STUDIES ON THE INFLUENCE OF DIET ON RESISTANCE TO INFECTION

I. THE EFFECT OF VARIOUS DIETS ON THE FERTILITY, GROWTH, AND SURVIVAL OF MICE

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(With 3 Figures in the Text)

THE observations described in this paper were carried out as a preliminary to a systematic study of the influence of dietetic factors on resistance to infection. The enquiry was initiated under the general direction of Prof. W. W. C. Topley of this School, and Prof. E. P. Cathcart of the University of Glasgow, and has been financed by a grant from the Medical Research Council. It was desired that all aspects of the problem, dietetic, immunological and statistical, should receive full and careful consideration at each stage of the investigation, and that any significant results, positive or negative, should be checked and controlled on a scale that would supply a firm foundation for further studies. To this end, a small advisory committee was formed, soon after the enquiry started, on which Prof. M. Greenwood, Prof. J. C. Drummond, Prof. Edward Mellanby, Dr Harriette Chick, Dr Bradford Hill and Dr Joyce Wilson kindly consented to serve. To all the above, I wish to express my thanks for help and advice. I wish also to acknowledge my indebtedness to Miss J. M. Hatswell for her assistance throughout the greater part of this work.

Mice were selected as the animals for study because their small size enables them to be housed and observed in sufficient numbers to yield statistically significant results, and because the same factor makes it possible to establish quarantine conditions that would be inordinately costly with larger animals. They have the additional advantage that several of the infective diseases to which they are naturally subject have been very extensively studied, both in regard to the sequence of events in the individual animal, and in herds submitted to the risk of contact infection.

It became clear, at the start of the enquiry, that more data were required with regard to the effect of various dietetic factors on the general health and well-being of mice before we could profitably proceed to the study of these factors on resistance to infection.

Although mice are bred extensively for use in experimental work of all kinds, a search of the literature dealing with this laboratory animal has revealed relatively little regarding its normal dietetic requirements, and the effect, on growth and fertility, of variations in the diet supplied.

Daniel (1912) has written fully on the breeding of mice for experimental purposes, and Parkes (1924) has put on record many facts concerning the fertility of mice, but in these two papers no mention is made of the diets on which the mice were fed. Kirkham (1920) also, in his study of the life of the white mouse, makes no reference to the diet given.

Wheeler (1913) found, in the course of feeding experiments with albino mice, that diets sufficient for adults allowed only a minimal growth in young animals. She concludes from her observations that mice, growing nearly twice as fast as rats, require a double proportion of bone- and flesh-forming food substances, and an inorganic salt concentration in the diet of about 7 per cent.

Robertson (1916), in experimental studies in the growth of young mice, gave to breeding mice a diet containing rolled barley, hard-boiled egg, dried bread, and lettuce, with water to drink, but the breeding experiments apparently were confined to this one diet. The same diet, except that raw egg was substituted for hard-boiled egg, was given to young mice after weaning. The young mice in his experiments showed three separate extra-uterine growth cycles. The first cycle reached its maximum velocity just prior to 7 days after birth and culminated at 14 days; the second attained its maximum velocity at 21–23 days and culminated soon after the twenty-eighth day; and the third attained its maximum velocity at about 6 weeks and thereafter decreased in velocity continuously but very slowly, so that growth still continued in the fiftieth and sixtieth weeks succeeding birth. He found great variability in the weight of the mice, and was of the opinion that because of this variability in weight a considerable number of mice must be used to obtain reliable data upon growth.

Beard (1925), in his investigations on the relation between diet and reproduction, gave to mice two diets, a "stock" diet consisting of skimmed milk powder, wheat bran, dog biscuit, and lettuce, with fresh milk during lactation, and a "standard" diet containing casein, starch, "Crisco" (a vegetable fat), cod-liver oil, salt mixture, and yeast. He found that the standard diet was unsuitable for breeding, but that the addition to it of fresh green lettuce thrice weekly was effective in curing sterility and in promoting normal reproduction, although insufficient to produce adequate normal lactation. Using the standard diet, which furnished approximately 5 calories per g. of which, allowing 91 per cent for utilization, 4·6 calories were available, Beard estimated the energy requirements of mice. He concludes from the results obtained in his experiments that, inasmuch as the relation between the calories-per-day and the two-thirds weight of the mouse is only very slightly logarithmic and almost linear, mice in so far as their total metabolism is concerned obey the surface area law. In young mice, the best growth was obtained when the protein concentration amounted to 31 per cent of casein (25·1 per cent of total calories) in the food mixture. Diets containing very large amounts of protein gave subnormal growth. At least 7 per cent of a well-balanced salt mixture was necessary for optimal growth; unbalanced salt mixtures resulted in poor growth.

Slonaker (1931 *a*, *b*) used a basic diet consisting of corn starch 5 parts, whole ground wheat 2 parts, whole ground corn 1 part, commercial skimmed milk powder 4 parts, ground alfalfa leaves 4 parts, commercial casein 2 parts, meat scrap 1 part, wheat germ 3 parts, unsalted butter 5 parts, yeast 2 parts, sodium chloride 1 part, calcium carbonate 1·5 parts. This diet contained 10·3 per cent of protein, of which approximately 57 per cent was vegetable and 43 per cent animal protein. To this diet he added meat scrap in varying amounts in order to obtain diets containing 14·2, 18·2, 22·2 and 26·3 per cent of protein. The principal varying factor in those diets was, therefore, the amount of animal protein. It is also noted that the amount of fat increased with the increase in the percentage of protein. All five diets had an energy value of 3·82 calories per g. Recently weaned albino rats were fed on these diets, and the course of events throughout their lives and the lives of their offspring observed. It was found that a diet containing just over 14 per cent of protein gave the best growth in weaned rats. A plus or minus deviation of 4 per cent produced no serious results. An increase greater than 4 per cent beyond the optimum caused progressive retardation in growth. The indications were that similar results would obtain if the percentage amount of protein was reduced progressively below the optimum. Rats fed on a diet with a protein content of 14 per cent showed the greatest fertility and the longest reproductive span. The same percentage of protein in the diet gave the greatest average number of litters, the greatest average number of young per litter, and the lowest mortality in unweaned young. As the protein content of the diet of the mothers increased, however, the birth weights of the offspring and the rate of growth of the unweaned young tended to increase, and were greatest in the young of does fed on a diet containing 26 per cent of protein. The data indicated that, as judged by the amount of weight lost by the does during lactation and the growth of the unweaned young, a diet with a high protein content was the best for lactating does and unweaned young.

The experiments of these and other observers show considerable disparity in rates of growth, form of growth curves, etc., and it seems reasonable to assume that a possible explanation of at least some of these differences might be found in the fact that the dietetic conditions of the experiments were dissimilar.

Experimental

In the course of the investigation here described breeding experiments with a variety of diets were carried out. These experiments have been grouped, for the sake of convenience, under the following headings:

I. "*Natural*" (*N*) diets in relation to fertility, survival, and growth. The diets in this group were composed mainly of natural food substances.

II. "*Synthetic*" (*S*) diets in relation to fertility, survival, and growth. In this group the diets were made up mainly from artificially purified food substances.

I. "NATURAL" DIETS IN RELATION TO FERTILITY, SURVIVAL, AND GROWTH

The ingredients of the diets in this group are given in Table I together with the average daily allowance of food per mouse. In the estimation of the protein content of the diets the analyses of Plimmer (1921) for oats and oatmeal and that of the manufacturer for dried separated milk were used. The protein content of bran varies in different samples and the figure taken here was 11 per cent. Yeastrel is a yeast extract.

Table I

Diets ...	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8
Whole oats	100	—	—	—	—	—	—	—
Coarse oatmeal	—	92	87	87	40	81	33	40
Dried separated milk	—	—	—	—	25	—	27·5	25
Granulated gelatin	—	—	—	—	—	4·5	2	—
Dextrine	—	—	—	—	23	—	25	—
Flour and water biscuit	—	—	—	—	—	—	—	23
Salt mixture no. 2	—	—	5	—	—	—	—	—
Salt mixture no. 3	—	—	—	5	—	6·5	—	—
Coconut oil	—	—	—	—	4	—	4·5	4
Cod-liver oil	—	1	1	1	1	1	1	1
Yeastrel (dry weight)	—	2	2	2	2	2	2	2
Wheat bran	—	5	5	5	5	5	5	5
Percentage of total protein in diet	8·2	11·87	11·25	11·25	14·66	15·01	16·71	17·42
Mouse ration per day in g.	12·5	6	6	6	6	6	6	6
Milk and water in equal parts to drink, approximately (c.c.)	2	2	2	2	2	2	2	2

Some mice, particularly nursing does, ate more than others, but in all the diet groups, with the exception of diet N_1 where ample allowance was made for the husk of the oats, the amount of food given was such as to be slightly in excess of the amount eaten. It is of interest to note, with regard to the amount of food consumed by a mouse, that in an experiment in feeding adult unmated mice with whole oats it was found that, although the number of oat grains eaten by different mice varied considerably, the same mouse ate practically the same number of grains each day, and that a mouse offered a double ration ate no more than when given a single ration.

The composition of the two salt mixtures is shown in Table II. Salt mixture no. 2, a modification of McCollum's salt mixture no. 185, was used in diet N_3, and salt mixture no. 3 in diets N_4 and N_6.

The work of Alexander & Bullowa (1910), McCollum *et al.* (1916, 1917), Osborne & Mendel (1918), Hawk (1923) and others has shown that gelatin is an efficient supplementary protein when added to diets containing oats protein. Diets N_6 and N_7, containing 4·5 and 2 parts of gelatin, were therefore compared with diets N_4 and N_5.

As will be seen from Table I, the mice on all diets were given, in addition to the dry food, a mixture of equal parts of water and pasteurized milk. This was

Table II

Salt mixture no. 2		Salt mixture no. 3	
Sodium chloride	10·38 g.	Sodium chloride	10·0 g.
Magnesium sulphate	32·7 ,,	Magnesium sulphate	30·0 ,,
Sodium acid phosphate	20·82 ,,	Potassium citrate	30·0 ,,
Potassium phosphate	57·24 ,,	Calcium lactate	70·0 ,,
Calcium phosphate	32·4 ,,	Iron lactate	7·0 ,,
Calcium lactate	78·0 ,,	Copper sulphate, 0·02 c.c. of a 10 % solution to every 100 g. of salt mixture	
Iron lactate	7·08 ,,		
Copper sulphate, 0·02 c.c. of a 10 % solution to every 100 g. of salt mixture			

given by day, in a drinking vessel of the inverted test-tube type, on 5 days in the week. The vessel was replaced at night by one containing water. On the remaining 2 days water only was given. The average daily allowance per mouse of the milk and water mixture was about 2 c.c.

In an experiment with diet N_5, which is not discussed here, the mice were given water only to drink, and it was found that, with this diet, the absence of fresh milk made no difference either to the fertility of the breeding mice or to the survival and growth of the young mice, the group of mice receiving water only giving results almost identical with those of the group receiving milk and water.

Cages. The results summarized in the tables in this section were all obtained from mice housed in glass cages. At the start of the enquiry the cages in use were made of zinc, of the type described by Topley (1923), but when the experiments had continued for a few months we were led to suspect that the zinc cages themselves, apart from the diets supplied to the mice in them, were affecting adversely both growth and fertility. The data in regard to this problem will be more conveniently discussed in the third section of this paper, and attention may for the moment be confined to the results obtained in the glass cages which, in all but the first few experiments, took the place of zinc cages.

Two sizes of cages were used. The larger cage, in which the breeding mice were mated and the young mice housed after weaning, was 10 in. in diameter and 5 in. in height; the smaller, or breeding cage, was 5½ in. in diameter and 4½ in. in height. The floor of each cage was given a thin covering of sawdust, and wood wool was provided in the breeding cages for nest making. The mice in large cages were changed to clean cages once a week, and the mice in small cages twice a week. All cages were sterilized by steam immediately after use and before being cleaned.

Mice. The mice selected as breeding stock in all the diet groups were drawn from the laboratory stock of mice. These mice are imported from several private breeders, all of whom breed particoloured mice exclusively for our use. The mice used in the breeding experiments were, therefore, of genetically different stocks. Young adult mice, mainly black and white in colour, were chosen, and before use the faeces of all mice were tested for the presence of *Bact. typhi-murium* and *Bact. gaertner*. These organisms were never found. In each set of experiments the mice were distributed in the experimental and control groups so as to give as nearly as possible the same number of mice from each dealer in each group. It was not possible to obtain the same number of mice from each dealer for all series of the experiments, but although the number of mice imported from any one dealer might vary in different series of experiments, the numbers in the same series were comparable.

Each experiment was set up with fifty-six mice, six males and fifty females, and this number was kept constant throughout the whole period of observation, all losses from death being made good by the addition of mice from stock. The mice, male and female, were given the diet under investigation for 3 weeks and then mated, and the diet given before mating was continued. The control mice were given the stock diet N_2 (oatmeal, bran, yeastrel, cod-liver oil). Mating of the mice was carried on throughout the experiment, bucks and does being caged together for 2 out of every 3 weeks. The number of females mated with one male was high—eight to ten does with one buck—but this arrangement was necessitated by the need to economize both space and labour. The does when pregnant were transferred to separate breeding cages and remained isolated until the young were weaned. Separation of the litter from the doe was usually made when the young mice were 28 days old, but the time of separation varied as it was the rule not to remove the young mice from the doe until they each had reached the weight of 10 g. It happened, therefore, and particularly in the diet groups in which the growth of the young mice was slow, that sometimes the does and litters were together for a longer period than 28 days. The does, after separation from their young, were given a resting period of 10 days before being re-mated. The same period of rest was given to mice with litters dead or eaten within a short time of birth.

The young mice, after weaning, were fed on the diet that their parents received. They were under daily observation, and were weighed at regular intervals from the age of 7 days.

Experimental period. The different series of experiments in the "N" diet group were not all commenced at the same time of year, but a group of mice fed on the control diet N_2 was invariably set up at the same time as an experimental diet group. Except for this seasonal variation and a variation in the length of time during which the experiments were carried on the experimental conditions were the same throughout. It may be noted that the temperature of the mouse rooms, which were heated, did not vary more than a few degrees. In most of the experiments the total period of observation was 64 weeks, i.e. the time taken to reach the age of 12 weeks by all young mice born within a period of 52 weeks from the first day of mating of the parents. Some of the experiments, however, were discontinued after a shorter period, and for this reason and because in some of the earlier experiments daily observation of the young mice ceased when they had reached the age of 8 weeks, the figures for young mice given in the accompanying tables are, with one exception, for a period of 34 weeks from the first day of mating of the breeding mice. The exception, Table VII, gives the figures for those experiments in which the young mice were under observation until the age of 12 weeks. Tables III and IV summarize the results obtained from all breeding does in a period of 26 weeks from the first day of mating. The figures in all tables refer in each case to an experiment commenced with fifty breeding does. In these and all subsequent tables percentages and average weights are given to the nearest half unit.

Influence of diet on fertility and litter-eating. The results obtained varied considerably between one diet group and another, and it would appear that the diets themselves were mainly responsible for the variation in results. The fertility of the breeding stocks and the survival and growth of the young mice all appeared to be influenced by the diets on which the mice were fed.

Fertility. When the experiments were set up each diet group contained fifty breeding does, but by the end of the period of observation the total number of does fed on each diet was greater than fifty, as from time to time mice from stock had been added to take the place of mice that died. Table III gives the average total number of does in each diet group and the number of does that did not give birth to litters in an experimental period of 26 weeks from the first day of mating. Does which were not members of a group for a sufficiently long time to give birth to litters have been excluded from the totals.

Table III

Type of cage	Diet	No. of tests	Average no. of does	Average no. of does without litters	Average % does without litters
Glass	N_1	2	70	34	48·5
	N_2	7	64	8	12·5
	N_3	1	70	13	18·5
	N_4	1	60	2	3·5
	N_5	2	54	2	3·5
	N_6	1	55	0	0·0
	N_7	1	57	2	3·5
	N_8	1	53	2	4·0

The mortality in the breeding mice was greater in some diet groups than in others, and in those diet groups in which the mortality was high the number of mice that did not give birth to young as a rule also was high. It is impossible to say, however, how much of the apparent sterility was due to true sterility, and how much due to resorption *in utero* of litters already conceived. Litter resorption is a recognized phenomenon in mice and other polytocous animals, and Evans & Burr (1917), from the results obtained in a detailed study of the causes of sterility in the rat, consider that the causal factor is the lack of vitamin E in the diet. In the preparation of the diets other than diet N_1, which consisted only of whole oats and milk and water mixture, provision was made for an adequate supply of both vitamins A and B, but the amount of vitamin E present was not estimated.

The fertility of the mice in most of the diet groups was at first fairly high, but in some of the groups the proportion of litters born to the number of does "at risk" became smaller and smaller as time went on. Some mice never showed signs of pregnancy, but others, although undoubtedly pregnant, did not give birth to young. Resorption of litters was extremely common in the mice housed in glass cages and fed on diet N_1. It was found also in the mice fed on diets N_2 and N_3 but to a much less extent than in the mice fed on diet N_1. It was relatively uncommon in the mice fed on diets N_4 and N_6, and apparently was rare in the mice fed on diets N_5, N_7 and N_8, at least in the later stages of pregnancy, for almost every mouse believed to be pregnant gave birth to a litter.

It might appear, from the fact that in some diet groups the time of separation of the litter from the doe was frequently delayed, that in those diet groups in which the growth of the young mice was slow the breeding does were given less opportunity to mate than in the diet groups in which the young mice were almost all ready for separation from the does at 28 days. In actual fact, however, this was not so. In those diet groups in which the growth of the young mice was slow, it was usual to find that a number of litters were born dead, or died or were eaten within a very short time of birth, with the result that a number of does were returned to the mating cages without much delay. Also, the mortality of the mice in these same diet groups was greater than that of the mice fed on the better adapted diets, and consequently the addition of mice from stock, mice younger and more likely to produce litters, was more frequent. Except in the early weeks of the experiments it was almost invariably found that the mice in the mating cages of the diet groups in which the growth of the litter mice was slow were of greater number than those in the mating cages of diet groups in which the young mice were separated from the does at 28 days.

Table IV

Type of cage	Time of year of first mating	Diet	Series	Total litters	% litters entirely eaten
Glass	Aug. 1933	N_1	(a)	110	42·5
	Dec. 1933		(b)	35	48·5
			Weighted average	**72**	**44·0**
	Aug. 1933	N_2	(a)	108	6·5
	Oct. 1933		(b)	88	3·5
	Oct. 1933		(c)	114	9·5
	Oct. 1933		(d)	116	11·0
	Oct. 1934		(e)	56	9·0
	Jan. 1935		(f)	77	11·5
	Sept. 1935		(g)	75	3·0
			Weighted average	**91**	**8·0**
	Aug. 1934	N_3		**74**	**9·5**
	Jan. 1935	N_4		**118**	**6·0**
	Jan. 1935	N_5	(a)	128	0·7
	Jan. 1936		(b)	79	5·0
			Weighted average	**103**	**2·5**
	Apr. 1935	N_6		**120**	**5·0**
	Apr. 1935	N_7		**99**	**7·0**
	Jan. 1936	N_8		**87**	**1·0**

Table IV sets out the results of each of the sixteen breeding experiments on the various diets in another form. It shows the date of first mating, the total litters born during the subsequent 26 weeks in each breeding group, and the percentage of these litters that were entirely eaten by the mothers. Each group was initially composed of six bucks and fifty does, and replacements of breeding mice that died were made as explained above. It will be seen from Table IV that, when several tests were made with any one diet, the number of litters born in the first 26 weeks after mating show a wide variation. This was so even when several tests were started simultaneously, or were commenced in the same month of different years. Such variations are, of course, to be expected in groups of only fifty does and six bucks, observed for a period as short as 26 weeks.

If Tables III and IV are considered together, however, there can be no doubt that diet N_1, consisting only of whole oats to eat and milk and water to drink, is grossly deficient as judged by its effect on fertility and litter-eating. The figure of 110 litters recorded in Table IV for group (*a*) on the N_1 diet is almost certainly misleading. For the reasons given above, only litters born within 26 weeks of first mating are recorded in this table, but many experiments, of which this was one, were continued for much longer periods. In this particular group not a single litter was born during the second 6 months.

It is much more doubtful whether any significance can be attached to the recorded differences between the groups fed on the remaining diets, so far as fertility and litter-eating is concerned; but we think it probable that diets N_4, N_5, N_6, N_7 and N_8 were superior to diets N_2 and N_3 in these respects.

In regard to litter-eating, there can be no doubt that the diet supplied to the does has an important influence on this habit; but it is certainly not the only factor. Anything that affected the comfort of the does was found to increase the proportion of litters eaten, while some does in each group were confirmed and habitual eaters of their young.

Influence of diet on the survival of young mice. The data with regard to the survival of young mice through the first 8 weeks of life are set out in Table V. It should be noted that the difference between the number of young mice alive at birth and the number alive at 4 weeks is accounted for not only by the number dying during that period, but also by the number eaten by their mothers. It is impossible to differentiate between young mice that have died and then been partially or wholly eaten, and mice that have fallen victims to cannibalism alone.

In all the diet groups the greater number of deaths occurred before weaning, but in some of the groups the number of mice dying between the ages of 4 and 8 weeks was considerable. In those diet groups in which there was a sufficient number of deaths on which to base an opinion there was a suggestion of critical ages in the lives of the young mice, for it was not uncommon to find that weaned mice from the same litter, of different sex and housed in different cages, died on the same day.

Taking the percentages of young mice alive at 8 weeks, it will be seen that diets N_1, N_2 and N_3 gave very unsatisfactory results. The average figure for survival given for diet N_1 is probably too high, since considerable experience with this diet, not recorded here, indicates that over 50 per cent of young mice

Table V

Type of cage	Time of year of first mating	Diet	Series	Young mice alive at birth excluding mice in litters entirely eaten at birth or later	% young mice alive at the age of 4 weeks	% young mice alive at the age of 8 weeks
Glass	Aug. 1933	N_1	(*a*)	319	69·5	58·5
	Dec. 1933		(*b*)	56	60·5	43·0
		Weighted average		**187**	**68·0**	**56·5**
	Aug. 1933	N_2	(*a*)	583	68·5	54·0
	Oct. 1933		(*b*)	541	59·5	42·5
	Oct. 1933		(*c*)	573	63·5	41·5
	Oct. 1933		(*d*)	695	61·5	43·0
	Oct. 1934		(*e*)	259	63·5	46·5
	Jan. 1935		(*f*)	370	76·0	54·5
	Sept. 1935		(*g*)	440	70·0	55·0
		Weighted average		**494**	**65·5**	**47·5**
	Aug. 1934	N_3		**335**	**61·5**	**51·5**
	Jan. 1935	N_4		**715**	**77·0**	**68·0**
	Jan. 1935	N_5	(*a*)	892	84·5	82·5
	Jan. 1936		(*b*)	506	93·5	89·5
		Weighted average		**699**	**87·5**	**85·0**
	Apr. 1935	N_6		**727**	**74·5**	**67·0**
	Apr. 1935	N_7		**657**	**90·0**	**86·0**
	Jan. 1936	N_8		**585**	**90·0**	**89·5**

tend to die within the first 8 weeks of life. Diets N_5, N_7 and N_8 give a much higher survival rate. Each of these diets, as will be seen from Table I, contained dried separated milk, and they were the only diets that did so. The differences between them are of a very minor kind, and probably quite unimportant. The figures for diets N_4 and N_6, in which there was no separated milk, but to which an alkaline salt mixture was added (see Table I), suggest that a diet consisting mainly of oatmeal may be improved in this way; but the survival rates in these groups were greatly inferior to those in which dried separated milk was added to the diet, but from which the alkaline salt mixture was absent.

In regard to all these survival figures it may be noted that there was never any evidence that mice were dying from any known infective disease, though bacteriological examination was carried out on all dead mice when this was possible.

Influence of diet on the growth of young mice. The data with regard to the growth of young mice are set out in Tables VI and VII and in Fig. 1. Table VI gives the weights for the mice of all groups up to 8 weeks. Table VII gives the figures for those groups that were observed for 12 weeks after birth. In Figs. 1, 2 and 3 a growth curve, constructed from the tables of Robertson (1916) for the normal growth of young male and female mice, is given for comparison.

Taking Table VI, it will be seen that diet N_1 is clearly grossly deficient. Diets N_2 and N_3, here, as in the case of comparative survival rates, are greatly inferior to diets N_5, N_7 and N_8 containing dried separated milk. Diets N_4 and N_6 again occupy a position intermediate between N_2 and N_3 on the one hand,

and N_5 and N_8 on the other. The figures for weights at 12 weeks (Table VII) follow much the same order. Here again diets N_5 and N_8 show the highest weights; but attention must be drawn to the discrepant figures for the group

Table VI

Type of cage	Time of year of first mating of parents	Diet	Series	Average wt. in g. of young mice at the age of 1 week	Average wt. in g. of young mice at the age of 4 weeks	Average wt. in g. of young mice at the age of 8 weeks
Glass	Aug. 1933	N_1	(*a*)	3·0	8·0	13·0
	Dec. 1933		(*b*)	3·0	6·0	9·0
		Weighted average		**3·0**	**7·0**	**11·0**
	Aug. 1933	N_2	(*a*)	3·5	10·5	18·0
	Oct. 1933		(*b*)	3·5	8·5	15·5
	Oct. 1933		(*c*)	3·5	8·0	15·0
	Oct. 1933		(*d*)	3·0	8·5	15·5
	Oct. 1934		(*e*)	3·0	9·5	17·0
	Jan. 1935		(*f*)	3·5	8·5	15·0
	Sept. 1935		(*g*)	3·5	9·5	15·0
		Weighted average		**3·5**	**9·0**	**16·0**
	Aug. 1934	N_3		**3·5**	**9·5**	**16·5**
	Jan. 1935	N_4		**3·5**	**10·5**	**19·5**
	Jan. 1935	N_5	(*a*)	4·0	13·5	23·0
	Jan. 1936		(*b*)	4·0	13·0	20·0
		Weighted average		**4·0**	**13·0**	**21·5**
	Apr. 1935	N_6		**3·5**	**11·5**	**18·5**
	Apr. 1935	N_7		**4·0**	**12·5**	**18·5**
	Jan. 1936	N_8		**4·0**	**12·0**	**20·0**

Table VII

Type of cage	Diet	Series	Young mice alive at birth excluding mice in litters entirely eaten at birth or later	% young mice alive at 4 weeks	% young mice alive at 8 weeks	% young mice alive at 12 weeks	Average wt. in g. of young mice at age of 1 week	Average wt. in g. of young mice at age of 4 weeks	Average wt. in g. of young mice at age of 8 weeks	Average wt. in g. of young mice at age of 12 weeks
Glass	N_2	(*f*)*	370	76·0	54·5	45·5	3·5	8·5	15·0	18·5
		(*g*)	440	70·0	55·0	48·0	3·5	9·5	15·0	19·0
Weighted average			**405**	**73·0**	**55·0**	**47·0**	**3·5**	**9·0**	**15·0**	**18·5**
	N_3		**328†**	**76·0**	**64·5**	**55·0**	**3·5**	**11·5**	**18·5**	**22·0**
	N_4		**715**	**77·0**	**68·0**	**63·0**	**3·5**	**10·5**	**19·5**	**23·0**
	N_5	(*a*)	892	84·5	82·5	81·5	4·0	13·5	23·0	27·0
		(*b*)	506	93·5	89·5	89·0	4·0	13·0	21·5	22·0
Weighted average			**699**	**87·5**	**85·0**	**84·0**	**4·0**	**13·0**	**21·5**	**24·5**
	N_6		**727**	**74·5**	**67·0**	**63·5**	**3·5**	**11·5**	**18·5**	**22·5**
	N_7		**657**	**90·0**	**86·0**	**84·5**	**4·0**	**12·5**	**18·5**	**22·5**
	N_8		**585**	**90·0**	**89·5**	**89·5**	**4·0**	**12·0**	**20·0**	**24·0**

* See Table V.

† The period of observation of this group from which these figures are drawn differs from that in Table V, and this accounts for the difference in the number of young mice.

N_5 (*a*) and the group N_5 (*b*) which were set up on exactly the same diet, but a year apart. It seems unlikely, in view of the findings that will be set out in the following paper, that this difference in growth rate was due to chance.

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Summary of Section I

Summarizing this series of experiments, we think it has been shown:

(*a*) That a diet consisting of whole oats, containing 8·2 per cent protein, and a mixture of milk and water (N_1) is grossly deficient for mice as judged by the lower fertility and frequency of litter-eating among the does, and the low survival rate and poor growth of the young mice.

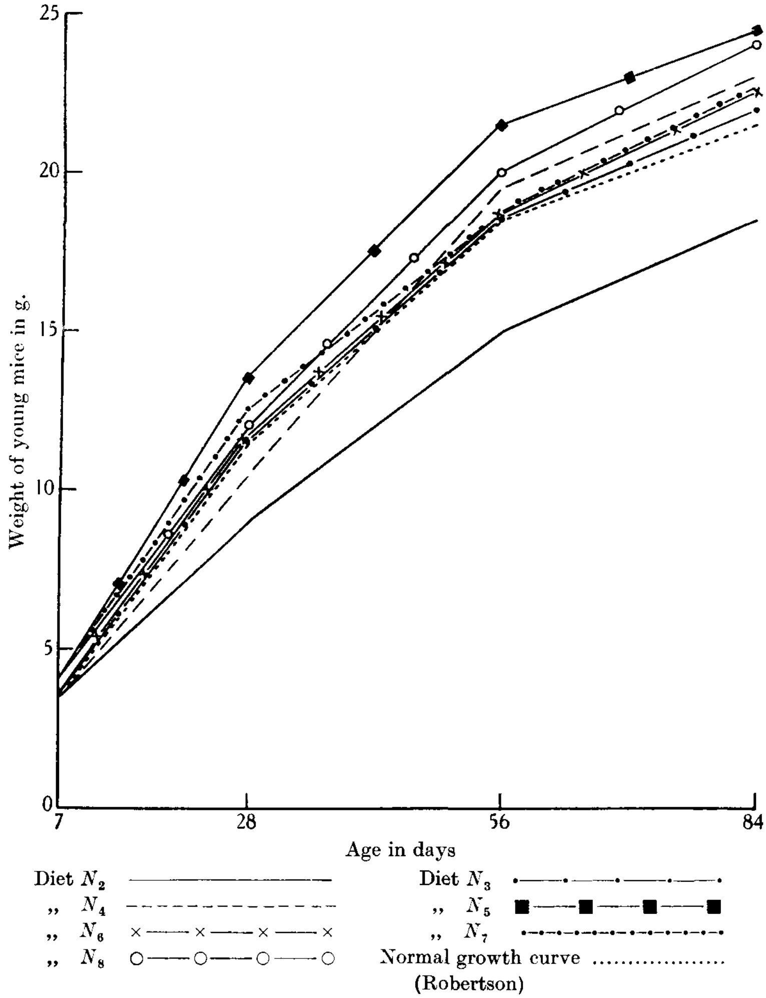

Fig. 1.

(*b*) That the substitution of coarse oatmeal for whole oats and the addition of cod-liver oil, yeastrel and bran (N_2) increases the fertility of the mice, and greatly decreases the frequency of litter-eating. This diet, which contains 11·87 per cent protein, supports growth in young mice but it is, however, a very poor diet as judged by the survival rate.

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(*c*) That the addition to the oats, cod-liver oil, yeastrel, bran and milk and water diet of an acid salt mixture (N_3) produces no significant change in the results obtained, as judged by any of the above criteria.

(*d*) That the addition to the oats, cod-liver oil, yeastrel, bran and milk and water diet of an alkaline salt mixture (N_4 and N_6) produces some improvement in the survival and growth rates of the young mice. Diet N_6 contains 15·01 per cent protein as compared with 11·25 per cent in diet N_4.

(*e*) That the reduction of the oatmeal to 33–40 per cent of the total diet, and its replacement by dried separated milk (25–27·5 per cent), and a ration of coconut oil, and dextrine (N_5 and N_7) or flour-and-water biscuit (N_8) gives a far more favourable diet. This is evidenced most strikingly by the survival rate among the young mice; but those diets also give very satisfactory results as judged by the fertility of the does, the relative infrequency of litter-eating, and the rate of growth of the young mice. Diet N_5 contains 14·66 per cent of protein, diet N_7 16·71 per cent, and diet N_8 17·42 per cent.

(*f*) That neither the alkaline salt mixture diet, nor the diet containing dried separated milk, appears to be significantly affected by the addition of a small amount of gelatin (compare N_6 with N_4, and N_7 with N_5).

II. "SYNTHETIC" DIETS IN RELATION TO FERTILITY, SURVIVAL, AND GROWTH

These "synthetic" diets were in point of time the first on which an attempt was made to rear young mice for resistance tests. They have been given second place here because it soon became obvious that they were greatly inferior to the "natural" diets, and one of our main objects in this investigation was to see whether any change in diet would induce a significant increase in resistance as compared with that observed in the diet now given to the stock mice in this laboratory (N_2).

Diets. The composition of the diets is shown in Table VIII, diets N_1 and N_2 being included as controls. The synthetic diets were three in number, and the only variation in them was in the kind of protein given, gluten only in diet S_1, caseinogen in S_2, and a mixture of gluten and caseinogen in S_3. In the first four of the seven series of experiments diet N_1 was the only control diet; in the fifth and sixth series diets N_1 and N_2 were both employed as controls; and in the seventh series the control diet N_2 alone was used.

When first made up, the three "synthetic" diets contained only 5 parts of bran, and the daily allowance of diet per mouse was 4 g. It was soon found that the bulk of the ration was insufficient to satisfy the breeding mice. In all three diets the allowance of food was eaten within a very short time of being given, and litter eating became almost universal. The proportion of bran in all three diets was increased to 55 parts, with a consequent increase to 6 g. of the mouse ration. The extra amount of food, readily eaten by the mice, caused a considerable diminution in, but by no means eliminated, the eating of litters.

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In addition to the dry food, the mice on the two control diets were given a mixture of equal parts of pasteurized milk and water. The mice on the three experimental diets S_1, S_2 and S_3 were given water only.

Cages. In the first two of the seven series of experiments the mice were housed in zinc cages; in the remaining five series the cages were of glass.

Table VIII

Diets ...	N_1	N_2	S_1	S_2	S_3
Whole oats	150	—	—	—	—
Coarse oatmeal	—	138	—	—	—
Gluten	—	—	20	—	10
Caseinogen	—	—	—	20	10
Dextrine	—	—	62	62	62
Salt mixture no. 2	—	—	5	5	5
Lard	—	—	5	5	5
Cod-liver oil	—	1·5	1	1	1
Yeastrel (dry weight)	—	3	2	2	2
Wheat bran	—	7·5	55	55	55
Percentage of total protein in diet	8·2	11·87	14·3	16·01	15·17
Mouse ration per day in g.	12·5	6	6	6	6

Salt mixture no. 2

Sodium chloride	10·38 g.
Magnesium sulphate	32·7 ,,
Sodium acid phosphate	20·82 ,,
Potassium phosphate	57·24 ,,
Calcium phosphate	32·4 ,,
Calcium lactate	78·0 ,,
Iron lactate	7·08 ,,

Copper sulphate, 0·02 c.c. of a 10% solution to every 100 g. of salt mixture

Mice. The mice selected for breeding were from the same mixed laboratory stock as those used in the "natural" diet groups. In each of the first five series of the experiments one hundred does and twelve bucks, fed on diet N_1 (whole oats), were mated. The does, as soon as pregnancy was definitely established, were transferred to one of the experimental diets, and thereafter received no diet other than the one to which they had been allotted. For each doe that was transferred to an experimental diet one remained on diet N_1 as a control. The litter rate in mice fed on diet N_1 was found to be too low to produce a sufficiency of young mice, and consequently in the sixth and seventh series of the experiments the breeding stock was fed and mated on diet N_2 (oatmeal, bran, yeastrel, cod-liver oil) instead of on diet N_1.

In the main series of experiments described in this section there are, therefore, no figures for fertility. Towards the end of the experiments, however, an attempt was made to breed from mice fed before mating on the three "synthetic" diets, and the results obtained may be briefly summarized.

The effect of the "synthetic" diets on fertility. Young male and female mice, bred from does fed on the stock diet N_2, were fed from shortly after weaning on diets S_1, S_2 and S_3, and when approximately 12 weeks old were mated. A control group of mice, similarly bred, was kept on the stock diet N_2 and mated. The experiment was carried on for 12 weeks from the first day of mating of the mice, and bucks and does were caged together throughout the entire period of experiment. The results, shown in Table IX, need no comment. Few of the mice on the "synthetic" diets gave birth to litters, and only one young mouse, bred from a doe fed with diet S_1, survived longer than 7 days.

Table IX

Diet	No. of does	No. of litters	No. of litters born dead	No. of litters eaten	No. of mice excluding litters eaten at birth or later	No. of mice alive 7 days after birth
N_2	30	15	1	2	69	54
S_1	50	2	0	0	14	1
S_2	50	2	1	0	1	0
S_3	50	4	3	1	0	0

The effect of the "synthetic" diets on resorption of litters and on litter-eating. As stated above, the does in the main series of experiments were transferred from one of the control "natural" diets (N_1 or N_2) to one of the "synthetic"

Table X

Diet	Series	Total litters	Litters entirely eaten	S_1, S_2 and S_3 (tests 1–6) for comparison with N_1		S_1, S_2 and S_3 (tests 5–7) for comparison with N_2	
N_1	(1)	30	12				
	(3)	23	15				
	(4)	21	11				
	(5)	19	6				
	(6)	23	10				
Totals		**116**	**54**				
% eaten litters			**46·5**				
N_2	(5)	12	0				
	(6)	24	0				
	(7)	37	7				
Totals		**73**	**7**				
% eaten litters			**9·5**				
S_1	(1)	27	24				
	(3)	10	3				
	(4)	20	12				
	(5)	14	4				
	(6)	11	1				
	(7)	35	12				
Totals		**117**	**56**	**82**	**44**	**60**	**17**
% eaten litters			**48·0**		**53·5**		**28·5**
S_2	(1)	29	22				
	(3)	12	3				
	(4)	16	9				
	(5)	10	1				
	(6)	12	0				
	(7)	38	12				
Totals		**117**	**47**	**79**	**35**	**60**	**13**
% eaten litters			**40·0**		**44·5**		**21·5**
S_3	(1)	29	23				
	(3)	11	6				
	(4)	13	7				
	(5)	12	0				
	(6)	13	2				
	(7)	37	17				
Totals		**115**	**55**	**78**	**38**	**62**	**19**
% eaten litters			**48·0**		**48·5**		**30·5**

Table XI

Diet	Series	Young mice alive at birth excluding mice in litters entirely eaten at birth or later	Young mice alive at the age of 4 weeks	Young mice alive at the age of 8 weeks	Young mice alive at the age of 12 weeks	S_1, S_2 and S_3 (6 tests) for comparison with N_1		S_1, S_2 and S_3 (tests 5–7) for comparison with N_2	
N_1	(1)	37	19	19	19				
	(3)	37	23	13	12				
	(4)	43	24	21	16				
	(5)	61	39	27	19				
	(6)	44	34	26	18				
Totals		**222**	**139**	**106**	**84**				
% total mice alive at birth			**62·5**	**47·5**	**38·0**				
N_2	(5)	76	70	60	47				
	(6)	127	103	74	57				
	(7)	164	83	62	50				
Totals		**367**	**256**	**196**	**154**				
% total mice alive at birth			**69·5**	**53·5**	**42·5**				
S_1	(1)	15	7	7	7				
	(3)	39	33	16	8				
	(4)	49	20	14	13				
	(5)	52	41	33	25				
	(6)	61	45	36	32				
	(7)	81	19	14	10				
Totals		**297**	**165**	**120**	**95**	**216**	**85**	**194**	**67**
% total mice alive at birth			**55·5**	**40·5**	**32·0**		**39·5**		**34·5**
S_2	(1)	44	24	19	18				
	(3)	48	35	25	23				
	(4)	44	20	11	11				
	(5)	44	38	35	35				
	(6)	57	51	41	35				
	(7)	128	24	21	20				
Totals		**365**	**192**	**152**	**142**	**237**	**122**	**229**	**90**
% total mice alive at birth			**52·5**	**41·5**	**39·0**		**51·5**		**39·5**
S_3	(1)	29	18	18	17				
	(3)	24	17	12	10				
	(4)	31	26	21	19				
	(5)	70	49	46	43				
	(6)	56	44	41	38				
	(7)	85	25	20	17				
Totals		**295**	**179**	**158**	**144**	**210**	**127**	**211**	**98**
% total mice alive at birth			**60·5**	**53·5**	**49·0**		**60·5**		**46·5**

diets (S_1, S_2 or S_3) as soon as pregnancy was definitely established. It was thus possible to study the effect of the "S" diets on the frequency of resorption of litters, and of litter-eating. Litter resorption was as frequent in mice fed on diets S_1, S_2 and S_3 as in mice fed with diet N_1, and three times more frequent than in mice fed on diet N_2. The relevant figures with regard to litter-eating are set out in Table X. There is no evidence that the "synthetic" diets are

Table XII

Diet	Series	Average wt. in g. of young mice at age of 1 week	Average wt. in g. of young mice at age of 4 weeks	Average wt. in g. of young mice at age of 8 weeks	Average wt. in g. of young mice at age of 12 weeks
N_1	(1)	2·0	6·5	11·0	13·5
	(3)	3·5	7·0	11·0	14·0
	(4)	3·0	9·0	14·0	17·0
	(5)	3·0	7·0	10·0	13·0
	(6)	3·0	6·5	8·5	11·0
Weighted average		**3·0**	**7·0**	**11·0**	**13·5**
N_2	(5)	3·0	9·5	16·5	21·5
	(6)	3·5	8·5	14·5	18·5
	(7)	3·0	8·0	15·0	20·0
Weighted average		**3·0**	**8·5**	**15·0**	**20·0**
S_1	(1)	4·0	10·0	15·0	22·0
	(3)	3·0	6·5	12·5	17·0
	(4)	3·0	8·5	15·0	22·0
	(5)	3·0	10·0	15·0	18·0
	(6)	3·5	9·0	15·0	19·5
	(7)	3·0	7·0	13·0	19·0
Weighted average		**3·0**	**8·5**	**14·0**	**19·5**
S_2	(1)	3·5	9·0	16·0	19·0
	(3)	4·0	9·5	17·5	21·5
	(4)	3·0	10·5	17·0	20·5
	(5)	3·5	14·5	20·0	24·5
	(6)	4·0	9·5	15·5	20·0
	(7)	2·5	9·0	14·0	17·5
Weighted average		**3·5**	**10·5**	**16·5**	**20·5**
S_3	(1)	3·0	9·5	17·0	22·5
	(3)	3·5	10·5	18·0	20·0
	(4)	3·5	9·5	17·0	18·0
	(5)	2·5	10·0	16·0	20·5
	(6)	3·5	9·0	15·0	18·5
	(7)	3·0	8·0	11·5	15·5
Weighted average		**3·0**	**9·5**	**15·5**	**19·0**

more unfavourable than the very unsatisfactory "natural" diet N_1, but they are definitely less favourable than diet N_2.

It should be noted that in Table X and the other tables summarizing the results obtained with this group of diets that there are no figures relative to the second series of experiments. The series was a complete failure. The breeding mice remaining from the first series, i.e. mice that had not become pregnant and that therefore had not been fed with any of the "synthetic" diets, were increased in number by the addition of mice from stock, and bred from. Only nine litters resulted, and of these eight were wholly eaten and one died within a few days of birth. For this reason, therefore, no record of the second experiment appears in Tables X–XII.

The effect of the "synthetic" diets on the survival and growth of young mice. The relevant figures are set out in Tables XI and XII, and in Fig. 2.

None of the diets, "synthetic" or "natural", gave good results as regards the survival and growth of young mice. The effects of feeding young mice with diets N_1 and N_2 have been discussed in the section dealing with these diets; and it is apparent from the figures in Tables XI and XII that the results ob-

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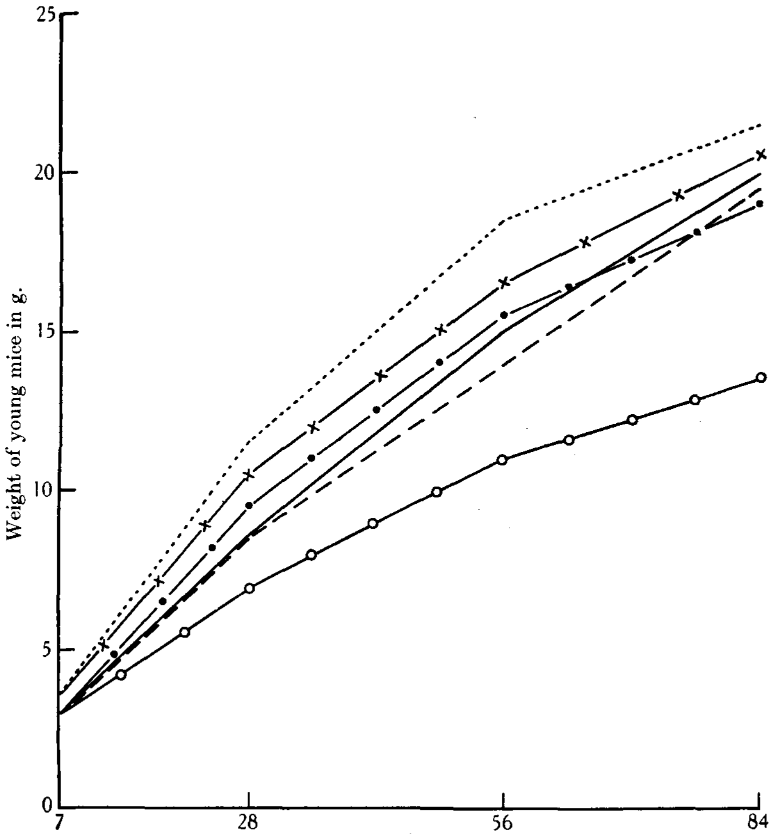
tained with the "synthetic" diets S_1, S_2 and S_3 are little, if any, better than those obtained with the highly unsatisfactory "natural" diets.

In all three "synthetic" diet groups many young mice died within the first few days of birth. In a few instances this early death was probably caused by neglect or inability to suckle on the part of the doe. In most cases, however, it was thought that death was due either to the lack of viability of the young



Diet N_1 o——o——o Diet N_2 ————————

" S_1 - - - - - - - - - " S_2 ×——×——×

" S_3 •————•————• Normal growth curve (Robertson) ....

Fig. 2.

mice themselves or to the poor nutritive quality of the nourishment provided by the mothers, as it was observed that the stomachs of the young mice, at this age visible through the abdominal wall when milk-filled, appeared to contain a sufficiency of milk.

The death-rate in mice over 4 weeks of age, although lower than in mice under that age, was high. Death frequently occurred with great suddenness in apparently healthy young mice, and post-mortem examination revealed no cause of death.

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Of the total young mice fed on the three experimental diets those fed on diet S_3 gave the best survival rate and those on diet S_1 the poorest. But the number of mice in individual experiments was very small and the variation in survival between successive experiments great, and the average figures can hardly be regarded as significant.

In the three "synthetic" diet groups there was no appreciable difference in the average weights of the young mice at the age of 12 weeks. In all the groups there were mice of good weight and mice of very poor weight, but a good weight was generally due, as was found at the post-mortem examination of mice which died, to an accumulation of fat.

The results obtained from the mice in the two control diet groups N_1 and N_2 were also poor, but they did not differ materially from the results obtained in other experiments with these same two diets.

Summary of Section II

In summarizing the results obtained in this section it is sufficient to note:

(*a*) That the "synthetic" diets reduced the fertility of the mice almost to zero, and induced, in those mice that became pregnant, resorption of litters and litter-eating.

(*b*) That, with regard to the survival and growth of the young mice, wheat gluten and caseinogen, the only variables in the "synthetic" diets, appeared to be equivalent in nutritive value, and that the diets containing them gave results of the same order as diets N_1 and N_2, the least satisfactory of the "natural" diets.

III. The type of cage in relation to fertility, survival and growth

As stated above, it was found, very early in these experiments, that mice throve far better in glass cages than in zinc cages. An attempt was therefore made to determine whether this difference was due to some constituent of the metal of which the cages were made, or to some other factor, such as the absence of light. It may be noted in this connexion that the frequent steam-sterilization of the zinc cages led to a considerable degree of corrosion and flaking.

Four types of cage were used: (*a*) zinc cages, (*b*) glass cages, (*c*) glass cages covered with black paper, and (*d*) glass cages with added scrapings from corroded zinc cages. Except for the difference in the housing of the mice the experimental conditions were the same in all four experiments, and were exactly similar to those in the N_2 diet groups already discussed. Each experiment was set up with fifty female and six male mice, and the mice in all types of cage were fed on the stock diet N_2 (oatmeal 92 parts, bran 5 parts, yeastrel 2 parts, and cod-liver oil 1 part). The relative data obtained from these experiments are set out in Table XIII and Fig. 3.

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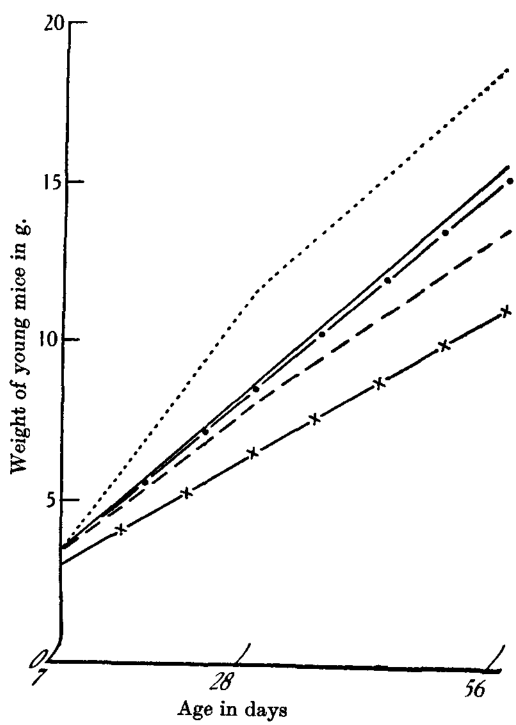
Table XIII

All mice fed on diet N_2	Total litters	% litters entirely eaten	Total young mice alive at birth excluding mice in litters completely eaten at birth or later	% young mice alive at 4 weeks	% young mice alive at 8 weeks	Average wt. in g. of young mice at 4 weeks	Average wt. in g. of young mice at 8 weeks
Zinc cage	27	40·5	82	38·0	27·0	8·0	13·5
Glass cage	88	3·5	541	59·5	42·5	8·5	15·5
Glass cage covered with black paper	87	6·0	522	67·0	40·0	8·5	15·0
Glass cage with added zinc scrapings	27	22·0	78	28·0	22·0	6·5	11·0

(*a*) *Zinc cages*. The fertility of mice mated in these cages, although never high, was at first fairly good, but the litter rate declined rapidly and resorption of litters was common. Few mice had more than one litter, and many never had a litter at all. Of a total of sixty does in the group 68·5 per cent did not give birth to a litter.



Zinc cage - - - - - - - Glass cage ————————
Glass cage and black paper •——•——• Glass cage and zinc scrapings ×——×——×
Normal growth curve (Robertson) ....

Fig. 3.

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The survival rate of the young mice born in the zinc cages was very poor, and their growth was retarded. Of eighty-two young mice alive at birth only 27 per cent survived to the age of 8 weeks and at that age the average weight was only 13·5 g.

(*b*) *Glass cages.* The results in this group were distinctly better than those obtained in the zinc cage experiment. The breeding mice remained in good condition, and the mortality was lower than in the zinc cage group. The fertility rate was high and resorption of litters was much less common than in the zinc cage mice. In this group the total does numbered 59, and of that number 7·0 per cent did not give birth to litters. Of 541 mice alive at birth 42·5 per cent survived to the age of 8 weeks. The average weight of the young mice at that age was 15·5 g.

(*c*) *Glass cages covered with black paper.* This experiment was carried out in order to test the possibility that light might be beneficial to mice. The mice, breeding stock and young, were housed in glass cages the sides of which were covered with thick black poster paper. By this means all light except that which entered through the perforations in the lids of the cages was excluded. The results of this experiment were very similar to those obtained from the mice housed in plain glass cages. The number of litters born was practically the same, although in this group 18 per cent of a total of sixty-six does did not have litters. There were 522 young mice alive at birth, and of these 40 per cent survived to the age of 8 weeks. The average weight of the young mice at the age of 8 weeks was 15 g.

(*d*) *Glass cages with added zinc scrapings.* The results of the paper-covered cage experiment suggested that the poor fertility and growth of the mice in the zinc cages was probably due to the toxic action of some constituent of the metal used in the construction of the cages. An experiment was set up in which scrapings from corroded zinc cages were mixed with the sawdust on the floor of the glass cages. The mice in these cages soon showed, and to an even greater degree, the loss of condition found in mice housed in zinc cages. As in the zinc cage experiment, few litters were born, and resorption of litters was common. Of a total of seventy does in the group 68·5 per cent did not give birth to a litter. Only seventy-eight young mice were alive at birth, and of these only 22 per cent survived to the age of 8 weeks. At that age the average weight was 11 g.

These results clearly support the conclusion that the poor condition of the mice in zinc cages was due to the toxic action of some constituent of the metal. The possibility that metal cages under certain conditions might prove harmful to mice was foreseen by Robertson & Ray (1916) who, in their breeding experiments, used cages made mainly of glass fitted into wooden frames because they considered galvanized iron cages unsuitable on account of the possible action of stale urine upon the metal, leading to the formation of metallic salts which might be absorbed by the mice and exert an unknown effect upon their growth and well-being.

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Zinc itself is stated to be present in animal tissues under normal conditions, and the addition of zinc to the diet of rats and mice would appear, in the opinion of those who have tested it, to be beneficial rather than harmful to the animals.

McHargue (1926) gave to rats housed in cages made of glass, monel metal wire and aluminium a synthetic diet to which he added small quantities of manganese peptonate, copper sulphate, or zinc lactate. He got results which he interpreted as indicating that compounds of manganese more definitely and possibly copper and zinc also have important biological functions in animal metabolism.

Thompson *et al.* (1927) found that the feeding of organic zinc salts (acetate, citrate, and malate) or of zinc oxide suspensions in doses of from 2 to 38 mg. of zinc daily to albino rats, not only for many weeks previous to mating, but during pregnancy and lactation as well, had no significant effect upon the health of the parent, upon fertility, or upon the health and early growth of the offspring. The average normal zinc concentration of albino rats did not vary significantly at different ages, and they regarded this constancy of zinc concentrations, regardless of age, as suggestive evidence of a functional importance on the part of this metal. Zinc compounds given by the mouth over long periods of time did not usually result in the storage of zinc in the rat in amounts above the normal. If storage in excess of the normal quota did occur, the amounts were very small and had no effect upon the health of the animal.

Hubbell & Mendel (1927) fed mice housed in metal-free cages of glass and porcelain on a zinc-low diet containing in the daily ration an average of only 0·005 mg. of zinc. The effect of the element on growth was then tested by adding to this zinc-low diet enough zinc sulphate to make the total intake either 0·02 or 0·04 mg. of the metal each day. Addition of the smaller amount was attended by growth very nearly normal, while the addition of the larger amount was less effective. The slightly more favourable effect of adding 0·02 mg. was more evident with females than with males. Hubbell & Mendel conclude that in an actively growing mouse there does not seem to be storage of zinc to any extent, and that while there is some evidence that the addition of a small amount may cause a very slight stimulation of growth it seems probable that the addition of zinc to a food mixture low in the metal is not sufficient to make the diet equal to standard. They consider it possible that any value that zinc may have lies not alone in the presence of the metal itself, but that it may in some way be associated in function with other metals present in small amounts, and that it is not unlikely that there is some variation in growth with varying amounts of zinc and that the metal is not merely an accidental factor in the nutrition of the mouse.

Bertrand & Bhattacherjie (1934) divided litters of newly weaned mice into two groups. One group was fed on a purified diet with a very low zinc content, and the other was fed on the same diet with the addition of a quantity of zinc equal to the amount that had been taken away by purification. Both groups of mice were kept in glass cages in order to avoid all metal contamination. From the results of these experiments Bertrand & Bhattacherjie conclude that zinc is of great importance in the nutrition of mice.

Batchelor *et al.* (1926), in an investigation on the health of zinc workers, found, although zinc workers absorb and excrete zinc in amounts considerably over the normal, and maintain constantly a blood zinc content slightly over the normal, no evidence that abnormal amounts of zinc entering and leaving the body over a period of years caused either symptoms or evidence of damage to the tissues.

From the findings of these and other workers it appeared probable that if the metal of the cages contained some constituent harmful to mice, a constituent other than zinc must be sought for. The scrapings from corroded zinc cages were analysed and found to contain lead, and in consequence the tissues of mice housed in different types of cage were examined for the presence of lead. Dr H. Heap, of the University of Manchester, very kindly examined for us over 200 mice that had been housed for varying periods in zinc or glass cages, or in glass cages with added zinc scrapings. The mice in the different groups were found to contain lead in amounts varying from less than 6 mg. per kg. skinned mouse to 57 mg. per kg. skinned mouse. In general, lead was found in greater quantity in the tissues of mice housed in zinc cages, or in glass cages containing zinc scrapings, than in mice housed in glass cages without added scrapings. But two groups of mice from glass cages showed appreciable amounts of lead; and the figures for all groups were very variable. While, therefore, we think that lead poisoning must be regarded as a possible cause of the differences observed, we do not think that any conclusions on this point can be drawn from these experiments.

Summary of Section III

Summarizing the results obtained in this section we think it has been shown:

(*a*) That zinc cages, corroded as the result of frequent steam-sterilization, lowered the fertility of breeding mice and raised the death-rate and retarded the growth of young mice housed in them. Analysis of the zinc showed the presence of lead, and lead poisoning of the mice was suspected but could not be proved.

(*b*) That mice, fed on the same diet as the zinc cage mice, but housed in glass cages showed greater fertility and higher survival and growth rates.

(*c*) That the almost entire exclusion of light from the glass cages made no appreciable difference to the survival and growth of young mice.

(*d*) That the fertility, survival and growth rates of mice housed in glass cages containing scrapings from corroded zinc cages were of the same order as those of mice housed in zinc cages.

Conclusions

The results obtained in these experiments have been summarized at the end of each section. The more important conclusions that emerge from them may be repeated here.

(1) Zinc cages are unsuitable for housing mice, at least for the type of experiment with which we are dealing here. There appears to be some toxic element in the metal, or solder, used in their construction that adversely affects the condition, growth, fertility, and survival of the mice. Glass cages give far better results.

(2) "Synthetic" diets containing wheat gluten, or caseinogen, or a mixture of these two proteins with the addition of bran, dextrine, lard, cod-liver oil, yeastrel and salt mixture, reduced the fertility of the mice almost to zero. They supported the growth of young mice, but growth and survival rates were both very poor.

(3) A "natural" diet consisting only of whole oats to eat, and milk and water to drink, gave results that, except in regard to fertility, were as unfavourable as those given by the "synthetic" diets. In regard to the growth of young mice they were even more unfavourable.

(4) Various other "natural" diets, all containing oatmeal, bran, cod-liver oil and yeastrel, with various modifications and additions, were found to vary widely in value. The best results, as judged by the fertility of breeding does, infrequency of litter-eating, and the growth and survival of young mice, were obtained with three diets each of which contained about 25 per cent of dried separated milk. On these diets the mice throve well in all respects.

REFERENCES

ALEXANDER, J. & BULLOWA, J. G. M. (1910). *J. Amer. med. Ass.* **55**, 1196.

BATCHELOR, R. P., FEHNEL, J. W., THOMSON, R. M. & DRINKER, K. R. (1926). *J. industr. Hyg.* **8**, 322.

BEARD, H. H. (1925). *Amer. J. Physiol.* **75**, 645, 658, 682.

BERTRAND, C. & BHATTACHERJIE, R. C. (1934). *C.R. Acad. Sci.*, Paris, **198**, 1823.

DANIEL, J. F. (1912). *Amer. Nat.* **46**, 591.

EVANS, H. E. & BURR, G. O. (1917). *Mem. Univ. Calif.* Vol. **8**.

HAWK, P. B. (1923). *Proc. Soc. exp. Biol.*, N.Y., **20**, 269.

HUBBELL, R. B. & MENDEL, L. B. (1927). *J. biol. Chem.* **75**, 567.

KIRKHAM, W. B. (1920). *Proc. Soc. exp. Biol.*, N.Y., **17**, 196.

McCOLLUM, E. V., SIMMONDS, N. & PITZ, W. (1916). *J. biol. Chem.* **28**, 483.

—— —— —— (1917). *Ibid.* **29**, 342.

McHARGUE, J. S. (1926). *Amer. J. Physiol.* **77**, 245.

OSBORNE, T. B. & MENDEL, L. B. (1918). *J. biol. Chem.* **34**, 521.

PARKES, A. S. (1924). *Brit. J. exp. Biol.* **2**, 21.

PLIMMER, R. H. A. (1921). *Analyses and Energy Values of Food.* H.M. Stat. Office, London.

ROBERTSON, T. B. (1916). *J. biol. Chem.* **24**, 363.

ROBERTSON, T. B. & RAY, L. A. (1916). *Ibid.* **24**, 347.

SLONAKER, J. R. (1931 *a*). *Amer. J. Physiol.* **97**, 322, 573, 626.

—— (1931 *b*). *Ibid.* **98**, 266.

THOMPSON, P. K., MARSH, M. & DRINKER, K. R. (1927). *Ibid.* **80**, 65.

TOPLEY, W. W. C. (1923). *J. Hygiene*, **21**, 226.

WHEELER, R. (1913). *J. exp. Zool.* **15**, 209.

(*MS. received for publication* 9. II. 1937.—Ed.)