FEEDING AND BREEDING OF LABORATORY ANIMALS

IX. A COMPLETE CUBED DIET FOR MICE AND RATS

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(With 5 Figures in the Text)

INTRODUCTION

Thomson (1936), in describing his cubed diet for mice and rats, stated that green-food and fresh milk were required as supplements. The only other cubed diet commercially available in this country until recently seems to have suffered from a similar disadvantage,* and one of the original objects of the work recorded in this series of papers was the elaboration of a complete diet in cubed form.

In Part I (Parkes, 1946), reference was made to two diets for mice and rats. One of these (diet 1, Table 1) was adequate for growth and maintenance, but inadequate for reproduction; the other (afterwards cubed as diet 20, Table 1) gave excellent reproduction, but was extremely expensive because of the high content of full-cream dried milk. Apart from the differences in the dried-milk content, the diets were dissimilar in the cereal components and in the content of yeast and of meat and bone meal. Prolonged experiments were made to find the reason for the difference in effectiveness between these two diets and to evolve a cheap complete diet for breeding mice and rats. It was soon found that the amount of full-cream dried milk could be reduced (diet 32, Table 1), and later work threw doubt on the need for this constituent. Results suggested, however, that a high wheat or a high yeast content was necessary for a breeding diet (Bruce & Emmens, 1948). These experiments, which included a large-scale test of factorial design, were extraordinarily laborious and time-consuming in relation to the results produced, and made little headway into the large number of possible variations in the nature and proportions of constituents. A simpler approach to the problem was therefore essayed.

Every schoolboy knows that white mice breed effectively on a diet of bread soaked in milk and water and supplemented with whole oats. Such a diet was used successfully from 1921 to 1931 for the albino mice described by Parkes (1926) and maintained by the Medical Research Council since 1932. This simple diet is the basis of the wet mash used in several laboratories. That in use at the Medical Research Council's Farm Laboratories in 1947, for instance, was made of wholemeal brown bread soaked in water, with crushed oats and small amounts of dried skimmed milk and fish meal added. This diet appeared to permit of fair reproduction, although the absence of records for the mass-breeding colonies precluded accurate estimates. A rough calculation was therefore made of the components of this diet and a cubed food was prepared of the composition shown in Table 1 (diet 39). Both the protein and the fat contents of this diet were much lower than those of diets 20 and 32 and somewhat lower

Table 1. *Percentage constituents of diets* 1, 20, 32, 39 *and* 41 *with theoretical composition*

(Calculated from *Bull. Minist. Agric., Lond.*, no. 124.)

	Diet no.				
	1*	20	32	39	41
Wholemeal flour	35	50	58	45	45
Ground oats	30	—	—	40†	40†
Dried meat and bone meal	10	6	6	—	—
Fish meal	—	—	—	11	8
Dried yeast	6	12	12	0·5	1
Full-cream dried milk	—	27	20	—	—
Dried skimmed milk	15	—	—	3	3
Cod-liver oil	2	3	2	—	2‡
Sodium chloride	1	1	1	0·5	1
Calcium carbonate	1	1	1	—	—
Digestible protein	16·9	19·3	18·4	15·0	13·6
Soluble carbohydrate	44·4	45·3	47·8	48·3	48·4
Fat	6·3	11·6	8·9	2·5	4·5
Fibre	1·1	0·5	0·5	1·4	1·4

* The original diet as first published (Parkes, 1946) contained 5 % dried yeast and 3 % cod-liver oil.

† Sussex ground oats were used for diets 39 and 41.

‡ Afterwards reduced to 1%.

* An undated brochure issued by Purina Mills, St Louis 2, Missouri, U.S.A., suggests that matters are more advanced in the U.S.A. For instance, a cubed diet, referred to as laboratory chow, is said to be nutritionally complete for mice, rats, hamsters and dogs. A chemical analysis but not a list of constituents is given for the diet in question.

than those of diet 1. Cubes of this composition were rather crumbly because of the lack of oil, but they were surprisingly effective for breeding mice and rats. When the value of diet 39 was certain, the formula was modified slightly by rounding off percentages and adding a small amount of cod-liver oil to ensure adequate vitamin A and D contents and particularly to improve the cohesion of the cubes (diet 41, Table 1). These modifications reduced still further the amount of digestible protein in the diet, and the fat content though slightly higher than that of diet 39 remained well below that of diets 20 and 32. A calcium determination was made on one batch of diet 41. The content, 0·58 %, was higher than that found adequate by Campbell & Sherman (1945), and indicates an effective surplus after allowing for the phytin of the oatmeal.

This diet, without any supplement other than water, proved extremely effective for growth, maintenance, and for intensive reproduction. It makes a first-rate cube, and requires only small amounts of difficult or costly ingredients. It is now the standard diet of all mice and rats maintained at the National Institute for Medical Research.

TECHNIQUE AND RESULTS

Mice

Mouse stocks and breeding methods were as described by Bruce & Emmens (1948). In a first experiment, diet 39 was compared with diet 32, the highly effective breeding diet described in the above paper (see Table 1). This comparison was made first over a 10-week period but the animals were maintained in a long-term experiment during which most of the females were simultaneously pregnant and lactating; 66 % of the females receiving diet 32, and 69 % of those receiving diet 39 gave birth to six or more litters during the period of test. When the adequacy of diet 39 was established a comparison of diets 39 and 41 was undertaken in a short-term experiment.

Comparison of diets 32 *and* 39. Details of the experiment are as follows:

Number of females per group at start of experiment	24
Test started	19. xi. 47
First litter born	9. xii. 47
Males removed	1. vi. 48
Last litter born	22. vi. 48

(i) Short-term comparison: all litters born during December and January. Females which failed to survive the full period, i.e. until 21. ii. 48 when the last litter was weaned, have been excluded from the results.

Two methods of mating:

Monogamous pairs	1 male 1 female
Polygynous groups	1 male 4 females

(ii) Long-term comparison: polygynous mating discontinued owing to the higher mortality associated therewith, and surviving females mated as monogamous pairs. Females which died before the end of the test have been included in the results up to the month in which death took place, but not for that month.

Results are given in Tables 2 and 3, and Fig. 1. Owing to the great variation in the performance of individual mice the differences between the two diets, although always in favour of diet 39, were not statistically significant.

Comparison of diets 39 *and* 41. Details of the short-term experiment are as follows:

Number of females per group at start of experiment	24
Test started	19. iv. 48
Males removed	8. vi. 48
Length of mating period	50 days

The mice used for this test were collected from the young born during February in the long-term comparison of diets 32 and 39. About two-thirds came from the group receiving diet 39. All were reared on diet 1 and then allocated at random to the various diet groups for the breeding experiment.

Results are given in Table 4 from which it will be seen that there is no difference in the reproductive performance of mice maintained respectively on diets 39 and 41.

Rats

Growth and food consumption on diets 1, 32 *and* 39

Wistar strain rats bred at Hampstead and derived from the colony maintained in the Nutrition Department of the Pharmaceutical Society of Great Britain were used for all the tests. They were mated as monogamous pairs, in wire-mesh cages 9 in. × 15 in. × 8 in., the male being kept continuously with the female as in the experiments with mice. All young born were left with the mother. The diets were given in the food baskets described in Part I of this series. Young females at weaning were sorted into three groups so that each litter provided an animal for each group. Young males were sorted similarly, giving three pairs of groups in all. The groups were then each made up to ten animals by the addition of two non-littermates to the female groups and three to the male groups. The collection of the sixty young rats for this test extended over 3 weeks. The three groups were reared respectively on diets 1, 32 and 39. The animals were weighed once a week. The average growth curves for the litter-mate animals only are given in Figs. 2 and 3. On these results diet 39 must be regarded as of the same order of adequacy as diet 32 for the promotion of growth.

After mating, at about 15 weeks old, the animals were maintained on diets 32 and 41. Details of their reproductive performance are given in the next section.

Table 2. *Comparison on mice of diets* 32 *and* 39. *Short-term*

Method	Diet	No. of surviving females	Total no. of young		Young weaned (21 days) (%)	Average no. of young per litter		Average weight of young weaned (g.)	Average production per female per month for Dec. and Jan.	
			Born	Weaned		Born	Weaned		Young born	Young weaned
Monogamous pairs	32	18	223	189	85	6·8	6·5	8·0	6·2	5·3
	39	18	277	249	90	7·7	6·9	8·4	7·7	6·9
Polygynous groups	32	13	128	93	73	5·3	4·9	7·9	4·9	3·6
	39	15	179	134	75	6·2	5·2	8·1	6·0	4·5
Combined totals	32	31	351	282	80	6·2	5·9	8·0	5·7	4·6
	39	33	456	383	84	7·0	6·2	8·3	6·9	5·8

Table 3. *Comparison on mice of diets* 32 *and* 39. *Long-term*

(Production of young per month in 3rd to 7th months of experiment)

Diet	Month of birth of litters	No. of surviving females	Total no. of young		Young weaned (21 days) (%)	Average no. of young per litter		Average weight of young weaned (g.)	Average production per surviving female	
			Born	Weaned		Born	Weaned		Young born	Young weaned
32	February	23	115	96	84	6·8	6·4	9·5	5·0	4·2
	March	22	139	85	61	6·0	5·3	9·4	6·3	3·9
	April	17	83	64	77	6·4	5·8	7·8	4·9	3·8
	May	13	96	81	84	6·9	6·8	9·2	7·4	6·2
	June	12	67	59	88	7·4	7·4	9·7	5·6	4·9
		—	500	385	77	6·6	6·2	9·1	—	—
39	February	27	229	190	83	8·5	7·9	9·4	8·5	7·0
	March	27	214	185	86	7·9	7·1	9·0	7·9	6·9
	April	23	183	168	92	8·3	8·0	9·1	7·9	7·3
	May	19	160	152	95	7·3	7·2	8·9	8·4	8·0
	June	18	113	71	63	7·5	5·1	10·5	6·3	3·9
		—	899	766	83	8·0	7·2	9·2	—	—

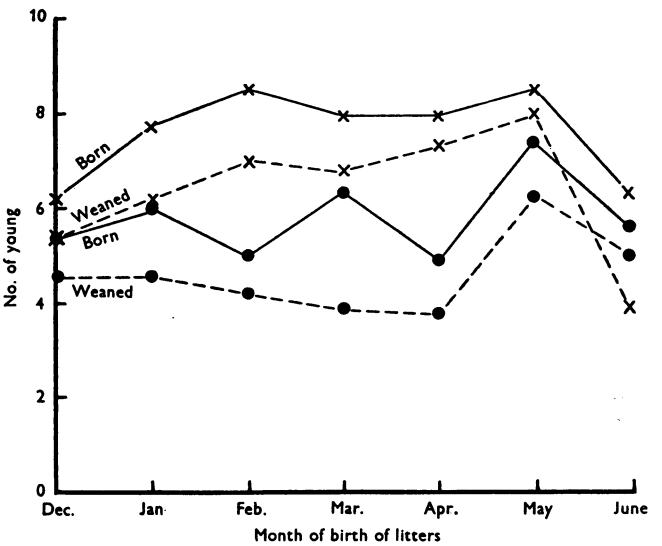

Fig. 1. Production of young mice per female per month on diets 32 and 39. ●—●, diet 32; ×—×, diet 39.

Table 4. *Comparison on mice of diets* 39 *and* 41 *for a mating period of* 7 *weeks*

Diet	No. of surviving females	Total* no. of litters born parity 1	2	3	Total no. of young Born	Weaned	Young weaned (21 days) (%)	Average no. of young per litter Born	Weaned	Average wt. of young weaned (g.)	Average no. of young born per female	Average no. of young weaned per female
39	20	20	18	5	265	218	82	6·5	6·4	8·4	13·3	10·9
41	24	24	22	11	343	242	71	6·4	5·5	8·2	14·3	10·1

* In five litters (diet 39, two; diet 41, three) the young were eaten before they could be counted. These have been omitted in calculating the average litter size.

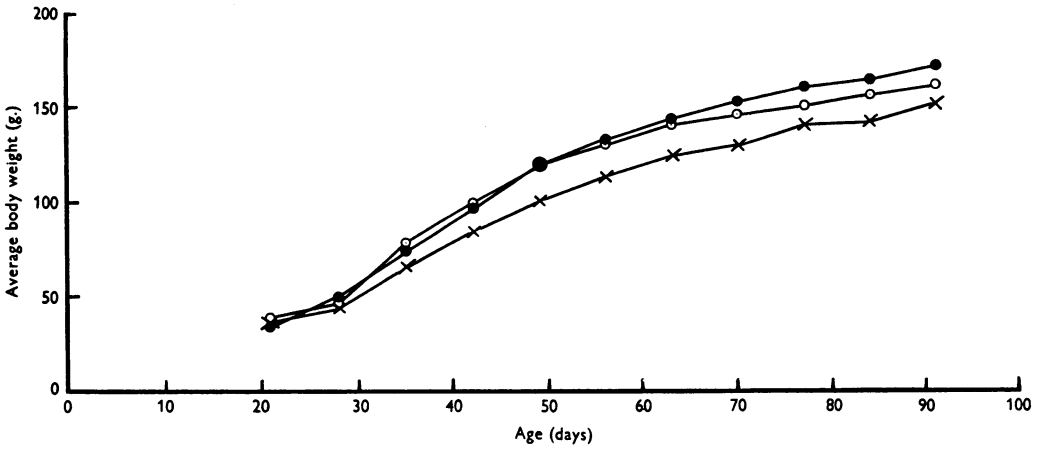

Fig. 2. Growth of young female rats on diets 1, 32 and 39. (Groups of eight triad litter-mates.) ×—×, diet 1; ●—●, diet 32; ○—○, diet 39.

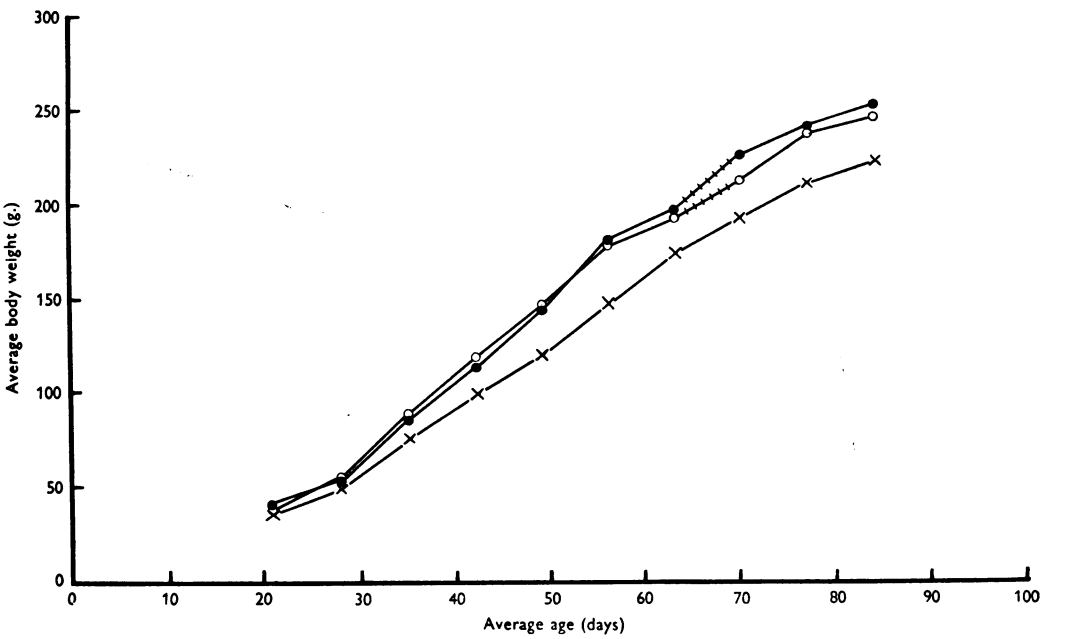

Fig. 3. Growth of young male rats on diets 1, 32 and 39. (Groups of seven triad litter-mates.) |–|–|, indicates death of an animal; ×—×, diet 1; ●—●, diet 32; ○—○, diet 39.

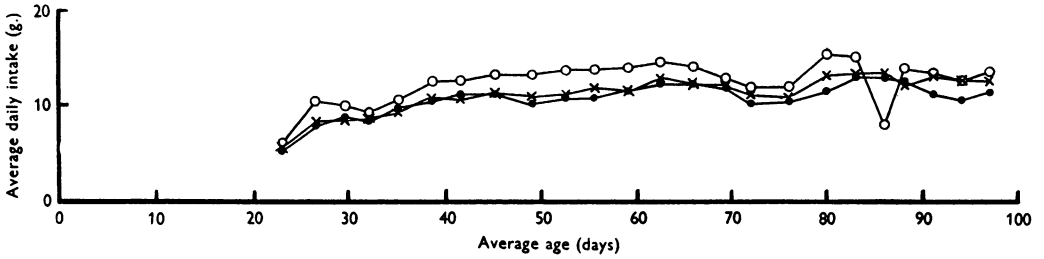

Fig. 4. Consumption of diets 1, 32 and 39 by female rats for 11 weeks between weaning and mating. ×—×, diet 1; ●—●, diet 32; ○—○, diet 39.

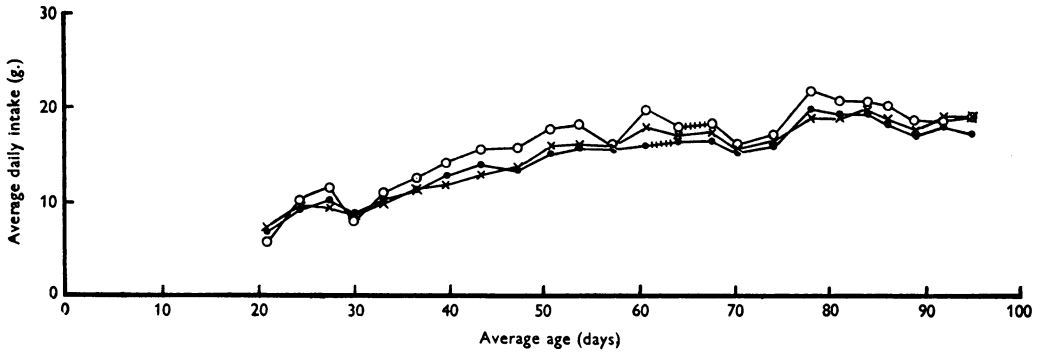

Fig. 5. Consumption of diets 1, 32 and 39 by male rats for 11 weeks between weaning and mating. ⊢⊣, indicates death of an animal; ×—×, diet 1; ●—●, diet 32; ○—○, diet 39.

Table 5. *Comparison on rats of diets* 1, 32, 39 *and* 41 *for a mating period of* 6 *months*

Mating group	Diet: Reared	Diet: Mated	No. of surviving females	Total no. of young: Born	Total no. of young: Weaned	Young weaned (21 days) (%)	Average size of litter weaned (young)	Average wt. of young weaned (g.)	Average no. of young born per female	Average no. of young weaned per female	Average production per female per month: Young born	Average production per female per month: Young weaned
A	32	39	10	460	321	70	7·6	34·6	46·0	32·1	7·8	5·4
	32	41	9	428	288	67	6·9	37·8	47·6	32·0	7·9	5·3
B	39	41	8	345	267	77	6·7	36·4	43·1	33·4	7·2	5·6
	32	32	6	264	159	60	6·6	37·0	44·0	26·5	7·3	4·4
	1	32	10	None	—	—	—	—	—	—	—	—

Total food intake for each of the six groups was measured throughout the period between weaning and mating. From Figs. 4 and 5 it will be seen that the consumption of diet 39 appears to have been slightly greater than that of diets 1 and 32. The difference, however, is not large and may be due to greater wastage of the crumbly cubes of diet 39 rather than to greater consumption. As young adults, the females appeared to consume 10–15 g. and the males 15–20 g. of the diets per day.

Reproduction on diets 32, 39 *and* 41

There were two groups differing only in age. Group A consisted of twelve litter-mate pairs of females reared on diet 32 and mated at 17–22 weeks old on diets 39 and 41. Group B comprised the rats from the growth experiment, except that two males died during the period of growth (Fig. 3) and were replaced by normal stock males. They were mated at about 15 weeks old as follows: eight pairs reared on diet 32 continued on diet 32 for mating; ten pairs reared on diet 39 were changed to diet 41 for mating; ten pairs reared on diet 1 were changed to diet 32 for mating.

Of these fifty-two mated pairs, three, one on each diet, consistently killed the young, and five pairs had no recorded matings in 10 weeks and were regarded as sterile for the purposes of this comparison. These pairs were broken up and re-mated. Three of the males and four of the females were fertile. In two

cases the members of a pair which had failed to mate together were both fertile with other partners. Thus, only three animals, two males and one female, were sterile. Neither the consistent killing of the young, nor the sterility, was associated with a particular diet; nor was the incidence of either unduly high in view of the small numbers on which the observations were made. One female on diet 41 died at the parturition of a second litter. Records are therefore available for forty-three pairs of rats over a period of 6 months. The results are given in Table 5. They show that the rats reared on diets 32 and 39, and mated on diets 32, 39 and 41, were all about equally fertile, there being no significant differences in number of young born and number or weight of young weaned. Chronological analysis of the results showed no falling off in production as time elapsed.

By contrast, animals reared on diet 1 failed to produce any young when mated on diet 32. The pairs were, therefore, broken up and the animals mated with partners of known fertility. Nine of the ten males reared on diet 1 again failed to sire offspring. The remaining one proved fertile after 3 months. The females reared on diet 1, however, all proved fertile with normal males.

DISCUSSION

The production of young rats in the experiments described above compared favourably with that recorded for other colonies. Kao, Conner & Sherman (1941) reported an improvement in growth and reproduction when the protein content of the diet was raised from 14·4 to 18·8 %, but there was no further improvement at a higher protein level. The average number of young weaned per female in a reproductive life of 319–378 days was 29–34. Campbell & Sherman (1945), in studies of the protein and calcium requirement of rats, found that the average number of young weaned per female on a diet containing 16 % protein was 29·6, 32·3 and 30·0 respectively at three different levels of calcium over an average reproductive life of 331–355 days. It is not clear to what extent, if any, post-partum mating was permitted in Campbell & Sherman's experiments, but even on the most favourable interpretation of their results those obtained by us with the use of diet 41 were at least as good and may well have been better.

The behaviour of the rats reared on diet 1 is suggestive of vitamin E deficiency, which causes testicular degeneration in young male rats by 8–10 weeks of age. The ensuing sterility is permanent. In female rats, on the other hand, the need for vitamin E arises only after the tenth day of gestation (Mason, 1940, 1944). It may thus be that the females reared on diet 1 were able to produce and rear normal litters when mated, on a complete diet, with normal males, whereas the males developed an irreversible sterility which was not overcome by the change to the adequate diet for mating. The fertility of male mice reared on diet 1 is not inconsistent with the idea that diet 1 was deficient in vitamin E, because male mice are extremely resistant to this deficiency (Bryan & Mason, 1940; Goettsch, 1942).

The failure of reproduction on diet 1, with symptoms suggestive of acute vitamin E deficiency in male rats, is difficult to explain in view of the fact that the contents of wholemeal flour and of total cereal are not greatly below those of diet 41. By contrast, it is difficult to know to what particular ingredient, or combination of ingredients, the success of diets 39 and 41 can be attributed. For instance, the dried yeast which proved one of the important ingredients in the series of diets discussed in Part VII (Bruce & Emmens, 1948) is reduced to a minimum in these diets, and the cod-liver oil can be omitted (diet 39), enough vitamin A being present presumably in the fish meal to satisfy even the requirements of intensive reproduction. The excess of vitamin A in diet 41 may be undesirable in view of its capacity to vitiate vitamin E, and in future batches of the diet (made after 1 March 1949) cod-liver oil will be reduced to 1·0 %. It is curious that the content of dried milk, thought by Watson (1937) to be an important constituent of her best mouse diets, is very low in diets 39 and 41. Schneider & Webster (1945) tried various cereal components in a diet consisting of whole grain cereal 66 parts, whole dried milk 33 parts, and sodium chloride 1 part. For general reproductive performance of Wistar Swiss mice the order of merit of the cereals tried was wheat, 'corn' and rye, rice, oats. Resistance to *Salmonella* infection was poor with oats and good with the other cereals. Specific nutritional factors involved in the resistance to infection were present in wheat but not in dried whole milk. These findings taken with our own make it likely that cereals, and especially wheat, have some particular virtue for the breeding of mice.

SUMMARY

A simple cubed diet is described, completely adequate to sustain rapid growth and intensive reproduction in mice and rats.

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