XXVI. THE TECHNIQUE OF BREEDING RATS FOR FEEDING EXPERIMENTS.

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(Received February 19th, 1923.)

Introduction. The importance of good stock for experimental purposes cannot be exaggerated. That control material should be identical with that used for experiment is axiomatic. The elimination of variations due to disease or nutrition is of such value to the experimenter on growth problems—so fundamental to his work—that we venture to publish the means we have found the best for producing good material. The work was begun by one of us (E. C. M.) in the Biochemical Laboratory in Cambridge; carried on by another (V. H. M.) in the Animal Nutrition Department at Lever Bros. and the experience gained¹, grafted on that of the third (G. A. H.) and brought to its present development by her at King's College for Women (Social and Household Science Department). Our observations and experiments have extended over more than six years.

That the present technique produces large animals is seen from the curves of growth of the animals compared with Donaldson's curves (Figs. 1 and 2). This size is not due to fat alone for it starts from birth. Nor does it interfere with reproduction, for the animals are prolific and have large sized litters. Only in the winter months does the number per litter fall below six and that rarely. In the height of the two breeding seasons it is often over twelve. Yet the mother rat is able to bring up all the litter with a high average weight (*e.g.* with 9 in litter the average weight at the 21st day is 27.6 g.; 11 in litter, 28 g.; 12 and 14 in litter, 24 and 25 g. Donaldson gives 21 g.). The animals are almost free from intercurrent disease—a fact readily acknowledged by those who take our surplus animals. Most important is that the growth curves for any one time in the year are nearly constant. The animals show the marked vivacity, the prompt reaction to auditory stimulus and the glossy thick coats of healthy rats.

Stock. The original plan* adopted was to buy mixed stock from the dealers, keep it under observation for some time and then to breed from the does, using large and powerful bucks. This we now consider unwise. The mixed

¹ Based as often as not on the results of mistakes as on expectations justified.

* Reliance on which together with too great an eagerness to get on with nutrition research before the stock was stabilised produced effects in the animal house at Spital which occupied much of the time of the successor of one of us in eliminating.

Fig. 1. Growth curves of suckling rats.

Open circles represent daily weight of rats bred by methods given in this paper.

Closed circles represent daily weight of rats as given by Donaldson.

* Animals began eating for themselves.

stock from dealers is often infected with disease which does not show up early in life and is almost impossible to eradicate. Long experience in our present laboratory (K. C. W.) backed by the similar experience of Dr C. E. Dukes, to whom we owe some of our stock, convinces us that the best way to proceed, is to begin by breeding from one or more pairs of adult animals, which have been obtained from a good source (*e.g.* another researcher) when quite young and kept on a good diet under strict observation. *Further animals should be obtained by inbreeding.* There is no evidence that inbreeding is of any danger

Fig. 2. Growth curves of male rats.

Closed circles are weights taken from Donaldson's book.

Open circles are weights of rats bred by methods given in this paper.

when the original stock is good. In fact inbreeding is of advantage in stabilising the stock and cutting down variations. We should be diffident in putting forward the value of inbreeding so prominently, were it not for the experience of the Wistar Institute lasting over several years, which confirms us in our impressions.

Coat colours. For preference we use black and white piebald rats as being most certain in temper and best in rearing young. Pure albinos rank next. They are less certain in temper and are not so good in rearing their first litter. When they are good mothers they are very good indeed. Other coloured rats are much more uncertain, the greys most of all. With black and white piebald rats brought up from birth or weaning by hand, so as to be used to handling, it is very rarely that one gets bitten. Whites sometimes bite when they have a litter, but much depends on how they have been handled in the past. Irish *a* and *b* rats frequently bite and the grey-piebald and self coloured have to be handled with care.

Coat colours influence the gross metabolism of the animal. In hot humid weather the whites do better than piebald black and white, and these, in turn, better than Irish *aa* and *bb* and self blacks. Such weather affects the growth and the tempers of rats very adversely. Fortunately it is not common in England.

In choice of coat colours it is well to eschew greys, self colours, Irish animals and fancy colourings and to depend on black and white piebalds or on albinos. The black and white piebalds are easier to handle and are more certain; the albinos stand heat, especially humid heat, better.

It is of advantage in experimentation to have animals variously marked. For instance in feeding experiments it is common to place pairs of the same sex in a cage together, for they like company. To know them apart it is convenient to have one albino and one piebald together, otherwise we must distinguish them by some artificial mark—ear piercing, tail clipping or what not. One of us used a simple Mendelian trick for getting equal numbers of piebald and albino rats. “Impure” piebald rats are crossed with albinos, with the result that 50 % of the offspring are albinos and 50 % piebald.

Housing. Probably most of the failure in rat breeding is due to housing. The first thing of importance is *constancy of temperature of the surroundings*. We try to keep the mean temperature 65° F. with no greater variation on either side than 5° F. Consequently we prevent, if possible, the temperature from rising above 70° F. or falling below 60° F. The animals will not breed at a low temperature. High temperature affects them adversely. Disease, especially pneumonia, is common if the temperature be not regulated. A north lighted room without great window space enables the temperature to be kept more constant. American paraffin oil stoves have been used to supplement the radiators at night with no observable detriment to the rats. Whether the duller light of a north-lighted room is an advantage or not we are unable to say. Many consider that the rat when normally fed does better in the dark than in the light. The problem of caging the animals is not difficult. Undoubtedly the animals prefer wood. It is warmer and more comfortable. But it has drawbacks in that it is more difficult to keep clean and it harbours parasites. Moreover rats are destructive and often gnaw wooden cages to pieces. Parasites, however, particularly that of scab, are easily dealt with as

described below. Some people believe that rats cannot live in metal cages. This is not true. One of us has reared generation after generation from birth to adult life in metal cages.

The present practice (G. A. H.) is to keep the pregnant and lactating does in separate individual wooden cages, $9'' \times 9'' \times 9''$, till it is time (21 days after birth) for weaning the young. Adult animals are kept in metal cages $2' 0'' \times 1' 6'' \times 1' 6''$. Not more than twenty are allowed in each cage, with three or four bucks to twelve or sixteen does. The growing animals are placed in proportionately smaller metal cages of smaller mesh. For the very young, square, and not oblong, wire meshing is used for the rats can climb on it and take exercise. A photograph of the latest design, with a platform to serve as "bedroom" is given (Fig. 3). For metabolism experiments the old round metal rat cage, opening at the side, has been discarded, for a square one on legs opening from above.

Fig. 3. Cage for adult rats $2' \times 1\frac{1}{2}' \times 1\frac{1}{2}'$. A. Tray in which the cage stands and forming its bottom. B, B. Palisade to prevent scattering of food and sawdust. C. Platform or "bedroom" on which the rats sleep. D. Drinking trough. E. Roof of cage, both sides shown open. The cage was made to our design by Messrs Baird and Tatlock (London), Ltd.

Disease. Some diseases among rats are regarded as inevitable by most researchers. Scab is an example. This disease is due to a parasite which burrows under the skin of the ears and tail. It may, unfortunately, be transmitted from animal to animal via wood. Infected shavings, wood wool or wooden cages hand on the disease. On this account one of us (V. H. M.) completely gave up the use of wooden cages and burned any shavings, etc. which had come in contact with infected animals. But we have found that wooden cages can be used and yet the disease stamped out.

The disease is easy to cure. The ears and tail of an infected animal are carefully anointed with the following mixture:

Spirit of tar	$1\frac{1}{2}$ parts
Olive oil	8 "
Paraffin oil	1 part

Add flowers of sulphur till the ointment is thick.

Instead of spirit of tar, creosote, in much smaller amounts, may be used and the other constituents may be varied in proportion with little harm.

Two days of application of this ointment should eliminate the disease from the animals. From the wooden cages we have got rid of the parasite by painting them within and without with a thin alcoholic solution of tar. Plenty of time was given for the alcohol to soak into the wood. Cages, disinfected thus, never harbour the parasite. In spite of severe infection prior to September 1920 when the trouble was first seriously tackled, no scab is

ever now seen, nor has it in the past ever recrudesced. Approximately 600 litters have been reared in wooden cages and no case of scab has been seen. *Scab is easily avoidable; in a well run animal house it should never occur.*

Mange, when it has occurred, has been treated with the same mixture successfully. Stock from animal dealers is likely to be mangy as well as scabby. Cure has been effected in a few days by the use of the ointment and the disease has not recurred. A loss of hair, which might be taken for mange, but is connected with diet, has been observed by one of us (G. A. H.) and will be reported on later. The common parasite, the louse, seen on elderly and sick animals, is best treated with flowers of sulphur.

An infection of the urogenital tract in does has been seen by two of us in the past in animals bought direct from a dealer. It causes a great deal of trouble. Attempts on our part, and on the part of the successor of one of us (Mr A. D. Stammers), to stamp it out, were unsuccessful. As none of the original stock, from which our present animals have been bred, suffered in that way, the disease is never seen. We should advise that any animals of unknown origin imported into an animal house, should be kept in pairs in quarantine until they produce litters, and that the litters be quarantined till they reach puberty.

Internal ear disease is sometimes seen. The animal holds its head on one side and later often starts circus movements. Such trouble seems irremediable. No correlation of it with inbreeding has been observed. It used to occur when the utmost pains were taken to secure cross-breeding. It occurs no more frequently now that the animals are intensively inbred. About twenty cases have occurred, in the last two years, in a colony which has never fallen below 300, and in summer is much larger.

Pneumonia is frequently seen. In fact it is the great danger in the rat house. The main factors affecting its incidence are temperature of the surroundings and diet. Since the surroundings of our animals have been made more equable in temperature and free from draughts; since the animals have had good food, pneumonia has greatly decreased. Outbreaks are usually referable to some unforeseen drop in temperature or a period when the "kitchen scraps" upon which the stock are fed contained too little protein.

The fact that diet causes a very appreciable difference in the frequency of the disease, makes it almost inevitable that during long-continued experiments on deficient diets, pneumonia will occur. Consequently, when possible, animals undergoing a long feeding experiment should be housed in quarters different from those of the stock. (This problem has not arisen in our present animal room, in any acute form, as the majority of the experiments have lasted three weeks only, and the diet has been adequate for every purpose except lactation.)

On the whole it does not appear to us worth while to quarantine any sick animal and try to nurse it back to health. Some vigorous, well-grown animals may recover from an incipient catarrh when quarantined and put on a good

diet. But usually the experiment does not succeed. The animal may recover temporarily, but usually succumbs again when returned to the stock cages. (This opinion must not, of course, be taken to apply to scab which can so easily and permanently be stamped out.)

Breeding. The does reach puberty at about the 90th day. On special diet they may reach it earlier. But it is not considered wise to allow impregnation till about the 6th month, when they have attained their adult stature. The present plan is then to place 16 females in a large cage with 4 well grown males. Other signs besides an increase in the size of the abdomen may help the inexperienced to judge of pregnancy, such as rapid increase in weight, uncertainty of temper, squeaking on handling, tetanic movements of forefeet, and nest building of chance chips and straws. Any of the signs, but not all, may fail. The last two occur very shortly before the birth of the young. Experience is the best guide in judging the stage of pregnancy.

The pregnant does are removed to separate quarters, wooden boxes 9" x 9" x 9", ventilated in front with perforated zinc and opening with a wooden lid from above, when pregnancy is well advanced. If the does have been used to being handled from early days (birth or weaning) by a person whom they get to know, there is no difficulty in that person's handling them when they are pregnant or after the birth of the litter. Nor should the handling of the litter result in loss. Ordinarily, mothers are apt to kill and eat their young if disturbed within ten days after parturition. But when they are used to being handled by any one person from weaning, they do not resent that person's handling them or their young. Experiments which one of us (G. A. H.) has been conducting now for some years, have necessitated the daily weighing of mothers and litter from birth. Over 600 litters have been handled and it has been rare indeed to have a casualty. The main breeding seasons are spring and autumn.

The young are usually eating for themselves by the 18th day and can readily be weaned on the 21st day. The best all-round diet for the mother while the young are dependent on her, is bread and milk. No such curves of growth and no such general fitness have been seen on other diets except bread, "food casein" and egg yolk; bread, "food casein" and whey; and bread, "food casein" and excess marmite.

After weaning, the general practice is to put twenty youngsters, with perhaps one adult male as foster parent, together in one of the cages with smaller squared mesh, promoting them to larger cages as they grow. The sexes are separated early. The diet given is bread and milk supplemented with kitchen scraps. Care has to be taken with the kitchen scraps lest they contain too high a percentage of vegetable material and not enough protein and fat. The best kitchen refuse for feeding purposes contains meat, milk puddings and fish. If it is noticed that the quality of the kitchen scraps is poor, it is usual to supplement it with fresh herrings or some cheap source of animal protein and fat on the market. Animals fed on this diet grow large

and healthy and produce large litters. Adult does under 200 g. rarely occur (when they do, they are used for histological purposes) and animals of 300 g. are frequent. The males sometimes weigh 400 g.

A final point is that personal supervision every day by the researcher who is using the animals is almost essential. However good paid assistants are, it has been our repeated experience that animals do better when the person in control of the experimental work sees them and works in the animal room every day. Daily supervision, with an inspection of every animal in the animal and experimental rooms at least twice a week, should be the aim of anyone who wants good animal material upon which to work.

Summarising our experience we would say that good rat stock may be best obtained by

- (i) housing at an equable temperature of 65° F. with no more than 5° variation in either direction;
- (ii) feeding on diets containing bread and milk and kitchen scraps with a high percentage of protein and fat;
- (iii) inbreeding from originally good stock using does of six months old;
- (iv) feeding the mothers during lactation with bread and milk;
- (v) inspection and handling of stock from birth.

Thanks are due to the Medical Research Council for grants defraying the cost of the majority of the experiments in preparation for which the above experience has been gained and to Messrs Lever Bros. Ltd. for permission to publish anything bearing on work done for them.