Observations on autoclaved, fumigated and irradiated diets for breeding mice

BY G. PORTER AND W. LANE-PETTER
MRC Laboratory Animals Centre, Carshalton, Surrey

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How to provide an adequate diet free from common murine pathogens is a problem facing the curators of all pathogen-free animal units. The commercial production of diets suitable for such units has not always been a complete success. There is the difficulty of obtaining complete sterility of the diet and the almost insurmountable obstacle of preventing contamination of the diet from the time it leaves the factory until it is placed before the animal.

EXPERIMENTAL

Diets

For the tests we used the following commercially produced diets for rats and mice:

No. 1. Dixon's F.F.G. Diet for medical research animals (untreated) (Porter, Lane-Petter & Horne, 1963).
No. 2. Dixon's F.F.G. autoclaved by steam at 120°, 15 lb/in^2 for 1 h.
No. 3. Dixon's F.F.G. fumigated by ethylene oxide at a gas concentration of 1500 ml/m^3 for 6 h.
No. 4. Dixon's F.F.G. sterilized by γ-irradiation, 2·5 Mrad.
No. 5. Dixon's 41 B, Bruce & Parkes (1956) formula for commercially cubed or pelleted diets (untreated).
No. 6. Dixon's 41 B autoclaved by steam at 120°, 15 lb/in^2 for 1 h.
No. 7. Wyatts Chardex (diet 1, Porter *et al.* 1963).
No. 8. Wyatts Chardex, autoclaved by steam at 120°, 15 lb/in^2 for 1 h.
No. 9. Spillers original laboratory animal diet autoclaved.
No. 10. Spillers autoclaved diet modified.
No. 11. Quaker Oats experimental rat and mouse diet.
No. 12. Quaker Oats, experimental diet.

The diets were manufactured and supplied by: nos. 1–6, E. Dixon & Sons, Crane Mead Mills, Ware, Herts; nos. 7 and 8, B. G. Wyatt Limited, Furnham Mills, Chard, Somerset; nos. 9 and 10, Spillers Limited, Old Change House, Cannon Street, London, E.C. 4, and nos. 11 and 12, Quaker Oats Limited, 139, Park Lane, London, W 1.

Table 1 gives a brief summary of the published vitamin requirements of the mouse.

The values given for diets 9 and 10 in Table 2 are the figures stated by the manufacturers, as also is the analysis of diet 12 in Table 3.

Table 4 shows the effects of autoclaving at 120° for 1 h on the nutrient content of diet 41 B. Table 5 gives the nutrient content of diet F.F.G. before and after fumigation by ethylene oxide. We are deeply indebted to Dr S. K. Kon and his colleagues, National Institute for Research in Dairying, Shinfield, Reading, and to Dr K. J. Carpenter, School of Agriculture, Cambridge, for providing the values given in these tables.

Table 1. *Vitamin requirements of the mouse*

Water-soluble			Fat-soluble		
Vitamin	Quantity/kg food	Reference	Vitamin	Quantity/kg food	Reference
Thiamine	3 mg	Morris (1947)	A	300 i.u.	McCarthy & Cerecedo (1953)
Riboflavine	1·5 mg	Morris (1947)	D	Not determined	Coward (1952)
	4·0 mg	Coward (1952)			
	4·0–6·0 mg	Fenton & Cowgill (1947)			
Pantothenic acid	1·5 mg	Fenton, Cowgill, Stone & Justice (1950)	E	20 mg	Cerecedo & Vinson (1944)
	3 mg as D-calcium pantothenate	Coward (1952)		80 mg	Lee, King & Visscher (1953)
Vitamin B_{12}	5 μg	Jaffé (1950)	K	1 mg	Cuthbertson (1957)
	10–20 μg	Bosshart, Paul & Barnes (1950)			
Nicotinic acid (amide)	10 mg; probably not required unless diet deficient in tryptophan	Cuthbertson (1957)			
Pteroyl-glutamic acid	1 mg	Cuthbertson (1957)			
Biotin	80 μg	Cuthbertson (1957)			
Pyridoxine	1 mg	Morris (1947)			
	5 mg	Fenton & Cowgill (1947)			
Inositol	10–100 g	Wooley (1941)			
Choline	1·0 g	Cuthbertson (1957)			

Table 2. *Manufacturer's stated figures for some components of diets* 9–10

Component	Diet 9	Diet 10
Oil (by hydrolysis) (%)	6·25	6·55
Vitamin A (μg/g)	9·15	9·45
Vitamin B_2 (μg/g)	6·00	6·95
Vitamin E (μg/g)	9·50	19·50

Animals

Seven strains of inbred mice and six F_1 crosses from three of these inbred strains were selected to test twelve diets. The inbred strains were A2G, BALB/c, CBA, C57BL/6, C57BR/cd, DBA/2 and 129 (Dinsley, 1961) subcultivated from the LAC inbred colony (Lane-Petter, 1961). The F_1 crosses were from A2G♂ × CBA♀, CBA♂ × A2G♀, A2G♂ × C57BR/cd♀, C57BR/cd♂ × A2G♀, CBA♂ × DBA/2♀, DBA/2♂ × CBA♀.

The method of selection, distribution and mating procedures, as well as the

Table 3. *Manufacturer's stated figures for some components of diet* 12

	%		%
Protein	29·5	Ash	9·00
Oil	8·5	Carbohydrate	42·75
Fibre	2·25	Moisture	8·00

Added minerals (mg/lb)		Added vitamins		
Cobalt	0·495	Vitamin A	1250	i.u./lb
Copper	2·500	Vitamin D_3	177	i.u./lb
Manganese	2·000	Riboflavine	1·0	i.u./lb
Iodine	0·451	Thiamine	0·41	mg/lb
Zinc	2·000	α-Tocopherol	22·0	mg/lb
Iron	48·500	Menaphthone	0·27	mg/lb
		Pantothenic acid	1·08	mg/lb
		Choline	529	mg/lb
		Folic acid	0·36	mg/lb
		Ascorbic acid	1·5	mg/lb
		Nicotinic acid	6·0	mg/lb
		Vitamin B_{12}	12·5	mg/lb

Table 4. *Effects of autoclaving at* 120° *for* 1 *h on the nutrient content of diet* 5

	Diet 5 (untreated)	Diet 6 (diet 5 autoclaved)	Diet 6 + 16 g L-lysine hydrochloride/kg
Water-soluble vitamins			
Vitamin B_{12} (ng/g)	12·5	2·75	—
Pantothenic acid (μg/g)	9·5	6·2	—
Nicotinic acid (μg/g)	32·4	33·0	—
Riboflavine (μg/g)	3·19	2·96	—
Pyridoxine hydrochloride (μg/g)	2·43	1·23	—
Biotin* (ng/g)	*c.* 180	*c.* 160	—
Thiamine (μg/g)	4·65	1·35	—
Fat-soluble vitamins			
Vitamin A (μg/100 g)	77	18	—
Carotenes (μg/100 g)	8·8	8·0	—
α-Tocopherol (μg/100 g)	1480	1100	—
Comparative nutritive values for *Streptococcus zymogenes* (diet 5 = 100)	100	45	55
Available lysine†	0·62 %	0·35 %	—

* Value is not precise, but there is no significant loss during autoclaving.

† The analysis was carried out by the procedure of Carpenter (1960). Internal recovery tests showed an average recovery of 73·5 % of added DNP-lysine, and the values given are corrected for the same recovery factor.

technique adopted for housing, caging and handling, were the same as those reported by Porter *et al.* 1963. The parent stocks of the F_1 crosses were selected from groups of surplus animals of three strains and thereafter treated as inbred animals.

Table 5. *Effect of fumigation with ethylene oxide on the nutrient content of diet* 1

	Nutrient content	
	Diet 1 (untreated)	Diet 3 (diet 1 fumigated)
Biotin (μg/g)	0·124	0·120
Nicotinic acid (μg/g)	31·7	16·0
Pantothenic acid (μg/g)	9·1	10·3
Riboflavine (μg/g)	8·9	8·9
Thiamine (μg/g)	4·3	4·1
Vitamin B_6 (pyridoxine) (μg/g)	3·58	2·75
Vitamin B_{12} (μg/g)	0·026	0·032
Folic acid (μg/g)	0·25	0·22
Vitamin A (μg/100 g)	97	120
Carotenes (μg/100 g)	36	36
α-Tocopherol (μg/100 g)	1300	1200
γ-Tocopherol (μg/100 g)	950	950
Methionine (g/16 g nitrogen)	2·3	2·3
Histidine (g/16 g nitrogen)	1·4	1·2
Comparative (untreated diet = 100) nutritive value for *Streptococcus zymogenes*	100	100
Available lysine as % of the crude protein	5·04	5·01
Crude protein (%)	19·5	21·2

Table 6. *Breeding performance of an inbred strain of mouse A2G/Lac on three different diets*

Diet no.*	No. of breeding pairs	Mean prenatal days (see below)	Mean no./litter			Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Q (see below)
			Born	Weaned	Lost			
1	20	24·9	6·4	5·45	0·95	30·05	274·0	21·9
3	20	28·3	7·25	6·70	0·55	28·65	264·9	23·7
10	20	26·5	7·50	6·75	0·75	31·20	256·3	25·47

* 1, F.F.G.; 3, F.F.G. fumigated; 10, Spillers modified.

Our criteria for measurement were:

(1) number of prenatal days for female mice monogamously paired for 110 days;

(2) number of young born, number weaned, and the preweaning mortality of the young;

(3) total number of young weaned per breeding pair;

(4) total weight of young weaned per breeding pair;

(5) *Q*, the index of productivity. This is a function of litter size and prenatal interval, that is the time between the first pairing and the birth of the first litter or between the births of successive litters in monogamously mated pairs that are never separated. It is calculated cumulatively from the date of the first pairing to the birth of the last litter and is given as the number of young weaned per 100 prenatal days (Lane-Petter, Brown, Cook, Porter & Tuffery, 1959).

RESULTS

It was impracticable to test every diet on every strain but a large number of comparative tests was made and the results are given below.

Comparison of strains

Strain A2G. This strain was used to test three diets, diet 1, diet 3 and diet 10. There was little difference in the results obtained, as is shown by the figures in Table 6. This was one of the few inbred strains that did slightly better on diet 10 than on diet 1, although the mean total weight weaned per pair was greater on diet 1 than on diet 10. The physical properties of diet 10 may have had some effect on the weaning weight and this is discussed further on p. 304.

Table 7. *Breeding performance of an inbred strain of mouse BALB/cLac on three different diets*

Diet no.*	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter			Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Q
			Born	Weaned	Lost			
1	20	27·85	6·05	5·55	0·5	24·2	228·7	19·92
9	20	29·3	7·0	5·9	1·1	25·4	210·8	20·13
10	20	30·9	5·15	4·25	0·9	17·3	150·3	13·75

* 1, F.F.G.; 9, Spillers original; 10, Spillers modified.

Table 8. *Breeding performance of an inbred strain of mouse CBA/Lac on twelve different diets*

Diet no.*	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter			Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Mean weaning wt of young	Q
			Born	Weaned	Lost				
1	20	23·75	7·5	6·8	0·7	29·35	218·63	7·44	28·63
2	20	26·35	7·5	6·85	0·65	30·9	225·65	7·30	25·99
3	20	27·65	6·75	6·0	0·75	26·12	207·85	7·95	21·69
4	20	26·85	7·35	6·3	1·05	27·95	205·20	7·34	23·46
5	20	25·75	6·8	5·45	1·35	25·0	186·14	7·44	21·16
6	20	30·6	6·0	5·25	0·75	21·5	153·44	7·13	17·15
7	20	29·65	7·35	6·25	1·1	26·2	188·45	7·19	21·08
8	20	28·5	7·5	6·9	0·6	29·8	230·55	7·73	24·21
9	20	27·5	6·5	5·2	1·3	21·6	149·0	6·89	18·90
10	20	27·0	5·5	3·85	1·65	17·05	118·2	6·93	14·25
11	20	31·45	6·4	6·0	0·4	22·35	190·2	8·51	19·07
12	20	32·3	7·0	4·9	2·1	19·2	148·55	7·73	15·17

* 1, F.F.G.; 2, F.F.G. autoclaved; 3, F.F.G. fumigated; 4, F.F.G. irradiated; 5, 41B; 6, 41B autoclaved; 7, Chardex; 8, Chardex autoclaved; 9, Spillers original; 10, Spillers modified; 11, Quaker Oats; 12, Quaker S.P.F.

Strain BALB/c. Diets 1, 9 and 10 were tested. Table 7 shows that diets 1 and 9 were almost equal, both being considerably better than diet 10. The mean total weight of young weaned was greater on diet 1 than on diets 9 or 10, which suggests that young inbred mice cannot deal effectively with the physical properties of some of these diets.

Strain CBA. Porter *et al.* (1963) found that this inbred strain was a sensitive indicator for assessing the adequacy of so-called standard diets. We did, therefore, test all twelve diets on this strain of mouse.

In order to make the comparisons reasonably simple, we have divided the diets into four groups and given a rank analysis of each group in Table 8. The results obtained from the four untreated diets confirm our earlier work (Porter *et al.* 1963) in which we found that F.F.G. (diet 1) was the best British commercial diet for this strain of mouse.

The breeding performance on F.F.G. sterilized by three methods, i.e. diet 2 autoclaved by steam, diet 3 fumigated by ethylene oxide and diet 4 sterilized by γ-irradiation at 2·5 Mrad, shows that the autoclaved diet gave the best results. The number of prenatal days was less with this diet than with the other two, and the other relevant criteria showed diet 2 to be better than diet 3 or diet 4.

Table 9. *Breeding performance of an inbred strain of mouse C57BR/cdLac on three different diets*

Diet no.*	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned (g)	Q
1	20	39·5	7·4	5·45	1·95	18·9	132·3	13·79
9	20	52·5	6·4	0·8	5·6	3·0	10·3	0·15
10	20	38·2	7·1	5·15	1·95	17·55	118·6	13·48

* 1, F.F.G.; 9, Spillers original; 10, Spillers modified.

Table 10. *Breeding performance of an inbred strain of mouse C57BL/6Lac*

Diet no.*	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Q
1	30	27·9	6·4	5·45	0·95	21·4	165·9	19·53
3	30	32·05	6·1	5·6	0·5	20·8	154·4	17·47
10	30	38·55	5·8	4·7	1·1	14·45	106·85	12·19
12	30	52·45	5·85	5·25	0·6	15·25	113·7	10·00

* 1, F.F.G.; 3, F.F.G. fumigated; 10, Spillers modified; 12, Quaker SPF.

The figures given for the three commercial diets that were steam-autoclaved show that diet 2 (F.F.G.) was better than diet 8 (Chardex), both being superior to diet 6 (41B).

Diets 9, 10 and 12 all appeared to be unsatisfactory for this strain of mouse. The preweaning loss per litter was high, especially on diet 12, and the numbers born per litter on diet 10 were considerably lower than for any of the other diets. Diet 12 also gave uneconomically long prenatal periods.

Strain C57BR/cd. M. Dinsley (1964, personal communication) found that it was necessary to provide this strain with supplements when fed on diet 1. Table 9 shows that diets 1 and 10 were suboptimal and that diet 9 was grossly inadequate. It again suggests that the physical properties of diets 9 and 10 were partly responsible for the poor reproductive performance. The high preweaning mortality indicated that the young mice were unable to manipulate these hard cubes. The figures for diets 9 and 10 show a complete reversal of those obtained for the other strains. This is a further indication that feeding inbred strains of mice is a more complex problem than feeding non-inbred strains.

Table 11. *Breeding performance of an inbred strain of mouse C57BL/6Lac on three different diets, showing a decline in the 2nd generation*

Diet no.*	Generation	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	*Q*
1	1st	15	26·1	6·1	5·2	0·9	21·8	172·2	19·92
10		15	28·5	5·8	4·9	0·9	18·8	141·0	17·19
12		15	35·5	6·6	6·3	0·3	20·2	165·6	17·74
1	2nd	15	29·7	6·7	5·7	1·0	21·0	159·6	19·19
10		15	48·6	5·8	4·5	1·3	10·1	72·7	9·25
12		15	69·4	5·1	4·2	0·9	10·3	61·8	6·05

* 1, F.F.G.; 10, Spillers modified; 12, Quaker experimental.

Table 12. *Breeding performance of an inbred strain of mouse 129/Lac on three different diets*

Diet no.*	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	*Q*
1	20	27·75	5·9	5·3	0·6	24·5	221·0	19·22
2	20	37·40	6·25	5·4	0·85	19·05	162·5	14·43
9	20	78·0	3·7	2·7	1·0	6·2	45·8	3·4

* 1, F.F.G.; 2, F.F.G. autoclaved; 9, Spillers original.

Strain C57BL/6. The four diets tested on this strain were diets 1, 3, 10 and 12. This strain does not normally have a high figure for *Q*. The results we obtained with diets 1 and 3 were average for this mouse. Diets 10 and 12 appeared to be grossly inadequate, as is shown in Table 10. We have confirmed (Table 11) the advantage of using an inbred strain of mouse to test the adequacy of diets. The first generation of animals on diets 10 and 12 appeared to show reasonable results, but the second generation clearly showed the inadequacy of these diets for this strain (diet 1 was included in this table for comparison).

Strain DBA/2. M. Dinsley (1964, personal communication) found that productivity was abnormally low in this inbred strain; it was, therefore, considered unsuitable for testing diets. Nevertheless, any inbred strain may have to be maintained, and it is essential to find a diet suitable for all such strains. Diet 10 was tested, and it was found that there was a significant lowering of the number weaned in the second generation. The mean number of young weaned per breeding pair was 5·6/100 prenatal days. Further observations with this strain of mouse had to be abandoned because of the poor condition of the few animals weaned in the second generation.

Strain 129. Diets 1, 2 and 9 were tested. Table 12 gives the results obtained. Diet 1 appeared to be adequate, but diet 2 considerably increased the number of prenatal days, thereby reducing the number of young weaned per breeding pair and the figure for Q. Diet 9 was found to be inadequate.

F_1 crosses. The ability of F_1 crosses to utilize a diet that had been found inadequate for some of the parent strains is shown in Tables 13–15. The relevant information for the parent strains has been included in these tables in order to simplify comparisons.

Strain A2G did better on diet 10 than any of the other strains, but C57BR/cd gave a poor breeding performance. The F_1 crosses of these two strains did well on diet 10, as shown in Table 12. When the female parent was A2G the results were slightly better than when the female was C57BR/cd. This is further demonstrated in Table 13, where it is shown that one of the parent strains A2G was better than the other parent strain, CBA. The F_1 crosses did better when the female of the parent strain was A2G. In Table 14 it is seen that F_1 crosses between CBA and DBA/2 gave good results, although both parent strains did poorly.

Table 13. *Comparisons between the breeding performance of parent strains A2G and C57BR/cd and their F_1 crosses on diet* 10 (*Spillers modified*)

Strain	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Q
A2G	20	26·5	7·50	6·75	0·75	31·20	256·3	25·47
C57BR/cd	20	38·2	7·1	5·15	1·95	17·55	118·6	13·48
F_1 (A2G♂ × C57BR♀)	20	24·95	9·85	9·25	0·6	42·5	382·0	37·07
F_1 (C57BR♂ × A2G♀)	20	24·3	11·2	10·0	1·2	51·5	473·8	41·15

Table 14. *Comparisons between the breeding performance of parent strains A2G and CBA and their F_1 crosses on diet* 10 (*Spillers modified*)

Strain	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Q
A2G	20	26·5	7·50	6·75	0·75	31·20	256·3	25·47
CBA	20	27·0	5·5	3·85	1·65	17·05	118·2	14·25
F_1 (A2G♂ × CBA♀)	20	26·9	9·0	8·4	0·6	36·0	338·4	31·22
F_1 (CBA♂ × A2G♀)	20	23·8	10·8	9·8	1·0	47·0	470·0	41·17

Table 15. *Comparisons between the breeding performance of parent strains CBA and DBA/2 and their F_1 crosses on diet* 10 (*Spillers modified*)

Strain	No. of breeding pairs	Mean prenatal days (see p. 298)	Mean no./litter: Born	Weaned	Lost	Mean total no. weaned/♀	Mean total wt of young weaned/♀ (g)	Q
CBA	20	27·0	5·5	3·85	1·65	17·05	118·2	14·25
DBA/2	20	35·0	4·6	3·65	0·95	9·85	69·28	10·25
F_1 (CBA♂ × DBA/2♀)	20	22·8	9·0	8·0	1·0	37·5	337·5	35·08
F_1 (DBA/2♂ × CBA♀)	20	24·1	8·7	8·1	0·6	39·1	332·3	33·60

Comparison of diets

Untreated commercial diets. Diet 1 (F.F.G.) gave better results than the other three diets tested. There was little difference between diet 5 (41 B) and diet 7 (Chardex). Diet 11 (Quaker) was the poorest for the strains of mouse tested. Diet 1 was unsatisfactory for strain C57BR/cd.

Autoclaved commercial diets. Diet 2 (F.F.G.) and diet 8 (Chardex) gave equally good results for the CBA strain of mouse and were superior to diet 6 (41 B). A supplement of lysine (0·33%, w/w) was far from effective in restoring the nutritive value of the autoclaved diet. On diet 2, strain 129 had a considerably higher number of prenatal days and a low figure for *Q*.

Diet F.F.G. treated by autoclaving, fumigation and irradiation. Diets 2, 3 and 4 all gave good results. Diet 2, (autoclaved) gave better results than diet 4 (irradiated), and for CBA mice both were better than diet 3 (fumigated). Strain A2G did equally well on diets 1 and 3 (F.F.G. before and after fumigation).

Diets recommended as pathogen-free. For strain CBA, diet 9 (Spillers original) gave the best results. There was little difference between diets 10 (Spillers modified) and 12 (Quaker experimental). The mean weaning weights of the young were greater on diet 12 than on diets 9 and 10, on which they were equal. Strain BALB/c did better on diet 9 than on diet 10. The mean weaning weight of the young was 1 g/mouse better on diet 1 (F.F.G.) than on diets 9 and 10. Diet 9 was inadequate for strains C57BR/cd, C57BL/6 and 129. Diet 10 was inadequate for C57BR/cd, C57BL/6 and DBA/2, but gave excellent results with all F_1 hybrids. Diet 12 was inadequate for C57BL/6.

Possible hazards of diets treated with ethylene oxide

Diets treated with ethylene oxide may have toxic effects under certain conditions. We accidentally killed a number of animals by feeding them on a treated diet that had been stored at about 6° after being removed from the fumigation chamber. Three strains of breeding mice, i.e. CBA, A2G and C57BL/6, were fed on the toxic diet. None of the males were affected, nor were the females of strain C57BL/6, but 50% of CBA and 33% of A2G lactating females died within 48 h of receiving the diet. The effects of this diet on two inbred strains of mice 19–28 days of age is shown below.

Strain	Sex	No. of animals	Deaths No.	Deaths %
CBA	♂	52	31	59·6
	♀	59	23	38·9
A2G	♂	21	12	57·2
	♀	24	7	40·5

DISCUSSION

Comparison of growth and reproduction shows that the feeding of inbred strains of mice presents more problems than the feeding of non-inbred strains or F_1 crosses of inbred strains.

Steam autoclaving of commercially produced diets in order to produce a sterile diet inevitably causes some damage to the constituents, but the results reported here suggest that one type of diet may suffer less from autoclaving than another. If autoclaving is unavoidable, consideration must be given to the raw ingredients of the diet.

The effects of an unpalatable diet on breeding and lactating animals cannot be completely ignored. Autoclaved diets are less palatable than non-autoclaved; diets fumigated by ethylene oxide have an obnoxious smell and a somewhat bitter taste. When a group of mice was given the choice between an untreated diet and the same diet fumigated by ethylene oxide, they always chose the untreated.

Some of the results could have been affected by palatability and not by a deficiency of nutrients.

The toxic effects of ethylene oxide appeared to affect the young males more than the young females. In the breeding stock, however, it affected only the lactating females of two strains, A2G and CBA; the C57BL/6 strain and the males of A2G and CBA were not affected. The higher percentage of deaths in young males and lactating females may have been connected with the higher metabolic rate of young males than of females, and of lactating females than of adult males. A small group of CBA mice of both sexes being kept for a longevity test was not affected.

We have since kept the diet treated with ethylene oxide at 21° and have had no further losses. We continue to investigate the effect of diets treated with ethylene oxide on strains A2G and CBA. At the time of writing both strains are in the fourth generation, but owing to our method of random selection these two strains can no longer be referred to as inbred. We are continuing tests of diet F.F.G. sterilized by γ-irradiation.

The difference between hardness of a cube and what may be described as 'gnawability' is a subtle one. A hard gnawable cube is acceptable to most mice and rats (even very young ones), but an equally hard ungnawable one is unacceptable. A cube with a high nutritional value is useless if the animals cannot eat it, and this is demonstrated in Table 8. The weaker inbred strains appeared to be incapable of dealing with diet 10, whereas the robust hybrids could. The special diets for pathogen-free animals were unsatisfactory for inbred strains of mice, but adequate for their F_1 crosses.

SUMMARY

1. Commercially produced diets before and after sterilization by autoclaving, fumigation or irradiation were studied for their effects on the breeding performance of inbred strains of mice. The animals were mated in monogamous pairs, remaining together for 110 days, during which time their prenatal days, litter size, preweaning loss, numbers weaned and the weaning weight of the young were recorded.

2. It has been shown that one commercial diet (F.F.G.) was superior to the other diets tested, and it was further shown that this diet when autoclaved supported reproduction, lactation and growth better than did the other two autoclaved diets.

3. Irradiation and fumigation were both found to be acceptable methods of sterilization, and fumigation used in the prescribed manner did not give rise to toxicity.

4. The physical properties of a diet had an important bearing on its efficiency.

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