

constituent that causes the low p_H in distilled water. The methyl red brom-thymol-blue indicator mentioned has given very good results with distilled water.

	p_H .	Indicator.
Caffeine	4.75-5.00-5.50-6.60	B. C. G.-B. T. B.-B. C. P.
Calcium chloride	7.45-7.90-8.10-8.90	P. R.-T. B.
Calcium glycerophosphate	8.20-8.30-8.35-8.40	P. R.-T. B.
Calcium lactate	6.85-7.00-7.50-7.90	P. R.
Dextrose	5.10-5.30-5.40-5.70	B. C. G.-B. C. P.
Hexamethylene-tetramine	7.70-8.00-8.20-8.40	P. R.-T. B.
Iron and ammonium citrate	3.10-3.50-4.00-4.60	B. P. B.-B. C. G.
Iron cacodylate	4.60-5.40-5.60-5.80	B. C. P.-B. C. G.
Magnesium sulphate	6.10-6.30-6.50-6.70	B. T. B. M. R. B. T. B.
Procaine	5.10-5.20-5.30-5.80	B. C. G.-B. T. B.
Potassium guaiacol sulphonate	7.45-7.60-7.70-7.90	P. R.
Sodium benzoate	7.70-8.20-8.50-9.30	P. R.-T. B.
Sodium cacodylate	7.85-8.00-8.30-8.45	P. R.-T. B.
Sodium citrate	7.70-8.00-8.30-8.40	P. R.-T. B.
Sodium glycerophosphate	8.60-8.70-8.90-9.00	T. B.
Sodium iodide	4.90-5.40-8.80-9.60	B. C. G.-T. B.
Sodium salicylate	6.90-7.05-7.10-7.40	P. R.-B. T. B.

For the p_H range 2.9 to 4.0 where bromo-phenol-blue has failed to give results dimethyl-amido-azobenzene has been used successfully. Under the title "A Universal Indicator for Hydrogen-Ion Concentration," Dr. Emil Bogen, in the *J. A. M. A.*, Vol. 89, 199, has suggested a mixture of phenolphthalein, 100 mg.; methyl red 200 mg.; dimethyl-amino-azobenzene, 300 mg.; brom-thymol-blue, 400 mg.; thymol blue 500 mg. Dissolve in 500 cc. of absolute alcohol and add tenth normal sodium hydroxide until the red disappears and the solution becomes yellow. This indicator will cover the p_H range from 1.0 to 10.0 and is very good to get the first estimate of the p_H ; however the writer has found that economy may be had by using half the quantities of indicators and half the alcohol, and after solution adding water to make up the volume.

Samples of soils and similar materials may be soaked in a small amount of distilled water and the p_H determined on the filtered extract. The quantities taken are open to the workers' option and should be stated with the results.

Observation of the details that have been mentioned as to the preparation and storage of solutions along with a little experience and care will result in very satisfactory determinations. The determination of hydrogen-ion concentration has afforded the explanation and elimination of many troublesome and undesirable reactions in pharmaceutical products. There is at present a rapidly growing list of pharmaceuticals that are being held to a very definite acidity or alkalinity, and there can be no doubt that they deserve a preference.

ELI LILLY AND COMPANY, INDIANAPOLIS, INDIANA.

CARE OF ANIMALS FOR BIOLOGIC ASSAYS.

(Concluded from p. 442, May JOUR. A. PH. A.)

BY PAUL S. PITTENGER.

THE ALBINO RAT.

Feeding.—The albino rat thrives on almost any food utilized for human consumption. The more varied the diet the more vigorous the rats. The common

foods which have been found to be acceptable to rats include fresh and powdered milk, whole wheat, whole wheat flour, cracked corn, rolled oats, corn meal, hominy grits, dried peas and beans, egg powder, salt, practically all kinds of vegetables and fruits such as oranges, apples and pears.

The mixed "scratch feed" supplied by poultry dealers for chickens contains a variety of food substances such as wheat, oats, cracked corn, barley, kaffir, milo, buckwheat and sunflower seed upon which the rats thrive. "Poultry Growing Mash" is also a very satisfactory food for rats. The more common brands contain dried buttermilk, oatmeal, corn meal, ground oats, wheat middlings, wheat bran, meat scrap, bone meal and fish meal.

Although a variety of food seems preferable, a mixture of Whole Wheat Flour 2 pounds, Powdered Whole Milk 1 pound and Sodium Chloride $\frac{1}{2}$ ounce includes all the necessary elements for sustaining life. This mixture is satisfactory for normal growing and adult rats, lactating females and young pups. The animals remain thrifty, grow rapidly and can be fed indefinitely upon this mixture alone.

Breeders and others who maintain large colonies should use more varied diets. Complete instructions for the preparation of varied diets have been published by Greenman and Duhring.¹

Water.—Fresh clean water should be available at all times. The rats will quickly foul water exposed in open vessels. Some breeders, therefore, supply water only in drinking fountains while others maintain that the rats keep themselves much cleaner and the odor is less marked in the colony house if the water is supplied in large open dishes in which the rats may bathe. If the latter method is used the water should be changed twice daily. The large open water container seems preferable in summer as the rats bathe frequently and apparently appreciate the cooling effect of the evaporation of the water from their bodies. This is demonstrated by the fact that the nursing mother will oftentimes quickly dip each of her pups in the water and carefully replace them in the nest. If the tops of the cages are made of wire screen the adult rats will oftentimes take a bath and then hang from the top of the cage by their teeth until dry. In extremely hot weather a small cake of ice placed in the water container is of value. The rats nibble off small pieces and seem to enjoy the procedure as heartily as all small boys enjoy taking small pieces from the neighborhood ice wagon.

During feeding experiments with known or unknown vitamins the water *must* be supplied in fountains. In such experiments only *distilled* water should be used and a few drops of tincture of iodine should be added to each litre to prevent the growth of bacteria, wild yeast, etc. Ordinary drinking water may contain extraneous mineral salts, bacteria, algae, wild yeasts and vitamins which may entirely upset the entire feeding experiment.

Handling.—"As in the case of other domesticated animals, it is not sufficient merely to feed and water albino rats and keep their cages clean."

"Individual attention, shown by handling and petting, is essential for the best growth of albino rats and for securing uniform reactions when used as research animals. They should have ample opportunity to know their caretakers.

¹ Greenman and Duhring, "The Albino Rat," 48-55.

Contentment soon replaces fear when they are placed in cages where they may receive some individual attention and where they may observe the various activities of the colony house, become accustomed to the noises of the place, and hear the voices of those engaged in the work of the colony. Under such conditions they eat and assimilate food in a more satisfactory manner. The absence of fear permits them to feed with pleasure and to digest food with none of the inhibiting influences of nervous tension which tend to restrain digestive secretions. Under such conditions, fertility is markedly increased."

"In handling rats, especially when they are unaccustomed to such treatment, one should approach the animals very slowly and quietly, making no quick or unexpected movements."

"Do not pick up a rat by its tail if this can be avoided."

"Grasp the rat gently about the body with its head towards the palm of your hand, closing the fingers under the abdomen, with the little finger under the rat's jaw. Any attempt of the rat to bite you may then be thwarted by closing your fingers firmly on its throat."

"Once held in your grasp for a moment or two, the rat will relax and very soon become quite contented in your hands. After such daily treatment for a brief period the rat will display no fear and will be quite satisfied to be handled. Albino rats are likely to exhibit greater fear when approached in the darker retreats of their cages. They seek shelter in such retreats and resent any attempt to remove them. The same animals may be approached with less risk of offense when they are out in open space." (Greenman and Duhring.)

Breeding.—If only occasional vitamine A assays are made it is preferable to buy the necessary animals from breeders who maintain large colonies. This is necessary in order to meet the U. S. P. requirements as to age and weight. Laboratories in which all kinds of vitamine and dietary deficiency experiments are conducted find it more satisfactory to breed their own animals in order that they may be certain as to the animals belonging to the same litter, the exact age of the animal, etc. For those who wish to breed their own animals the following information is of value:

The span of life is about three years.

The female mates when about eighty days of age.

The period of gestation is from twenty-one to twenty-three days.

The female may mate within two days after casting a litter.

One female may produce one hundred pups in a year.

Brothers and sisters may be mated with no unfavorable results.

The female usually ceases breeding when about 450 days of age.

Healthy females average seven to eight pups per litter.

One male may be caged with two or more females.

Heat or rut takes place every five days.

The male should not be removed from the cage when the female becomes pregnant or after the litter is born.

The nursing litter should be removed just before the succeeding litter is born.

Diseases.—The most common diseases of the albino rat are pneumonia, middle-ear disease and various deficiency diseases.

The deficiency diseases may be cured by supplying the proper diet. The best remedy for the so-called pneumonia is hot cocoa administered every hour by means of a pipette.

Rats suffering with middle-ear disease usually carry the head turned slightly to one side. All such rats should be eliminated as they do not represent average normal rats and are, therefore, unsatisfactory for most research experiments.

Parasites.—Fleas and lice are the most common of the parasites which annoy rats. They should be treated the same as described under Cats.

ROOSTERS.

(Cockerels and Cocks.)

A *cockerel* is a male fowl less than *one* year old while the term *cock* is applied to a male fowl one year or more old.

There are many breeds of cocks only one of which (the White Leghorn) is official for Biologic Assay purposes.

The leghorns belong to the Mediterranean class which had their origin in countries bordering on the Mediterranean Sea. There are eight varieties of Leghorns, as follows: Single and Rose Comb White, Single and Rose Comb Brown, Single and Rose Comb Buff, Single Comb Black and the Silver.

The official cock for U. S. P. ergot assays is the *Single Comb White Leghorn*, less than eighteen months of age, and weighing approximately two Kg.

The Leghorns were originally imported by America from Italy.

Housing.—For *cocks kept indoors* the cages shown in Fig. 7 are very satisfactory both for housing stock fowl and for caging during the fasting and test periods. Two units of the cage, without the partition between are used for housing each five cocks. Two roosts are placed across the end of one of the units. This allows sufficient room for exercise between tests. In order to prepare for the fasting period all of the cocks are placed in the one unit and the partition inserted between it and the other unit. The tray is then removed, emptied, washed and about $\frac{1}{4}$ filled with clean sawdust. The partition is then withdrawn and the cocks driven into this unit. The partition is replaced while the tray of the other unit is emptied and the sawdust renewed. The partition is then removed. By this process the possibility of the cocks obtaining food from the bedding is entirely eliminated. During the fasting period the mash hoppers are closed. If only one or two of the cocks are to be injected at a time the partition is again inserted in order that those injected may be separated from the uninjected ones which greatly facilitates their removal for observation during the test period.

For *cocks kept outdoors* it is necessary to build some type of poultry house. The location and design of the poultry house and its construction determine in a large measure the condition of the cocks. A loose, dry, sandy, quickly draining soil is to be preferred. Avoid if possible heavy clays or land where rain water stands in puddles.

Bio-assayists, however, usually have practically no choice as to where they can keep their fowls. It is in most cases the college or factory yard. The principle condition, therefore, is the proper construction of the house. The essential requirements are comfort to the fowls and convenience for the attendant. It should be dry, cheerful, well ventilated, free from drafts, warm in winter, cool in summer, and easy to clean and keep in a sanitary condition.

Cocks have a relatively high body temperature (106° to 108°) and are, there-

fore, very sensitive to drafts. The best type of house for preventing drafts have the back and two ends closed with a properly constructed open-front. The open-front insures sunlight which in warm climates can be left open all the year and in cold climates the openings may be fitted with frames covered with burlap or unbleached muslin. This allows free passage of air but breaks up drafts.

The *floor* may be of concrete, cinders, earth or boards and should be a foot or more above the ground level to insure dryness. Dryness is one of the first essentials of a poultry house. Cold wet floors result in a very humid, unhealthy atmosphere. Cement floors should be covered with 4 to 8 inches of dry litter.

Sunlight is essential and it is important, therefore, that the house contain windows, preferably in the south side.

Eight to twelve square feet of floor space per bird should be allowed in small flocks and not less than four or five square feet per bird in larger flocks. Where birds are kept in cages in a large animal house the space per bird may be materially reduced.

Roosts.—About 50% of the cocks' time is spent roosting and it is, therefore, important that the roosts be properly constructed. Very often the mistake is made of constructing roosts of lumber 1" by 1" or 1" by 2". Roosts should be made of 2" by 2" or 2" by 4" material with the upper corners slightly rounded.

Feeding.—Cocks for ergot assays whether housed in cages indoors or kept in outdoor houses should be fed mixed scratch feed, growing mash with buttermilk, greens, charcoal and grit. These materials may be obtained from dealers in poultry supplies. The *growing mash* contains dried buttermilk, feeding oat meal, corn meal and ground oats, wheat middlings, wheat bran, meat scrap, bone meal, fish meal. The *scratch feed* contains wheat, oats, cracked corn, barley, kaffir, milo, buckwheat and sunflower seed.

The growing mash, charcoal and grit should be before the cocks at all times except during the starvation period immediately preceding each assay. These materials should be placed in 3-compartment *hoppers* constructed as shown in Fig. 8. Hoppers for outdoor houses should be provided with a drop lid which should be closed during the night. Open mash hoppers attract rats which will consume large quantities of mash.

The *function of the mash* is to supply proteins and fat in a form which is quickly and easily digested. When feeding dry mash always keep the supply of drinking water close to the mash hopper.

The *function of the scratch feed* is to supply carbohydrates. These supply body heat and induce the cocks to exercise. The scratch feed should be scattered in the litter so that the birds will exercise in searching for it.

Green food should be part of the daily diet of all caged or housed birds. Grass clippings, oat sprouts, celery tops, alfalfa, young wheat, clover, red beets, etc., are very acceptable.

Grit is a valuable aid to digestion. Cocks have no teeth and the grit is necessary in order that it may aid in grinding up the food in the gizzard. Charcoal has been found to be a valuable or perhaps necessary adjunct to the diet of fowls.

Feeding Methods.—The quantity of feed to be given depends largely upon the appetite and number of the flock. In the morning only sufficient scratch feed should be scattered in the litter to induce the birds to come down off the roost

and keep them busy for an hour or two. If fed too much in the morning they will sit around for many hours while the feed is slowly digested.

If greens are to be fed give about noon and open the mash hoppers so they may help themselves during the afternoon.

Late in the afternoon give sufficient scratch feed so that each cock will go to roost with a full crop. If a little grain remains in the litter, it will be scratched out early in the morning and keep the cocks busy until feeding time.

Moisture.—Although the house must be free from dampness a small percentage of moisture must be present or the birds will suffer from their legs becoming too dry. When they are kept in indoor cages, it is advisable, once a day, to slightly dampen the sawdust with water by means of a small sprinkling can such as used for watering flowers.

Beak and Nails.—As stated in the introduction, the beak and nails of caged birds will sometimes become so long and curved that it is difficult for them to walk or eat. It is necessary, therefore, at regular intervals to clip the beak and nails with bone clippers.

Water.—A supply of fresh water should be kept before the birds at all times. It should never be given in open dishes or hoppers. One of the commercial sanitary water containers should be employed. Open water dishes and hoppers are one of the greatest sources of spreading disease.

Sanitation.—If the cockerels are to be kept free from parasites and disease the most sanitary conditions must be maintained. Rational sanitary measures must be taken to prevent or delay the appearance of disease, to limit its spreading and to eradicate a disease which has already gained foothold. Cages should be cleaned at least semi-weekly. The trays should be scalded and washed with an antiseptic solution. The entire cage should be steamed or washed with an antiseptic solution at least once a month. Outdoor houses should be sprayed. Tray supports in cages and all cracks and crevices in houses should be sprinkled with a mixture of Sodium Fluoride 50% and Sodium Borate 50%.

Parasites.—Parasites by their irritation and blood sucking lower the vitality of the cocks and make them more susceptible to disease. The common external parasites which infest cockerels are *lice and mites*. Lice usually live, breed and have their complete cycle on the bird whereas mites usually originate elsewhere and go to the bird for feeding. When found dust the birds with a mixture of Talcum 60% and Sodium Fluoride 30%. Disinfect and if possible sterilize all cages or houses. The author has been able to entirely prevent infection by using cages as shown in Fig. 7 which are sterilized once a month with live steam applied by means of a hose.

Scaly Legs is a condition common in some localities so called because of the accumulation of scales or scabs upon the legs. This is a form of scabs caused by the parasite *Sarcoptes mutans, variety gallinae*. This condition should be treated by soaking the scabs with warm water and removing as many as possible by means of a small brush. Apply antiseptic solution. Caged birds if kept under proper conditions are seldom troubled with the internal parasites such as round worms, gizzard worms, gape worms and tape worms.

Diseases.—Cockerels are susceptible to the following diseases: The non-contagious diseases include obstruction of the beak, pip, sore mouth, diseases of the crop, limber neck and colitis. The contagious diseases include blackhead, fowl cholera, white diarrhea, tuberculosis, chicken-pox and roup.

In bio-assaying laboratories cocks are used only for ergot assays. The birds are, therefore, of little more value than their purchase price. It is usually more advisable, therefore, to discard afflicted animals than to try to cure them.