two compartments communicate through a circular opening 3" in diameter, located near the rear of the cage. The object of this division is to increase the number of more or less sheltered positions where the rat may build its nest or protect itself from direct lights. It affords the rat an opportunity to escape from one compartment to the other if frightened. This simple shifting of location appears to satisfy the animal that it has protected itself. Furthermore, it

<sup>1</sup> Greenman and Duhring—"Breeding and Care of the Albino Rat for Research Purposes."

is sometimes desirable to close the opening in the dividing partition in order to confine the rats in one compartment while the other is being cleaned."

"The cleats used in the construction of the cage, across the ends, and on the lower edge of the dividing partition are all without the cage, thus avoiding wood angles within the cage."

"The circular opening from one compartment to the other presents the only angle or edge within the cage which the rat may gnaw. This edge is protected by a metal band."

"Everywhere else within the cage the wood presents only flat, smooth surfaces which the ordinary albino rat will not attempt to gnaw. Special protection must be provided in cages which are intended to contain extracted albino rats."

"For the cage floor in each compartment, a removable galvanized wire-cloth screen  $15\frac{3}{4}$ " x  $15\frac{3}{4}$ " (No. 22 wire  $\frac{1}{8}$ " mesh) having a folded edge  $\frac{3}{4}$ " wide, of No. 22, galvanized sheet steel is provided. This screen floor is supported along the front and along the rear by  $\frac{3}{4}$ " x  $\frac{3}{4}$ " x  $\frac{1}{16}$ " galvanized steel angles. Wood or fiber floor may be used if desired for protection against cold. Beneath the removable floor is a galvanized sheet steel drip pan or tray,  $15\frac{3}{4}$ " x  $15\frac{3}{4}$ " x  $\frac{1}{2}$ " deep. This pan catches the drips and finer particles of dirt falling through the cage floor."

"At the right-hand end of each cage is the drinking fountain carrying a one-quart water bottle. This drinking fountain consists of a galvanized iron pocket opening into the side of the cage. Into this pocket projects the metal tube outlet of the inverted water bottle. A fresh drop of water always hangs on the slightly constricted outlet of this tube. Here the rat may drink, but it cannot foul the water supply. Furthermore, the outer end of this iron pocket carries a wire screen to admit light. This inhibits the rat from filling the space with food, litter, or other materials, and thus interfering with the proper working of the drinking fountain. The drinking fountain is so constructed that all excess water dripping from the water bottle flows out the end of the cage, thus preventing the wetting of the cage and the bedding on the cage floor."

"The two doors of the cage are made of galvanized wire cloth,  $\frac{1}{4}$ " mesh, with a folded edge  $\frac{3}{4}$ " wide of No. 22 galvanized sheet steel riveted at the corners. In the middle of one side a stove bolt with suitable brass sleeve and lock-nut furnishes both a handle for the door and a part of the locking mechanism. It is well to paint the wire-cloth door with some hard black enamel. This renders the contents of the cage more plainly visible."

"The doors are not hinged to the cage, but set into the front openings of the cage, so as to protect all exposed edges of wood and so they may be easily removed. In feeding, the caretaker may unlock and open a cage door with one hand while serving food with the other."

"At the rear of the cage and along its entire length at the upper part, a  $2\frac{1}{2}$ " opening covered with  $\frac{1}{4}$ " mesh galvanized wire cloth affords ventilation for the rats."

"The cage is so constructed that one may set upon the top of another in banks of four or five as may be desired. No projecting part interferes with this arrangement. Or they may be supported on a rack with projecting arms which would come just under the overhanging upper edges of the ends. With such a supporting rack, each cage may be removed without disturbing others."

"Our own practice is to pile the cages five high on an angle iron frame suspended from the ceiling so that the lowest cage is 14 inches from the floor."

"The light weight of such cages makes it possible for a person with a small amount of physical strength to handle cages. Such a cage, stripped of its accessories, weighs  $14\frac{1}{2}$  pounds."

"In cage construction the economy of operation should be considered if there are many cages in service. Light-weight cages can be cleaned and sterilized more quickly than heavy ones. The lighter the cage, the less heat units will be required to sterilize it."

"The dormer cage here described is intended to accommodate one breeding pair

of rats and a litter of young. Not more than eight or ten adult albino rats should be kept in a cage of this size."

The construction of the dormer cage with a turntable exerciser in place is shown in Fig. 11.

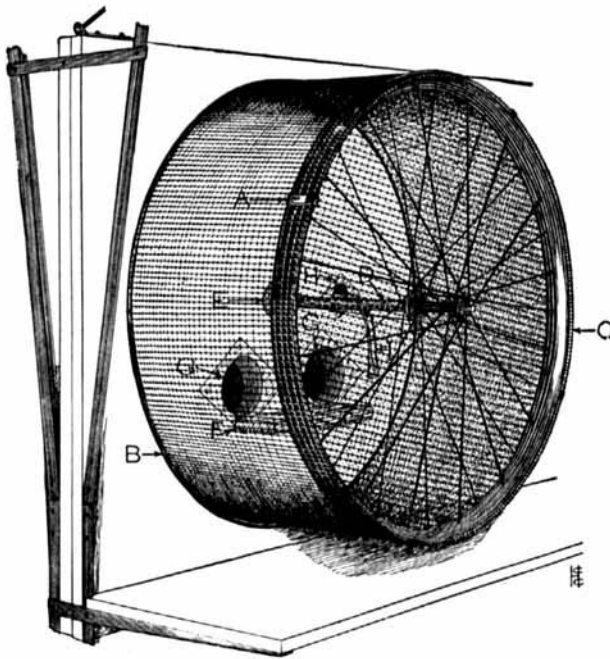


Fig. 9.—Exercising or revolving cage. (Greenman and Duhring: "The Albino Rat.")

#### EXERCISING OR REVOLVING CAGE.

"The exercising cage is an essential part of the colony equipment if fertility is to be maintained and vigorous rats are desired."

"The cage which we have found very satisfactory is constructed upon a 21-inch bicycle wheel. The excellent ball bearings of a bicycle wheel are essential, for revolving cages are subjected to a very considerable

daily use. The recording mechanism frequently registers 5000 revolutions in the twenty-four-hour period."

"Each revolving cage is provided with a galvanized steel pan,  $18" \times 10" \times \frac{1}{2}"$  deep, so placed on a shelf beneath the drum as to catch the drips and refuse falling from the drum. Most of this material passes out between the free edge of the drum and the background."

"The nest box (Fig. 10), located on the rear face of the background, is  $21\frac{1}{2}"$  long,  $8\frac{1}{2}"$  high, and  $7\frac{1}{2}"$  wide, and is constructed of  $\frac{1}{2}"$  thick white pine. It is divided in the middle by a solid wood partition into two compartments. Each compartment communicates with the revolving drum through a  $2\frac{3}{4}"$  circular opening, already shown in Fig. 9 (G, G'). The nest is accessible through the top, which is closed by a lid extending its whole length. In the lid over each

compartment a 2-" circular opening covered by a wire screen affords ventilation for the nest box (Fig. 10, A)."

"One side of the nest box is closed by the background which carries it. Across this side of the nest box, a 1/2" x 1/8" flat iron bar (B) is screwed to the ends and middle of the box, and is utilized to carry the box on two flat hooks, secured to the background. This arrangement makes it possible to remove the nest box easily for cleaning."

"Each compartment of the nest box is supplied with a wire-screen floor (C), similar to the floor in the dormer cages. These are removable."

"Beneath the screen floor a galvanized sheet-steel pan (D) serves to catch the drips and particles of dirt falling from the nest box. Above the nest box is located the water bottle (E), with its extra long metal tip leading through the background into the revolving drum. The bottle rests upon a small bracket and is held in a vertical position by a wire loop at the top. The label holder (F) is fixed on the rear face of the background."

"We have used the revolving cages mounted singly on individual stands and in batteries of twelve or less mounted on one large background."

"The revolving cage here shown is one of a series of twelve mounted in a double row on a vertical background suspended from the ceiling. Beneath each row of six cages is a projecting shelf 10" wide to carry the drip pans under the cages."

"The Turntable.—As a less expensive substitute for the revolving cage, we have used the turntable mounted in a dormer cage. Its position and construction is shown in Fig. 11. The turntable consists of a wooden disc 1/2" thick and 14" in diameter."

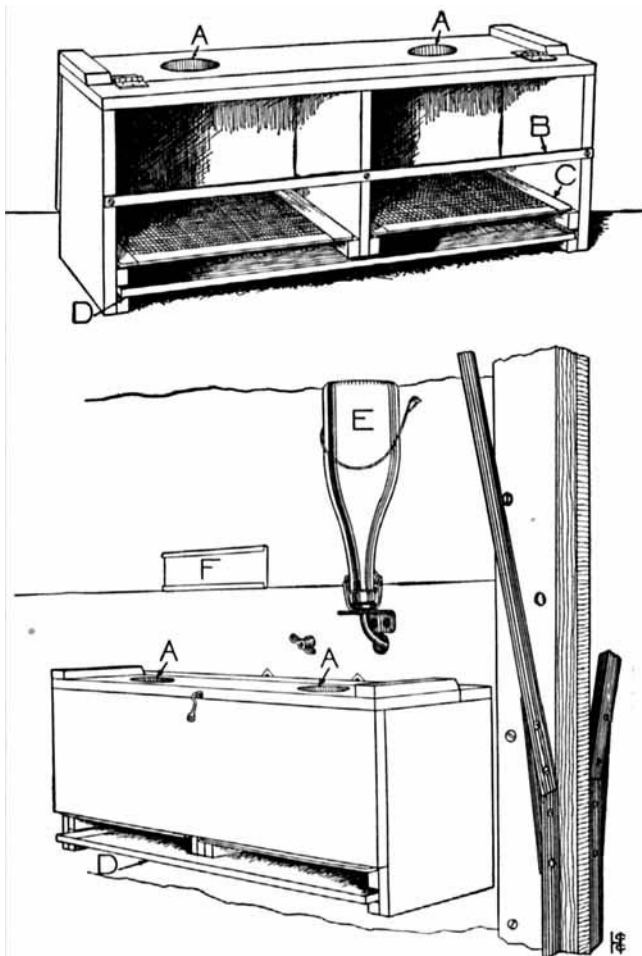


Fig. 10.—Shows construction of nest box for revolving cage shown in Fig. 9. The nest box is attached to one side of the background and the revolving cage to the other side. (Greenman and Duhring: "The Albino Rat.")

"A modified bicycle wheel hub and axle (A) are used to carry the revolving table."

"Secured to the lower end of the bicycle wheel hub is a brass disc 4" in diameter and  $\frac{1}{8}$ " thick which carries the larger wooden disc."

"The upper end of the axle is provided with an elbow-joint (B), the position of which is controlled by a bolt and wing-nut permitting the turntable to be adjusted to any desired angle."

"A stud bolt (C) projecting from the upper portion of the elbow-joint passes through the ceiling of the cage and, held in place by a wing-nut, acts as a mounting for the turntable. By removing the wing-nut at the elbow-joint the turntable may be easily removed for cleaning purposes."

"While the turntable occupies considerable space within a cage and makes

cage cleaning a little more difficult, we are inclined to the belief that it stimulates the rats to a greater variety of exercises than the revolving cage already described."

"A female appears to appreciate the advantages of the exercising wheel. When her pups are able to go out into the wheel, she will operate the wheel while they cling to the wire of the revolving drum."

"Occasionally a female will carry her pups from the revolving cage to the nest box. This happens when the pups are twelve to fifteen days of age. She apparently thinks they are too young for exercise. Later, the female will sometimes take one pup at a time in her mouth and run with it in the revolving drum, then return this one to the nest box and take another

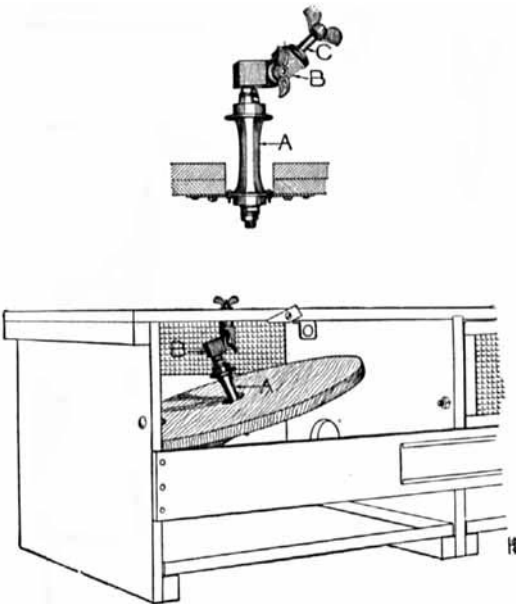


Fig. 11.—Turntable exercising cage. Made by placing turntable in dormer cage. (Greenman and Duhring: "The Albino Rat.")

out for similar treatment until the entire litter has been with her in the revolving wheel."

(To be concluded)

#### EXPORT OF EPHEDRA VULGARIS FROM CHINA TO THE UNITED STATES.

A phenomenal increase in the exports of *ephedra vulgaris* from China to the United States is disclosed in reports of Vice-Consul A. J. Ward, Tientsin, China. Among the crude drugs declared for export to the United States during the first seven months of 1926 and 1927, figures for *ephedra vulgaris* were 8317 pounds valued at \$741 and 477,218 pounds worth \$53,652, respectively.

Of the 477,218 pounds shown for the first seven months of 1927, more than one-third or 147,582 pounds, value \$15,667, was exported in July.