

## FEEDING AND BREEDING OF LABORATORY ANIMALS

### I. RAT AND MOUSE CUBES, AND CUBE CONTAINERS

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(With 7 Figures in the Text)

#### INTRODUCTION

A general review of the methods of feeding and breeding laboratory animals is in progress at the National Institute for Medical Research, and the present series of papers is designed to record any conclusions which may be of general interest. As regards food and feeding, the object has been to increase standardization and efficiency, to decrease cost, wastage and labour, and to eliminate diets or ingredients which are variable, inadequate, difficult to obtain, non-keeping, or unduly costly. Particular attention has been paid to avoiding the necessity for supplements, which though fashionable are an unmitigated nuisance in the large-scale husbandry of laboratory animals. It has been borne in mind that the cost of feeding an animal should not be taken as the daily cost, but as the total cost of the food required to bring the animal into proper condition for experiment. Thus, where an animal has to grow to a certain size before it can be used, a diet that costs little per day may be expensive in the end if it produces only a slow rate of growth. Such a diet, moreover, decreases the capacity of the animal house and increases labour costs per experiment.

Much attention has also been paid to wastage, which in rat and mouse diets is possible at four points:

(a) When diets are mixed on a comparatively large scale without proper equipment.

(b) When, as often happens, wet diets, which will not keep, are not all used on the day of mixing.

(c) When much of the food put into the cage is wasted by the animal. This seems inevitable when mash or meal in an open container is used in a cage with a floor grid or sawdust.

(d) When the animal eats more than it needs to maintain optimum condition.

#### RAT AND MOUSE DIETS

In 1942 the stock diet of the mice maintained at Hampstead was a daily ration of whole oats and a piece of wholemeal bread soaked in water, without additional water. This diet gave indifferent results and was most unhygienic, owing to the wet bread

being put on to the sawdust bedding. The stock diet of the rats at the same time was:

<i>Full-cream diet</i>	
(diet RBSS 4 of Glaxo Laboratories)	
	%
Wholemeal flour	50
Dried full-cream milk	27
Dried yeast	12
Meat and bone meal	6
Cod-liver oil	3
Mineral mixture*	2

This diet, which was mixed by hand and used as meal in conjunction with a water bottle, gave very good results, but it was extremely expensive and very wasteful. The use of different diets for the mice and the rats was administratively cumbersome, and the unsatisfactory nature of the one diet, and the expensiveness of the other led to a consideration of whether it would not be possible to use a single reasonably cheap labour-saving diet giving good results with both species. It should be noted that the animals at Hampstead are almost all awaiting or under short-term experiments and include few breeding animals, the main colonies of which are maintained at the Field Laboratories, Mill Hill.

A large number of diets suitable for one or other, or both, species have been described or are in use in different laboratories, and the convenience of having stock diets pelleted or cubed is gradually becoming recognized. Thomson (1936) described a cubed diet for rats, which contained a large number of ingredients and which, he recommended, should be supplemented with milk and greens for breeding stock. This diet or a derivative thereof, in cube form, is marketed by the North-eastern Agricultural Co-operative Society. Another cubed rat diet is made by Joseph Thorley Ltd. Before the war a cubed diet was available from the United States, and another, in pellet form, is now advertised. There is an obvious disadvantage, especially under

\* Mineral mixture: calcium carbonate 2400 g., sodium chloride 2400 g., iron citrate 480 g., potassium iodide 13 g.

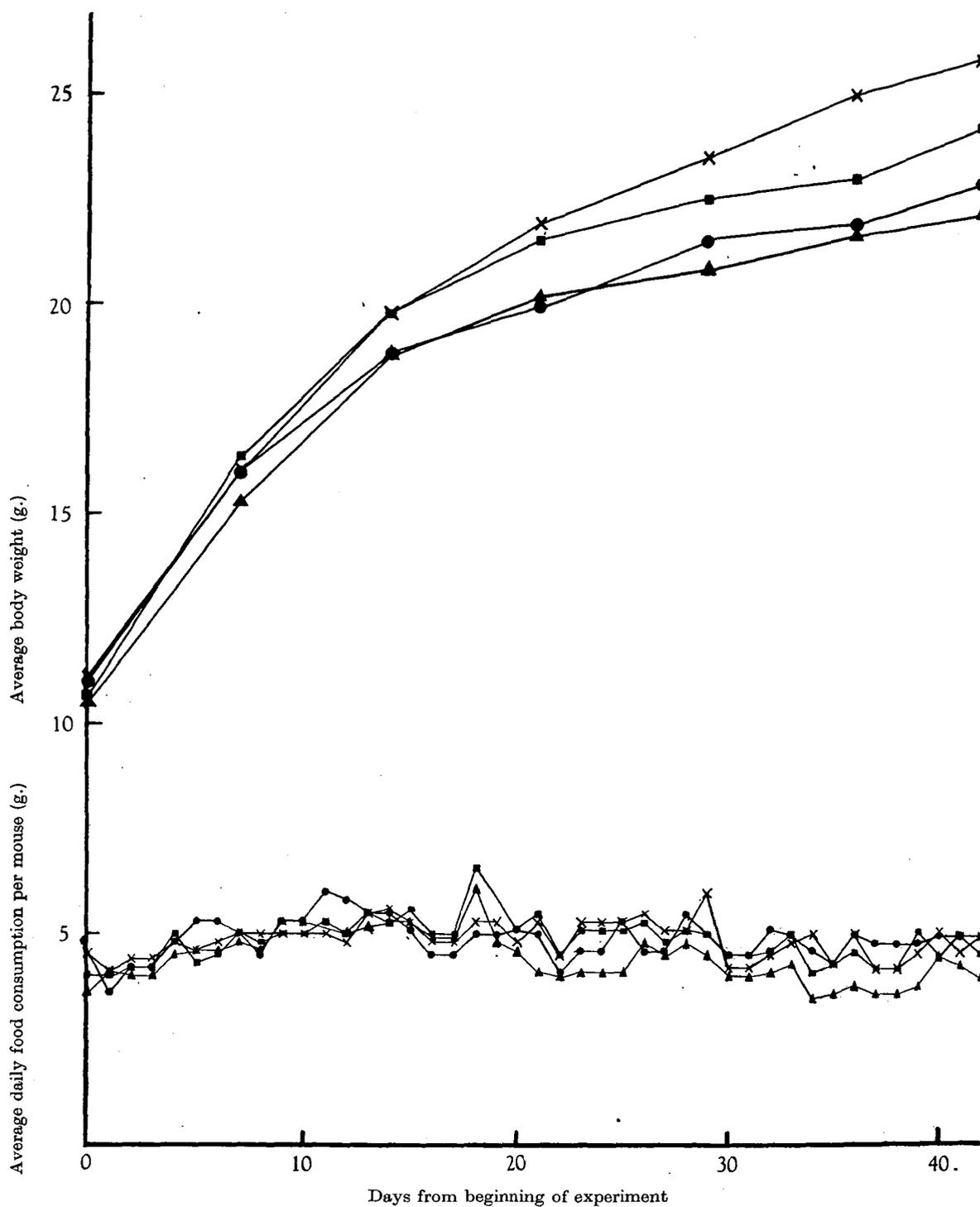


Fig. 1. Growth and food consumption of young male mice on diet 1 cubes. Six mice per cage.  
 ● Cage 1; × Cage 2; ▲ Cage 3; ■ Cage 4.

war-time conditions, in relying on a commercial cube of perhaps variable composition, and it was decided to have a diet especially mixed and cubed for use at the Institute. After consideration of the diets used by Watson (1937) for mice and of those used in several well-known colonies of rats and mice,

A 1-ton batch of this diet was prepared and pelleted by Messrs J. and H. Robinson Ltd. Subsequent batches in which the cod-liver oil was reduced to 2%, and the minerals simplified to 1% sodium chloride and 1% calcium carbonate, were cubed. This diet has given excellent results on the

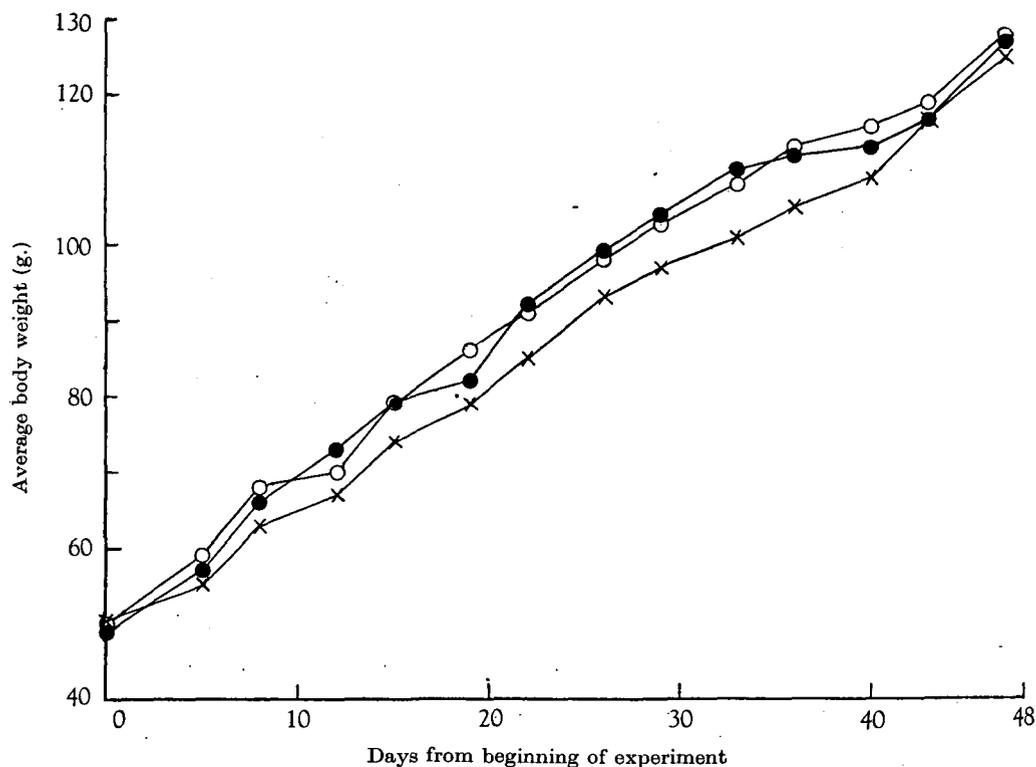


Fig. 2. Growth of young male rats on diet 1 and on full-cream milk diet. ○ Diet 1 (pellets), 30 rats. ● Diet 1 (ground pellets), 28 rats. × Full-cream milk diet, 29 rats.

a. trial was made with a diet of the following composition:

	%
Diet 1. Rolled or ground oats	30
Wholemeal flour	35
Dried yeast	5
Dried skimmed milk	15
Meat and bone meal	10
Cod-liver oil	3
Mineral mixture*	2

This formula gave a theoretical digestible content of:

	%
Protein	16.9
Oil	6.3
Soluble carbohydrate	44.4
Fibre	1.1

Mineral mixture as for full-cream diet.

growth and maintenance of rats and mice and is now made for us in batches of 5 tons. For some reason not yet determined it is not suitable for breeding stock. It may be noted that for cubing or pelleting by commercial methods, a total oil content of 3-4% is necessary, and that the size of the commercial machines is such that it is highly uneconomical to process a batch of less than 1 ton. The final water content of batch 1 was about 7%.

*Growth of young mice and rats on diet 1 compared with that on full-cream milk diet*

*Mice.* Diet 1 in cube form was tried on mice in conjunction with a water bottle of the usual type. Four groups, each of five mice averaging about 12 g., were used. The results (Fig. 1) show that a good rate of growth was obtained. Similar results were obtained by Mr Glover in a trial at Mill Hill; in his

experiment the weight increase in young mice maintained on the cubes and water was more than double that of mice fed the stock diet of oats and wet bread.

*Rats.* The first experiment carried out with the pelleted diet 1 on rats was to compare its adequacy for growth with that of the full-cream milk diet.

Diet 1 pellets were then compared with the same diet in cube form, six groups of five rats, averaging 40 g. in body weight, again being used on each diet. After 33 days the supply of pellets was exhausted, but the cubes proved to be equally good and maintained vigorous growth up to 140 g. when the experiment was stopped (Fig. 3). At this stage the

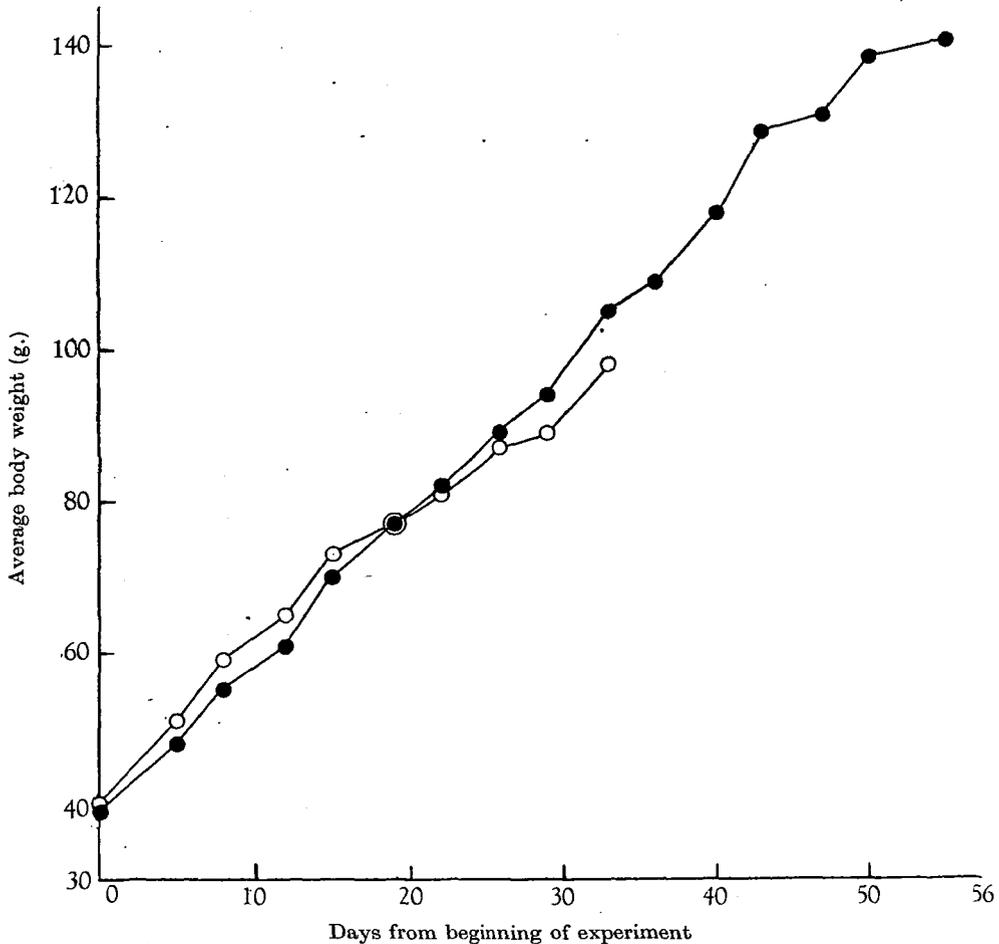


Fig. 3. Growth of young male rats on diet 1 pellets, and diet 1 cubes. ○ Diet 1 (pellets), 27 rats. ● Diet 1 (cubes), 28 rats.

Six groups, each of five young rats averaging 46 g. in body weight, were put on each diet; a similar number were put on ground pellets to control any effect of the form of the diet. At the end of 47 days, one rat on the full-cream diet, and two rats on diet 1 meal had died. The growth curves are shown in Fig. 2, from which it will be seen that diet 1, pellets or meal, gave growth as good as or better than the full-cream diet.

manufacture of pellets for rats and mice was abandoned, since they are more difficult to make than cubes and much less adapted to use in a food basket.

#### CUBE BASKETS

In the experiments recorded above, the diets were fed in open pots placed in the cage. Cubes fed in this way tend to be dragged about the cage and

though not completely lost, as in the case of meal spilt from pots, they are soiled with sawdust and excreta, and those not eaten are often thrown away when the cage is cleaned. Moreover, pots are easily broken. It was therefore decided to investigate the use of wire containers so placed and designed that the animals could not get inside or on top. The wire mesh or bars of such a basket, through which the animals nibble the food, must be so arranged as to make eating (a) sufficiently easy to ensure that the animals get enough, (b) sufficiently difficult to ensure that any partly eaten cube which falls out is finished up before a further attack is made on the basket. Moreover, the baskets, if detachable, must be so designed that they can easily be fitted, removed and filled, and should have a flat bottom so that they will stand upright on a table for filling. It should be explained that the present problem was to design baskets to fit the existing cages which had not been made for this method of feeding. If new cages were being designed it would be possible, though perhaps not altogether desirable, to have built-in cube baskets which could be filled from outside without opening the cage.

*Mice.* Two types of mouse cage are in use for ordinary purposes at the Institute. The first is a box made of solid sheet zinc (11 × 8 × 4 in.), covered with a coarsely perforated zinc lid held in position by a rim. Several different patterns of wire basket, each capable of holding several days' supply of food for five mice, were made and tried out, the one finally chosen being semi-circular in cross-section, 4 in. along the flat side and 3 in. high, hooked through holes in the end of the mouse box at such a height that the lid of the box closed the top of the basket. An experiment was carried out to see whether the use of these baskets had any inhibiting effect on food consumption and, therefore, on growth. Two types of mesh were tried out. The first was  $\frac{5}{16}$  in. square, the second had vertical bars  $\frac{5}{16}$  in. apart with horizontal tie wires 1 in. apart. It was thought that the latter might give greater range of movement to the animal's jaws without greatly increasing the ease with which the half-eaten cubes fell out. In practice the mice (fifteen in each group) were apparently able to obtain fairly adequate rations through either kind of mesh, though both the growth and food consumption were rather greater with the mice fed from open pots. The growth and food consumption curves given in Fig. 4 suggest that there was more waste of diet with the small-square mesh, probably due to ineffective gnawing, and for this reason, and also to decrease cost, the barred type was selected. These baskets hold about 175 g., a supply for 35 mouse days.

The second type of mouse cage was a large flat box 20 in. long, 18 in. wide, and 5 in. high, covered with a wire-mesh lid. With these cages the rim of

the lid fitted too closely and was too deep to allow of baskets being hooked through the side. A basket was therefore designed to stand on the floor in the middle of the cage. This basket had the  $\frac{5}{16} \times 1$  in. mesh selected as the result of the first experiment. It was 4 in. in diameter and had four 1 in. legs so that the container itself had a height of 4 in. The top was closed by the lid of the cage. Two wires projecting 1 in. from the top of the basket fitted through the mesh of the cage lid to prevent the basket being upset. These baskets hold 500 g., or a supply for 100 mouse days. Since the cages usually contain 20–30 mice, one basket needs to be refilled only every second or third day.

*Rats.* Two types of rat cage had to be considered. The first was a wire-mesh cage 9 × 15 × 8 in., with detachable tray, and doors at the front end and at the back of the top. A basket, semi-circular in cross-section, was designed to hang on the inside of the cage close under the roof. It was 4 in. across the flat side and 5 in. high. An experiment was done to compare growth rates of animals fed on cubes (a) in open pots, (b) in baskets of  $\frac{5}{16}$  in. square wire mesh, and (c) in baskets with vertical bars  $\frac{5}{16}$  in. apart and horizontal tie wires 1 in. apart. Three groups of five rats were used for each variation. Similar results were obtained to those with the mice. At the end of 4 weeks the rats with the open pots had grown slightly more than the others, and those with the barred baskets slightly more than those with the square-mesh baskets (Fig. 5). All three growth curves, however, were satisfactory. The barred type of basket was selected for routine use. These baskets hold 300 g. of cubes, a supply for 20 rat days. As the usual complement of these cages is five young rats, the baskets need to be filled only every 2 or 3 days.

The second type of rat cage to be considered was a large wire-mesh run 40 × 15 × 8 in. high. A basket similar in general design to that described for the small rat cage, but larger (6 in. high × 6 in. across the flat side), was designed. This basket holds 500 g., and, as the cage contains up to thirty growing rats, the use of two baskets per cage reduces filling to about every other day.

## FOOD CONSUMPTION

The consumption of cubes fed in open pots can be estimated fairly well if the amounts put in and the amounts remaining in the pot or about the cage as whole or partly gnawed cubes are compared. Estimation of food consumption is much easier when baskets are used, since, if the latter are properly used, there should be no food left among the sawdust.

To test out the suitability of the baskets as routine equipment, food consumption records were

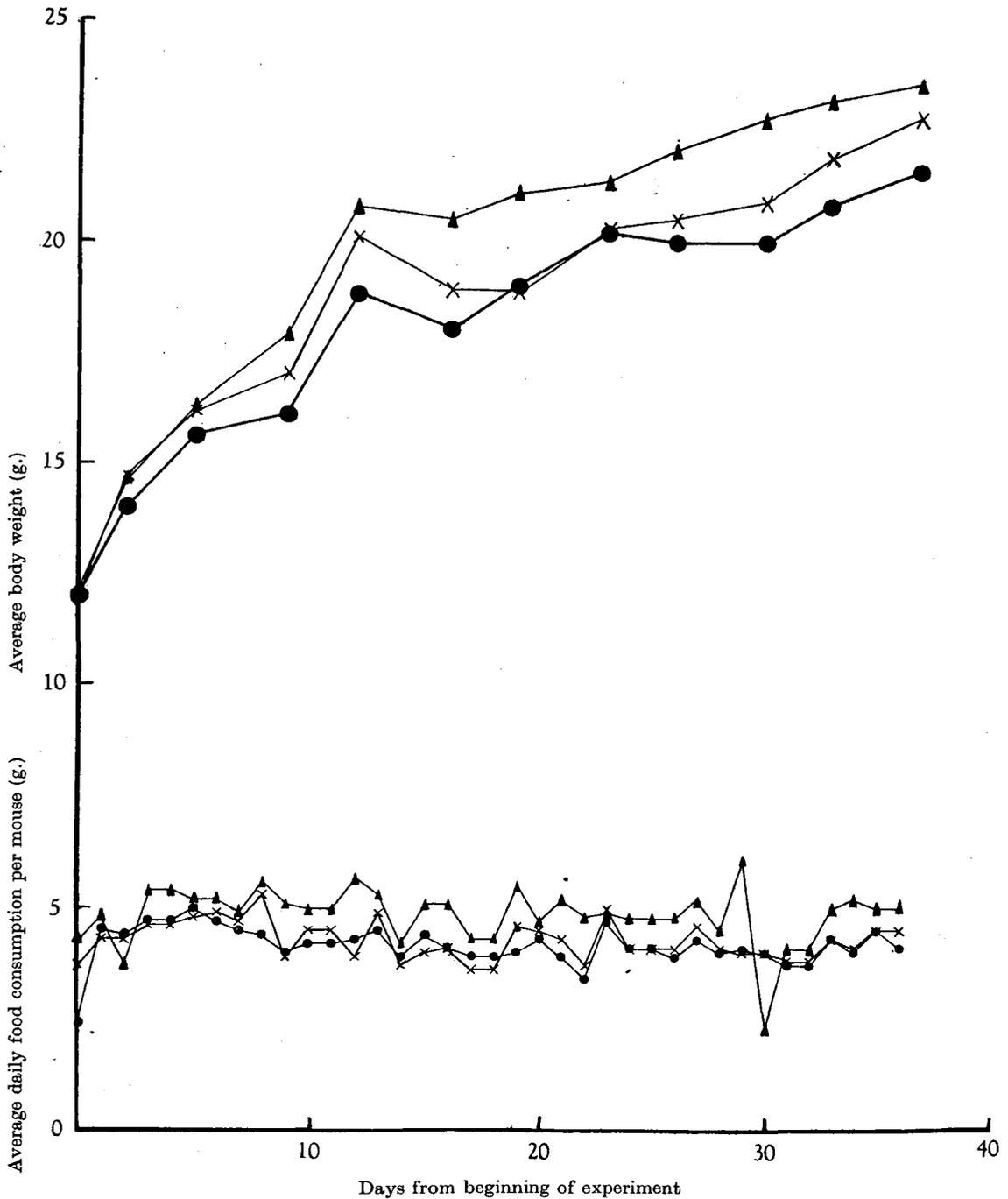


Fig. 4. Growth and food consumption of young mice on diet 1 cubes in baskets and open pots. ● Square-mesh basket. × Barred basket. ▲ Open pots. *Note.* Body weights on the 12th day, and food consumption on the 29th and 30th days, were recorded by an untrained assistant and are probably inaccurate.

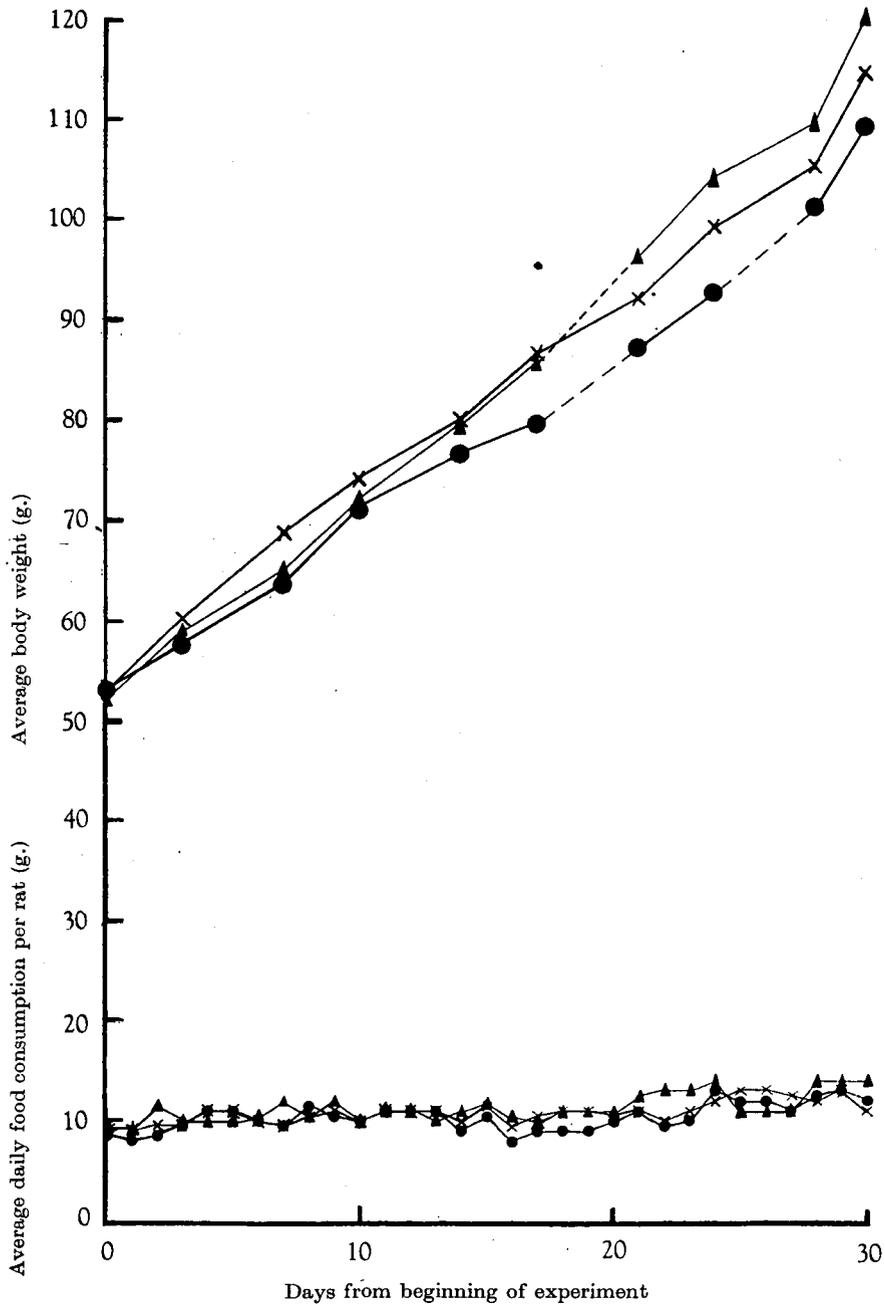


Fig. 5. Growth and food consumption of young rats on diet 1 cubes in baskets and in open pots. ● Square-mesh basket. x Barred basket. ▲ Open pots. Dotted lines indicate the death of one of the group.

obtained in the comparison of square and oblong mesh baskets referred to above.

*Mice.* In a preliminary experiment in which the cubes were fed in open pots, adult male mice were found to consume about 5 g. of cubes per day each. This information gave a basis for calculating a suitable size for the basket, remembering the intention that a basket should hold several days' supply for the number of animals usually housed in the cage. In the next experiment, growth curves of which are given in Fig. 1, food consumption records were kept separately for each of the four groups of

*Rats.* A preliminary experiment showed that small male rats weighing about 70 g. consumed some 12–14 g. of cubes a day when fed in open pots, and that female rats double this size ate but little more. In the experiment on the type of mesh most suitable for the basket, food consumptions were also kept (Fig. 5). These results again show a slightly greater apparent consumption from open pots, but the effect on growth was negligible. As with the mice, there was little increase in food consumption, while the body weight increased to double its initial figure.

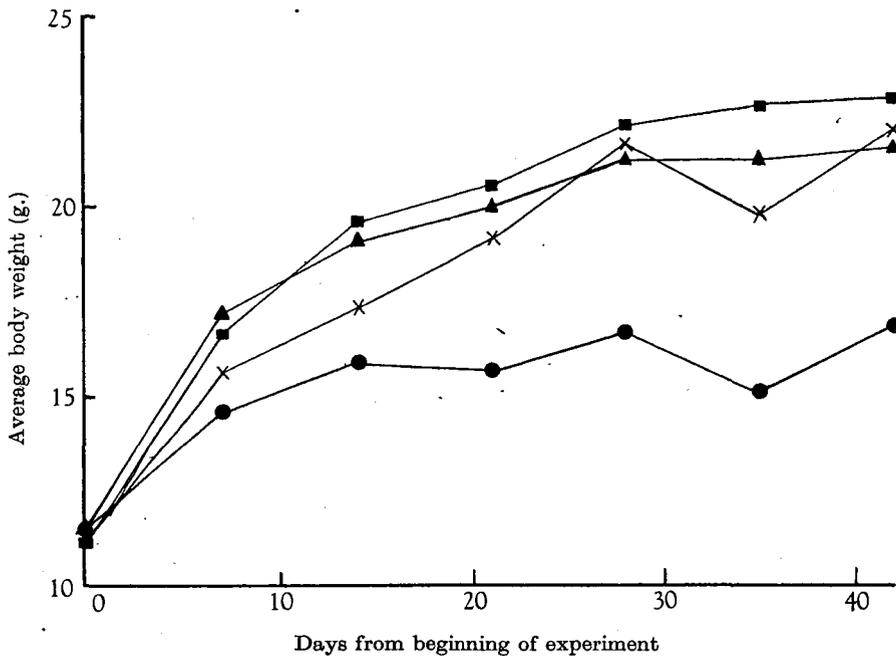


Fig. 6. Growth of mice on limited rations of diet 1 cubes. ● Cages 1 and 5; 2 g. daily (10 mice). × Cages 2 and 6; 3 g. daily (10 mice). ▲ Cages 3 and 7; 4 g. daily (10 mice). ■ Cages 4 and 8; 5 g. daily (10 mice).

mice, and are given in Fig. 1 in comparison with the growth curves. It will be seen that there was little increase in food consumption with increasing size of mice, and that the overall average daily consumption was approximately 5 g. The next experiment related to the food consumption from the two types of basket in comparison with that from open pots (Fig. 4). Here again consumption showed little change with increasing size of mouse. Consumption from open pots appeared to be slightly greater than from either type of basket, but it should be remembered that complete recovery of the residue is much more difficult with the open pots, and that unrecovered residue will appear as consumption in the record. For all types of container the consumption varied between slightly over 4 g. and slightly over 5 g. per day.

#### FOOD REQUIREMENT

The food consumption recorded above seemed to be very large in relation to body weight, especially in mice. It seemed possible that the animals were eating above their requirement, and experiments on limited rations were therefore planned. In each of the experiments the animals were kept five per cage, the total ration for the five animals being put into the cage each day. It was not possible to ensure that the consumption by individual animals in the cage was the same.

*Mice.* Ten young female mice averaging a little over 11 g. were put on each of four diet allowances, 2, 3, 4 and 5 g. per day per mouse, fed in open pots. At the 2 and 3 g. levels the ration was invariably eaten completely. There was occasionally a very

small residue from the 4 g. allowance, and more often from the 5 g. ration. The growth curves of these mice during a 6-week period are shown in Fig. 6. Although only one mouse died, the group receiving 2 g. per day per head showed poor growth, only about one-half the proper increment. This ration is therefore quite inadequate. The remaining groups all grew fairly well and there were no deaths. The mice on the 5 g. ration, however, showed the

ration, 6, 8, 10, 12 and 14 g. per head per day. Growth curves are shown in Fig. 7. The 6 g. ration was quite inadequate and several deaths occurred in the group. 8 and 10 g. were also inadequate to maintain growth after the first 2 weeks, and again there were several deaths. The 12 and 14 g. rations gave good growth curves over the 25 days of the experiment, though the large ration gave slightly the best result. From these various data it may be

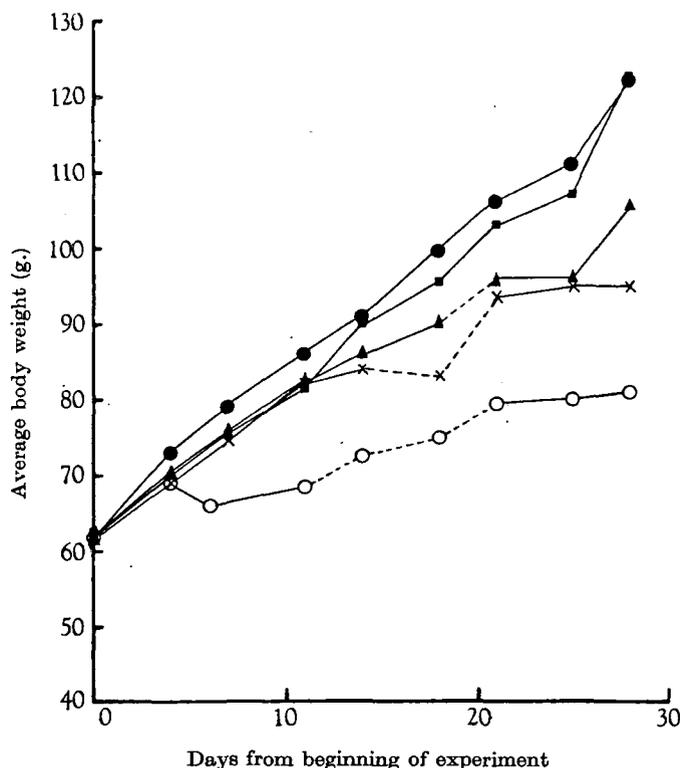


Fig. 7. Growth of young rats on limited rations of diet 1 cubes. O Cages 1 and 6; 6 g. daily (5 rats per cage). × Cages 2 and 7; 8 g. daily (5 rats per cage). ▲ Cages 3 and 8; 10 g. daily (5 rats per cage). ■ Cages 4 and 9; 12 g. daily (5 rats per cage). ● Cages 5 and 10; 14 g. daily (5 rats per cage). Dotted lines indicate the death of one of the group.

best growth curve at all stages. It is noticeable that whereas a 50% increase in ration from 2 to 3 g. gives a marked increase in growth rate, a 66% increase from 3 to 5 g. is nothing like so productive, and is probably partly wasted by excretion. It is evident, none the less, that 5 g. per day per head is not a grossly excessive ration, and this amount has been taken as the figure for general purposes.

*Rats.* A similar experiment was carried out on rats maintained on a restricted diet. Groups of ten male rats (five per cage) were put on each of five

concluded that 12–14 g. is a suitable daily ration for rats of the weight considered. 15 g. might perhaps be taken as an average figure for all sizes of rats. It seems, therefore, that in relation to body weight, the requirement of mice is greater than that of rats.

#### COST OF MAINTENANCE

The cost for food of rats and mice maintained on the cubes can easily be calculated. The present

(June 1945) price of the diet is about £30 per ton, or 7*d.* per kg., so that the daily ration of 5 g. for mice costs about  $\frac{1}{30}$ *d.*, and the 15 g. for rats about  $\frac{1}{10}$ *d.*

#### SUMMARY

1. General considerations relating to the feeding of laboratory animals are discussed.

2. A cubed diet suitable for growing and stock rats and mice is described, together with wire-mesh containers adapted to various types of cage.

3. The consumption and requirement of these cubes by growing rats and mice is recorded.

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