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STUDIES ON THE INFLUENCE OF DIET ON RESISTANCE TO INFECTION.

I. The Effect of Various Diets on the Fertility, Growth, and Survival of Mice.

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 animals, 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_5.

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 cc. of a 10% solution to every 100 g. of salt mixture.	
Iron lactate	7·08 "		
Copper sulphate, 0·02 cc. of a 10% solution to every 100 g. of salt mixture.			

The work of Alexander and Bullowa (1910), McCollum et al. (1916, 1917), Osborne and 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 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 and Burr (1927), 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		(a)	108	6·5
	Oct. 1933		(b)	88	3·5
	Oct. 1933		(c)	114	9·5
	Oct. 1933	N_2	(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	8·0
	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 tend to die within the first 8 weeks of life. Diets N_6, 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

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 8 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

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_8, 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 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 part of this paper, that this difference in growth rate was due to chance.

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	19·5
	Jan. 1936	N_8		4·0	12·0	20·0

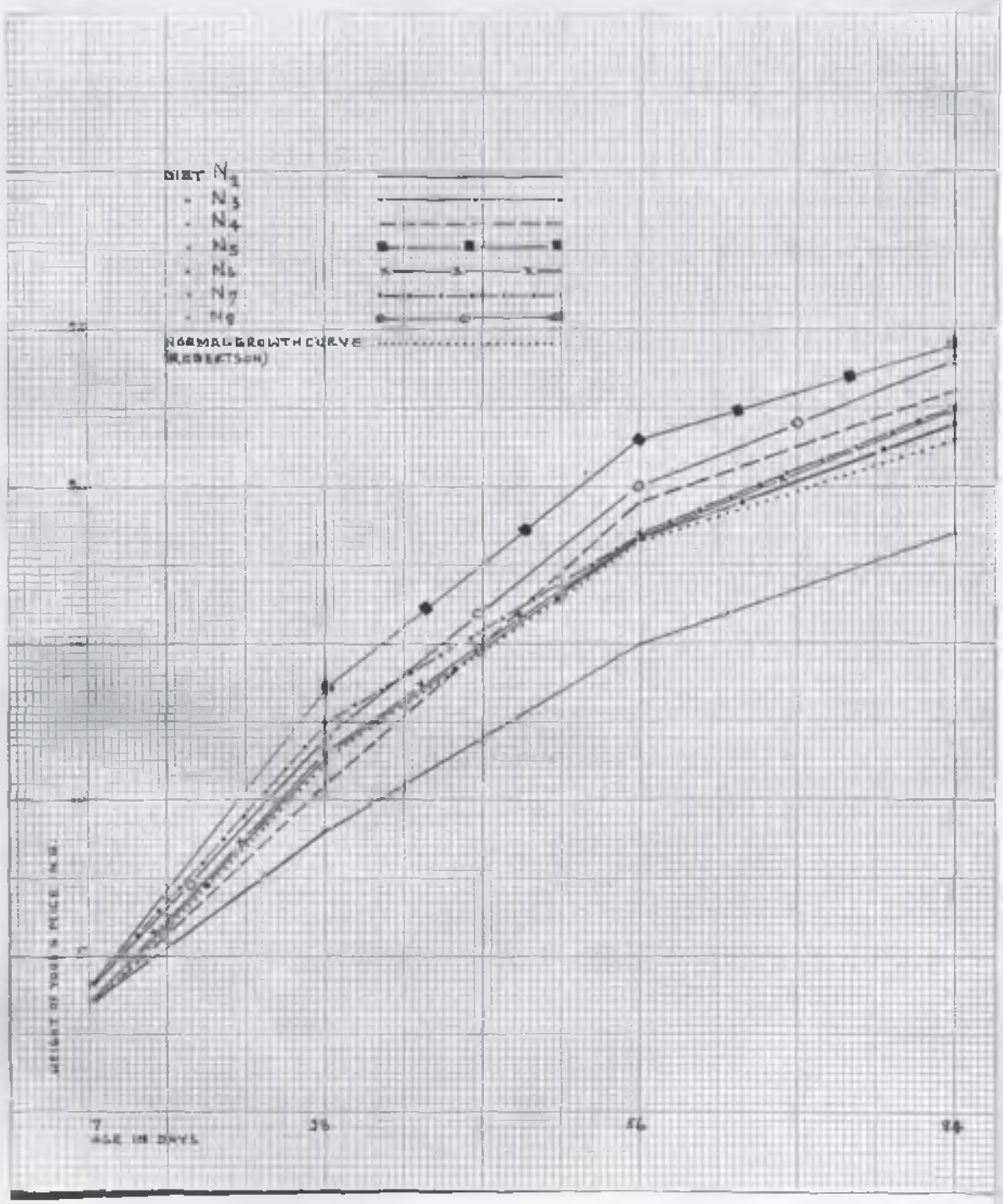
Table VII

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.

Fig. I.


DIET N1
N3
N4
N5
N6
N7
N8
NORMAL GROWTH CURVE
(ROBERTSON)
WEIGHT OF YOUNG MICE IN G.
AGE IN DAYS
7
28
56
84

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.

(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.

(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.

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.

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	

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.

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.

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

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		49·5		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

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

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" 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 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

Table XII

Diet	Series	Average wt. in g.of young	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 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

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 obtained 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.

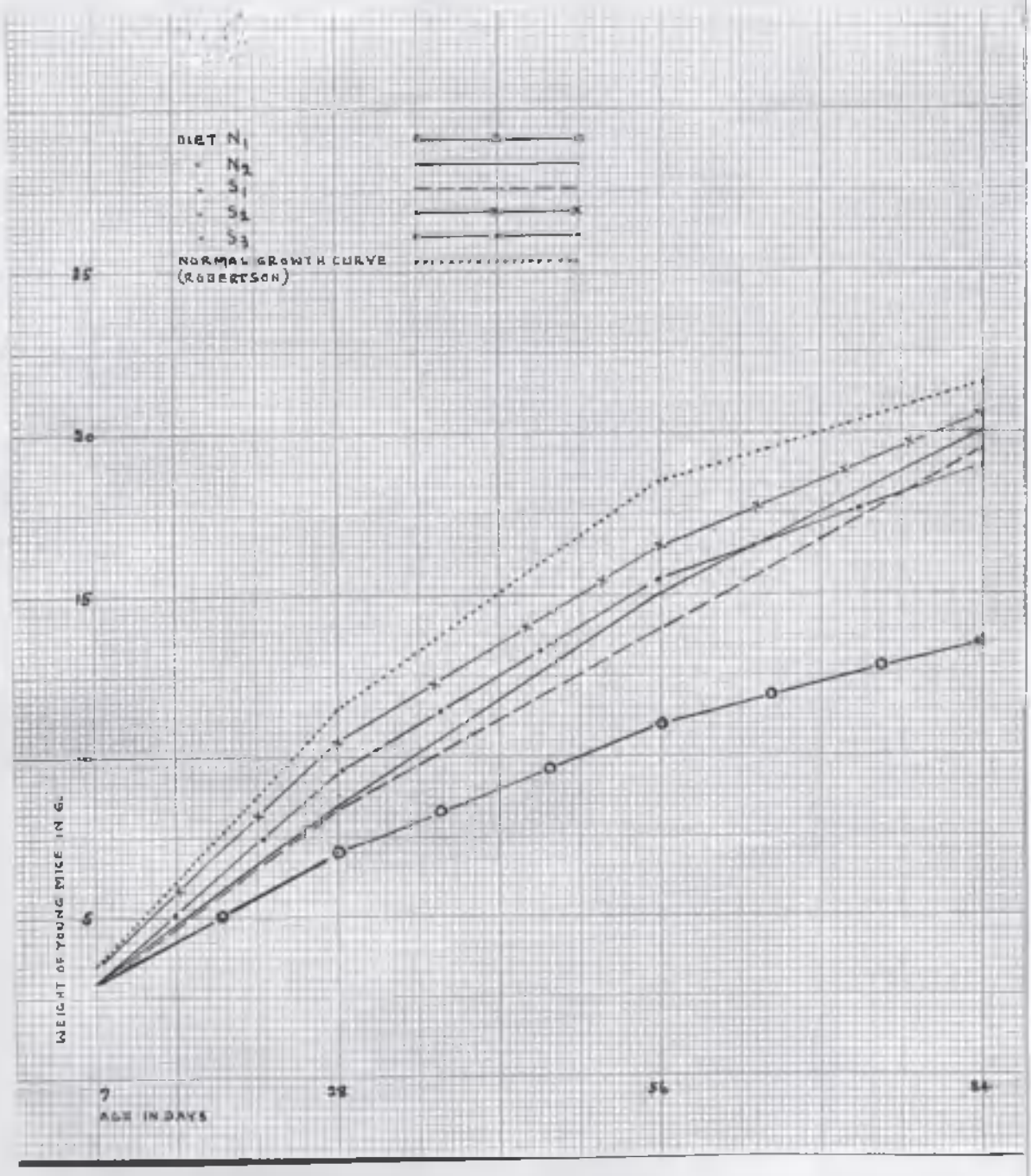
In all three "synthetic" diet groups many young mice died within the first few days of birth. In a few instances this early death was probably caused by neglect or inability to suckle on the part of the doe. In most cases, however, it was thought that death was due either to the lack of viability of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

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

Fig. 2


DIET N_1
" N_2
" S_1
" S_2
" S_3
NORMAL GROWTH CURVE (ROBERTSON)
35
30
15
5
WEIGHT OF YOUNG MICE IN G.
7
28
56
84
AGE IN DAYS

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.

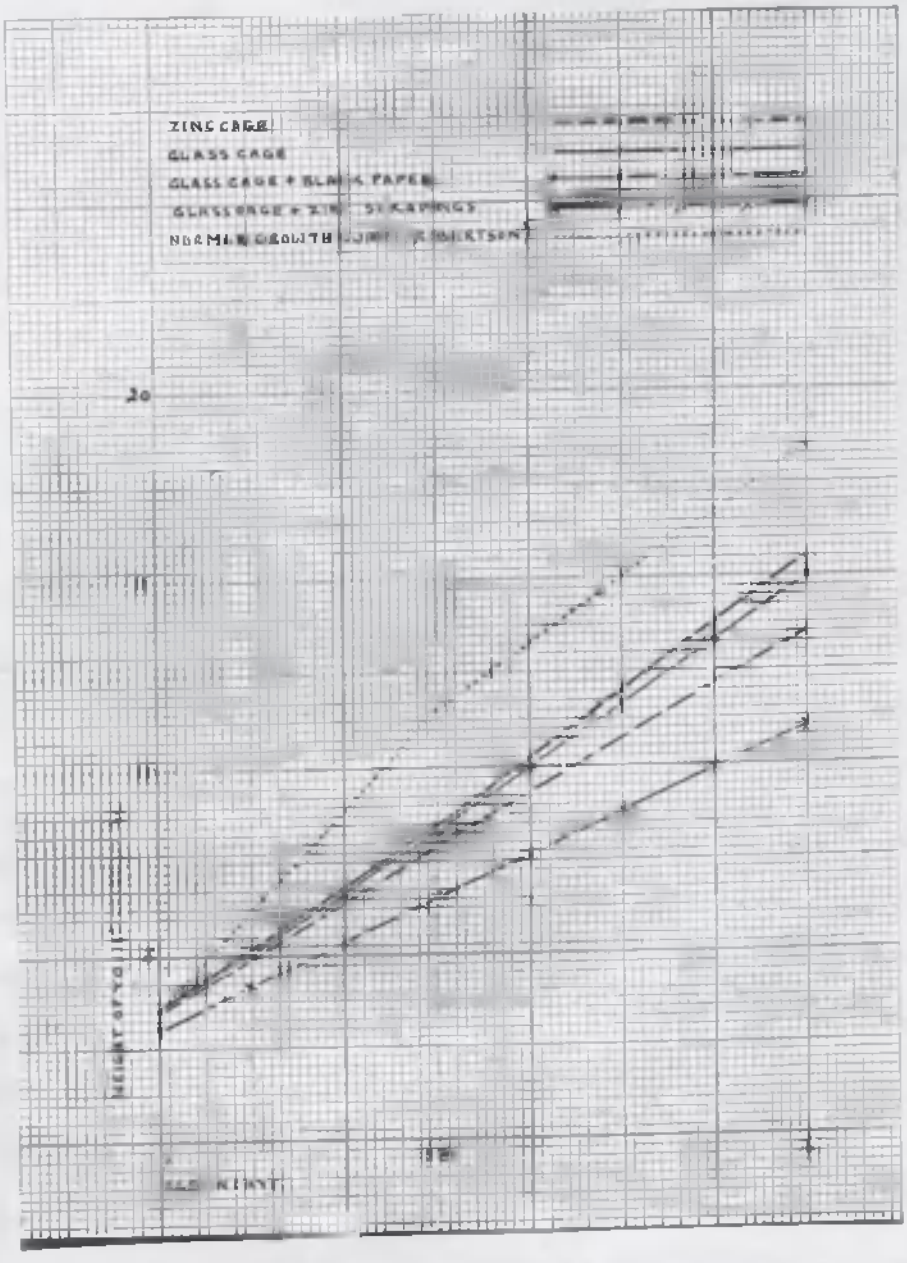
Four types of cage were used: (a) zinc cages, (b) glass cages, (c) glass cages covered with black paper, and (d) glass cages with added scrapings from corroded zinc cages. Except for the difference in the housing of the mice the experimental conditions were the same in all four experiments, and were 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.

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 weight in g. of young mice at 4 weeks	Average weight 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

Fig. 3


ZINC CAGE
GLASS CAGE
GLASS CAGE + BLANK PAPER
GLASS CAGE + ZINC SHAVINGS
NORMAL GROWTH (JOHN ROBERTSON)
20

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.

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 and 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.

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 and 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 and 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 and 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 and 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.

II. The Effect of Various Diets on the Resistance of Mice to Bacterial Infection.

The preceding part of this paper recorded experiments on the influence of various "natural" and "synthetic" diets on the fertility of breeding does, and on the growth and survival of young mice. The present part records the relative resistance of mice, bred and reared or fed for shorter periods on these diets, to Bact.typhi-murium, or to its endotoxin.

Although the investigation has been in progress for several years, a considerable portion of the time has been spent in breeding mice for infection experiments, and consequently that part of the work which deals with resistance to infection is still in its infancy. The results set forth here have been obtained from a comparatively small number of experiments, and many more observations will be necessary before conclusions that are beyond question can be put forward.

The problem of the effect of diet on resistance to infection is one that has attracted the attention of many investigators, but the experimental work has been confined mainly to studies of the influence of the vitamins, and in particular of vitamin A, on resistance to bacterial infection. The

experiments carried out by Drummond (1919), Hess et al. (1921), Cramer (1923, 1924, 1927), Werkman (1923), Gross (1924), Wolbach and Howe (1926), Goldblatt and Benischek (1927), Green and Mellanby (1928, 1930), Hotta (1928), Reiter (1929), Lassen (1930, 1931, 1932), Gudjónsson (1930), McClung and Winters (1932), Greene (1933), and others have shown that animals fed on diets lacking, or grossly deficient, in vitamin A are less resistant to spontaneous infections and more susceptible to experimental infections than are animals given an adequate amount of this vitamin.

Whether any of the other known vitamins, B and its various components, C, D and so on, have any significant effect on resistance to infection is far more doubtful. Reference to the voluminous literature, and especially to such reviews as those of Clausen (1934) and Robertson (1934), will reveal a mass of confusing and contradictory statements based on experiments carried out on an inadequate scale, and without regard to simple statistical requirements. The available evidence, taken as a whole, would seem to suggest that vitamins B and D have little, if any, influence on resistance, while in the case of vitamin C the observations recorded are particularly confusing and difficult to interpret.

In the present enquiry it was desired, at least during the preliminary stages, to avoid this particular dietetic problem, and the diets employed have, with one exception (N_1), contained, in approximately equal concentrations, an ample supply of vitamins A, B and D, in the form of cod-liver oil and yeastrel.

Before recording our own observations we may briefly review the available evidence, with regard to the influence on resistance to experimental

infection, of dietetic factors other than vitamins. It will be necessary, in some instances, to introduce the question of vitamins, since this has been specifically raised by the authors of the papers referred to.

Lange and Simmonds (1923) found no significant difference in the reaction of rats fed on diets of varying protein content to subcutaneous infection with bovine tuberculosis, either in the general condition, the weight curves, or in gross or microscopic autopsy findings. Rats fed on a diet deficient in salts but otherwise adequate are stated to have shown a more diffuse and extensive local reaction at the site of inoculation, and a slower dissemination and elimination of the lesions than did rats fed on a diet containing a larger amount of salt mixture.

Kligler and Geiger (1928) fed rats on a synthetic diet of varying salt content. The resistance of the rats was tested, not by a bacterial agent, but by the intraperitoneal injection of Trypanosoma evansi, and the duration of the infection was used as the index of individual resistance. The results of the experiments suggested a decreased resistance in rats fed on a salt-deficient diet, as compared with those maintained on a full or standard diet.

Hotta (1928) carried out a series of dietary experiments on mice, and concluded that either a salt-deficient or a partial starvation diet was effective in lowering the resistance of the mice to the intraperitoneal injection of mouse typhoid bacilli.

Webster and Pritchett (1924) tested the resistance to infection of mice fed on a modified McCollum diet consisting of 67·5 per cent whole-wheat flour, 15 per cent casein, 10 per cent milk powder, 5 per cent butterfat, 1 per cent sodium chloride, and 1·5 per cent calcium carbonate. The control mice, with

which the modified McCollum diet mice were compared, were fed on the ordinary stock diet of the Rockefeller Institute. This diet, which had proved adequate over a period of years for the breeding and rearing of successive generations of mice, consisted of a daily ration of baker's bread soaked in fresh, pasteurized Grade B milk warmed to at least 60-70°C., supplemented by two weekly feedings of an oatmeal and buckwheat mixture and one weekly feeding of dog biscuit. Three experiments, in which the mice were infected by stomach tube with Bact.typhi-murium, were carried out. In the third experiment the McCollum diet differed from that given in the first two experiments in that it was prepared from commercial materials instead of from carefully purified materials. In all three experiments pregnant mice, previously fed on the Institute diet, were given the modified McCollum diet or kept on the control diet, and the young, after weaning, were fed on the same diet that their mothers had received. The young mice, when they had attained the weight of 16-18 g., were infected per os, in groups of ten to thirty-six mice, with 2-5,000,000 Bact.typhi-murium. After infection the young mice were housed in separate cages. No tabulated protocols are given, but Greenwood et al (1936), in reviewing the experiments of Webster and Pritchett, constructed a table from the data available, and it is this table which is reproduced below.

	McCollum diet		Institute diet	
	No. tested	No. died	No. tested	No. died
Exp. 1.	10	1	10	8
Exp. 2.	26	6	26	26
Exp. 3.	33	4	36	22
Total	69	11	72	56

The figures give a mortality of 15·9 per cent among the mice fed on the McCollum diet as compared with 77·8 per cent among the mice fed on the Institute diet. These results quite clearly suggest, although only three experiments are recorded and the groups were not large, that the mice fed on the McCollum diet were more resistant than those fed on the Institute diet.

In two further experiments reported in the same paper, mice fed on the same two diets were compared in their resistance, in one case to mercuric chloride administered by stomach tube, and in the other to botulinum toxin injected intraperitoneally. In both these experiments the mice fed on the modified McCollum diet showed a delay in the time of death as compared with the mice on the Institute diet, but the difference between the death-rates of the two groups was not nearly so great as in the mice injected with living organisms. In the opinion of Webster and Pritchett the results from these last two experiments establish further evidence that the so-called general resistance of the host may be largely non-specific in character.

Pritchett (1927), in a further series of experiments with the same two diets, attempted to determine the relative value of the various constituents of the modified McCollum diet. The technique followed in these experiments differed in an important particular from that employed in earlier series. The mice tested were bred and fed, until the age of 6-8 weeks, on the Institute diet. At that age the mice were either transferred to the test diets or kept on the Institute diet as controls, and after 10-14 days' further feeding were infected per os, in groups of eighteen to fifty mice, with 4-5 x 10^6 Bact. typhi-murium. In the first experiment the diets compared were the Institute diet and the McCollum diet minus butterfat.

Here again no tabulated protocols are given, but the percentage mortalities, read from the small-scale curves, would appear to be 87·5 in twenty-four mice fed on the Institute diet, and 52·5 in twenty-five mice fed on the butter-free McCollum diet. The resistance of the butter-free McCollum diet mice, therefore, appeared to be greater than that of the Institute diet mice, though not so great as that of the mice fed on the complete McCollum diet. In the second experiment the diets tested were (a) Institute diet, (b) Institute diet plus 10 per cent whole-milk powder, (c) Institute diet plus 10 per cent casein, (d) Institute diet plus 10 per cent whole-wheat flour, and (e) McCollum diet minus butterfat. The percentage mortality figures, again read from the small-scale curves, were as follows:

Diet	No. tested	% died
Institute	47	74
Institute + 10% dried milk	20	85
Institute + 10% casein	18	83
Institute + 10% wheat flour	20	70
McCollum - butterfat	20	40

The addition to the Institute diet of the various constituents of the McCollum diet other than butterfat and mineral salts did not appear, therefore, to induce a resistance equal to that conferred by either the complete or the butter-free McCollum diet. In the third experiment the mice were fed on (a) Institute diet, (b) Institute diet plus 10 per cent butterfat, (c) Institute diet plus 10 per cent cod-liver oil, and (d) McCollum diet minus butterfat. The percentage mortalities, read from the small-scale curves, were as follows:

Diet	No. tested	% died
Institute	24	82·5
Institute + 10% butterfat	22	28·5
Institute + 10% cod-liver oil	22	50
McCollum - butterfat	24	50

In this experiment the lowest mortality was given by the mice receiving butterfat, and the author concludes that butterfat is evidently the most important single constituent of the complete McCollum diet, and that the protective action of butter may perhaps be found in cod-liver oil also.

The later experiments in this series were undertaken to determine the relative efficacy of fat-containing substances, especially with regard to their vitamin A content. Because of the possibility that the seasonal fluctuations in resistance noted in previous experiments might be due in some way to seasonal changes in diet, three fats of known vitamin A content were used in the test diets. These fats were butterfat, a fat known to vary seasonally in its content of accessory food factors; cod-liver oil, a fat known to be relatively constant in its content of such factors; and "Crisco", a vegetable fat thought to be free of vitamins. Three of the diets tested consisted of the Institute diet plus 5 per cent of one of these three fats. The other two diets in the experiment were the McCollum diet minus butterfat, and the Institute diet in which the milk was exposed to the direct light of a small mercury vapour lamp for 1 hour. Tests on mice fed on these diets were carried out from January to June inclusive, as at this time of year the mortality rates were likely to be highest. The mortalities in the groups on these diets as compared with the mortalities in the groups on the Institute

diet are given in the table, a modification of that of Greenwood et al.(1936).

Diet	No. tests	No. mice tested	% mortality
Institute	11	434	69·4
Institute + cod-liver oil	11	435	53·3
Institute	5	139	61·2
Institute + butter	5	138	49·3
Institute	5	139	61·2
Institute + Crisco	5	137	53·3
Institute	3	119	81·5
McCollum - fat	3	93	47·3
Institute	3	90	61·1
Institute + rayed milk	3	90	45·5

Taking the successive experiments with these diets as a whole, the mortality shown by the mice fed on the Institute diet was higher than that shown by the mice fed on any of the other diets. The Institute diet with rayed milk gave the lowest mortality, though it differed but little from that given by the McCollum diet without butterfat. The "Crisco" diet mice gave the most variable mortality, the mortality in successive tests being sometimes higher, sometimes equal to, and sometimes lower than that of the mice on the control Institute diet, but the average mortality was lower than that of the Institute diet mice, and was no higher than that of the mice fed on the cod-liver oil diet. The mortality of the mice fed on the Institute diet plus butterfat lay between that of the McCollum diet mice and that of the "Crisco" diet mice. Pritchett, in the discussion of the experimental

results, though noting the low mortality shown by the mice on the McCollum diet minus butterfat, puts forward the view that the addition of 5 per cent of an active animal fat to an apparently adequate diet increases the resistance of mice fed on it to *per os* infection with mouse typhoid, and that such a diet tends to stabilize the death-rate of animals so infected at a relatively low level, and so reduce the seasonal variation in mortality, the occurrence of which she considers to be established on the basis of the secular records of these and earlier experiments. The fact that the butter-free McCollum diet gives the most favourable comparison with the control, combined with the fact that the "Crisco" diet conferred only slightly less benefit, as compared with the control, than those diets containing cod-liver oil or butterfat, would seem, however, to be in disagreement with Pritchett's view that the vitamin A content is the determining factor in the influence exerted by these diets on resistance to *Bact. typhi-murium*.

Taking the experiments of Webster and Pritchett as a whole, there seems little doubt that the giving of the modified McCollum diet raised the resistance of the mice to infection with *Bact. typhi-murium*. The constituent of the McCollum diet responsible for the increase in resistance seems far more doubtful. From the results obtained in the earlier experiments Webster and Pritchett formed the opinion that butterfat, i.e. the vitamin A-containing constituent, was responsible, and this opinion was supported by Pritchett on the basis of her later experiments. But a study of the experimental results reveals certain facts which weaken this supposition. The McCollum diet without butterfat, though less effective in conferring resistance than the complete McCollum diet, gave a greater resistance than

the apparently adequate, except for its vitamin A content, Institute diet, and the Institute diet plus butterfat produced no better resistance than the McCollum diet minus butterfat. The addition, in Pritchett's series of observations, of vitamin A, in the form of either butterfat or cod-liver oil, to the diet caused a small but suggestive decrease in the mortality rates of the mice fed on the Institute diet alone, but the mice on the diet containing "Crisco", a fat apparently devoid of vitamin A, gave a mortality, calculated from the average death-rate in all the tests with this diet, no greater than that shown by the mice fed on the diet containing cod-liver oil, and a mortality insignificantly less than that of the mice fed on the diet containing butterfat. No direct comparison of the McCollum diet with or without butterfat is reported in the Webster and Pritchett papers, and it would appear that without a comparison such as this the true value of the vitamin A-containing constituent of the McCollum diet must remain in doubt.

Viewed in the light of our own findings, the results obtained by Webster and Pritchett assume a significance that is not suggested by them; but it will be more convenient to discuss this point after our own observations have been described.

The earlier experiments of Webster, and of Webster and Pritchett, were designed to test the resistance to infection of individual mice and did not deal with epidemic spread. In a later experiment Webster (1930) compared the resistance to Bact.typhi-murium of herds of mice fed on the Institute diet with that of herds fed on the McCollum diet. The mice were fed for a time on the Institute diet and then transferred to the McCollum diet, or vice versa, and the observations were carried on in four herds recruited by the

daily addition of two normal mice for a period of 2 years. Webster found that a change from the Institute diet to the McCollum diet was followed by a fall in the mortality rate, while a change from the McCollum diet to the Institute diet had the reverse effect. The McCollum diet, therefore, apparently was effective in increasing herd resistance in addition to increasing the individual resistance of mice. The four herds, however, were not comparable in all respects for the entire experimental period of 2 years, for in two herds, within a few months of the change over from the McCollum diet to the Institute diet, the daily additions of normal mice were made from a relatively susceptible breed instead of from a relatively resistant breed as had formerly been the case.

Another series of experiments was carried out by Topley et al. (1931). In some of these experiments, the method adopted was that of the closed epidemic. In each trial twenty-five mice were infected intraperitoneally with approximately 1000 Bact. typhi-murium. They were immediately added to 100 normal mice in a large cage, and the happenings in the cage were observed over the following 60 days. In comparing the course of events in the different cages, attention was confined to the 100 normal mice exposed to risk. In this set of experiments five experimental diets, in addition to a control diet consisting of whole oats and the provision, in drinking vessels, of a mixture of equal parts of water and pasteurized milk, were tested. Each diet group was set up in duplicate. The diets were designed, mainly in view of the results recorded by Webster and Pritchett, to test the effect of various fat constituents, in varying amounts, on the resistance of

mice to contact infection. They were as follows:

	A	B	C	D	E
Whole-meal flour	60	60	20	20	60
Casein	20	20	20	20	20
Butter	5	-	40	-	5
Lard	-	-	-	33	-
Vitamin A concentrate	-	5	-	-	-
Sodium chloride	1	1	1	1	-
Calcium lactate	2	2	2	2	-

The results of these experiments appeared to be at variance with those recorded by Webster and Pritchett. Each of the herds on the test diets showed a higher mortality than the two on the control diets. The basal diet A which, apart from the absence of milk powder, did not differ greatly from the modified McCollum diet employed by Webster and Pritchett, was no exception, but this diet, and the same diet without the salt mixture, gave the lowest mortalities apart from those shown by the two control herds. The addition of vitamin A concentrate, or of excess lard, was associated with a definite increase in mortality. In the second set of experiments, in which the mice were housed in separate cages, the technique bore a greater resemblance to that employed by Pritchett. Four groups, each of fifty mice, were fed for 14 days on the control diet, diets A (basal), B (basal plus vitamin A), or C (excess butter). On the 14th day each mouse of each group was injected intraperitoneally with 1000 Bact.typhi-murium. The diets given to the mice before inoculation were continued. The results were as follows:

	% mortality	S.E.	Mean survival time limited to 14 days	S.E.
Control	82·0	5·43	6·9	0·70
Basal	74·0	6·20	7·9	0·70
Basal + vitamin A	78·0	5·86	8·6	0·61
Excess butter	74·0	6·20	8·5	0·61

There is here no suggestion, as in the earlier closed epidemic experiments, that the replacement of the control by any of the three test diets was associated with an increase in mortality; indeed, the mice on the test diets suffered a slightly lower mortality, and lived on the average slightly longer, than the control group; but the difference is in no case statistically significant. The authors suggest the possibility that some factor, other than the effect of the diet in raising or lowering resistance, and operating in the epidemic cages, may have been responsible for the results obtained in the closed epidemic experiment, and conclude that, if any benefit at all was derived from the diets tested, it was so slight as to be more than counterbalanced by other influences that may have increased the facilities for contact infection.

In a third set of experiments, this time of the closed epidemic type, twelve herds were tested. Three of the herds received the control diet of whole oats and milk and water mixture, three the control diet plus the daily addition of cabbage, three the control diet plus the daily addition of mangolds, and three the control diet plus the daily addition of carrots. The experimental results showed that, taking the results obtained in the different herds on the same diet as a whole, the addition to the control diet of cabbage, mangolds, or carrots did not raise the resistance of the mice.

Experimental.

In the experiments described here a comparison was made of the resistance to Bact.typhi-murium of mice fed on eight different diets. These eight diets fall into two groups. In the first, or "synthetic", diet group the diets were composed mainly of artificial food substances, and the resistance experiments were carried out on young mice bred from does that, mated on one or other of the control diets, had been transferred when pregnant to the test diets. In the second, or "natural", diet group the diets were mainly made up of natural food substances, and the young mice infected were bred from mice which had been transferred to the test diets 3 weeks before mating. The experimental conditions in the two diet groups were therefore not in all respects comparable, though in both series the does had been fed on the test diets during pregnancy, and the mice tested for resistance had been fed on them from birth.

Influence of "synthetic" (S) diets on resistance to infection.

The constitution of the "synthetic" diets has been given in Table VIII of the preceding part, and need not be repeated here. It may, however, be recalled that diet S_1 contained gluten as its protein constituent, diet S_2 contained caseinogen, and diet S_3 a mixture of gluten and caseinogen in equal amounts. These synthetic diets were controlled by two "natural" diets, one of which (N_1), consisted only of whole oats to eat, and a mixture of milk and water to drink, while the other (N_2) contained oatmeal, cod-liver oil, yeastrel and bran, with the same mixture of milk and water.

Six experiments were carried out, and all six contained mice fed on the

three experimental diets. Diet N_1 was the only control diet in the first three resistance tests, but with this diet there was difficulty in rearing a sufficiency of young mice for an adequate control, and in the fourth and fifth tests mice bred on diet N_2 were added as a second control. In the sixth experiment diet N_2 was the only control diet.

The young mice infected in the first four experiments were bred from does which had been mated on diet N_1 and transferred, as soon as pregnancy was definitely established, to one of the three test diets. The control young mice were bred from does fed on diet N_1 only. The litter rate in mice mated on diet N_1 was found to be too low to provide an adequate number of young mice, and consequently in the fifth and sixth experiments the young mice tested for resistance were bred from does mated on diet N_2 instead of diet N_1. Except for this change in the diet on which the breeding mice were mated the young mice in Exps. 5 and 6 were in all respects comparable with the mice tested in the earlier experiments.

The young mice were fed after weaning on the diets that their mothers had received during pregnancy and lactation. When approximately 12 weeks old they were infected per os with 100×10^6 Bact.typhi-murium, each mouse being infected separately. After infection the mice were housed in separate cages, and the diet given before infection was continued. Mice that died were examined post-mortem, and cultures taken from the heart and spleen. The survivors were killed on the 28th day after infection and were likewise examined.

The results of these experiments are set out, in different forms, in Tables XIV and XV. Taking the latter table first it will be seen that there

is a suggestion that the mice receiving their protein, other than that contained in the bran, entirely (S_2) or partly (S_3) in the form of caseinogen were slightly more resistant than mice receiving all their protein in the form of gluten (S_1). Taking the average figures the S_1 mice were no more resistant than mice fed on the "natural" diet N_2, and little, if at all, more resistant than the mice fed on diet N_1; but it must be noted that those latter averages are not strictly comparable, since they do not cover identical series of tests.

Table XIV.

	Diet....	N_1	N_2	S_1	S_2	S_3
Exp. 1	No. of mice infected	19	-	7	18	17
	% survivors	21·0	-	43·0	44·5	47·0
Exp. 2	No. of mice infected	12	-	8	23	10
	% survivors	41·5	-	62·5	17·5	70·0
Exp. 3	No. of mice infected	16	-	13	11	19
	% survivors	12·5	-	0·0	9·0	42·0
Exp. 4	No. of mice infected	19	47	25	35	43
	% survivors	16·0	32·0	40·0	65·5	39·5
Exp. 5	No. of mice infected	18	57	32	35	38
	% survivors	15·5	21·0	22·0	48·5	39·5
Exp. 6	No. of mice infected	-	49	10	19	17
	% survivors	-	26·5	20·0	26·5	35·5
All experiments	No. of mice infected	84	153	95	141	144
	% survivors	20·0	26·0	28·5	41·0	42·5

Table XV

No. of mice infected	Diet	% mortality in 28 days	Mean survival time limited to 28 days
84	N_1	80 S.E. = 4·36	14·18 S.E. = 0·92
153	N_2	74 S.E. = 3·55	16·42 S.E. = 0·66
95	S_1	71·5 S.E. = 4·63	16·88 S.E. = 0·82
141	S_2	59 S.E. = 4·14	19·16 S.E. = 0·70
144	S_3	57·5 S.E. = 4·12	19·11 S.E. = 0·71

The actual significance of the recorded differences in mortality between the mice receiving caseinogen and those receiving only vegetable protein is, however, very doubtful. Omitting the results obtained with diet N_1, the ratios of the observed differences to their standard errors are not large. Moreover, reference to Table XIV, in which each experiment is recorded separately, shows how great was the variability in the results recorded in successive tests. It is true, of course, that considerable variability is to be expected with such small groups as these; but the fact that in only two of six tests did mice fed on diet S_2 appear to be much more resistant than mice on diet S_1, while in one test they appeared to be more susceptible, raises grave doubts as to the significance of an apparent advantage shown by the averaged mortalities.

The fact that lends some support to the view that the observed differences are not likely to have been due entirely to sampling errors is that an apparent increase in resistance is shown by both groups of mice that were given caseinogen in their diet as compared with any of the three groups receiving only vegetable protein. Even so, we should attach little if any importance to the results of these experiments taken alone. We think, however, that they may be accorded some significance in relation to the far more striking observations recorded in the following section.

Influence of "natural" (N) diets on resistance to infection

We have noted, in the preceding paper, how inadequate were the "synthetic" diets as judged by the growth and survival of young mice receiving them. It was this inadequacy that rendered it impossible to rear groups of suitable size for the resistance tests. Since certain of the "natural" diets had proved greatly superior from this point of view, the "synthetic" diets were abandoned, while the effect of certain of these "natural" diets on resistance was studied more extensively, and in greater detail.

The constituents of these diets, which have already been given in the preceding part of this paper, are repeated for convenience in Table XVI.

Table XVI

Diets...	N_2	N_4	N_5	N_8
Coarse oatmeal	92	87	40	40
Dried separated milk	-	-	25	25
Dextrins	-	-	23	-
Flour and water biscuit	-	-	-	23
Salt mixture no. 3	-	5	-	-
Coconut oil	-	-	4	4
Cod-liver oil	1	1	1	1
Yeastrel (dry weight)	2	2	2	2
Wheat bran	5	5	5	5
Percentage of total protein in diet	11·87	11·25	14·66	17·42
Mouse ration per day (g.)	6	6	6	6
Milk and water mixture per day approximately (c.c.)	2	2	2	2

Salt mixture no. 3

Sodium chloride	10 g.
Magnesium sulphate	30 "
Potassium citrate	30 "
Calcium lactate	70 "
Iron lactate	7 "

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

Of these four diets, one was the control diet N_2, and the others were the three diets which, in the fertility, survival and growth experiments described in another paper, had given the best survival and growth in young mice. The first series of resistance experiments was carried out on mice bred on those four diets.

In these experiments, the mice tested were the offspring of bucks and does that from 3 weeks before mating had all been fed on no other than the test diets. The young mice, after weaning, were given the diet which their parents received, and when approximately 12 weeks old were tested for resistance. After infection with Bact.typhi-murium the mice were housed in separate cages, and the diet given before infection was continued. Mice that died or were killed on the termination of the experiment were examined post-mortem, cultures being taken from the heart and spleen.

In one group of experiments mice bred and reared on one or other of the test diets were infected per os, each with 100×10^6 Bact.typhi-murium; in another group of experiments they were infected intraperitoneally with 100,000 Bact.typhi-murium; in a third group of experiments they were injected intraperitoneally with a toxic fraction isolated from Bact.typhi-murium. The experiments with Bact.typhi-murium were terminated 28 days after infection, and those with the toxic fraction 5 days after injection.

Results of Experiments.

(1) Per os infection with Bact.typhi-murium.

The first three of the experiments in which the mice were infected per os contained mice from each of the diet groups N_2, N_4 and N_8. In the

fourth and fifth experiments no mice from diet N_4 were included as at the time of infection the mice on this diet were not of comparable age with the mice on the other diets. The fifth experiment contained, for the first time, mice bred on diet N_8.

The results of this series of experiments are set out in Tables XVII-XIX and in Figs. 4-8.

Table XVII. Bred mice. Per os infection.

Date of infection	No. of B. typhimurium infected	Diet	No. of mice infected	No. of mice dying	% survivors on 28th day	Mean survival time limited to 28 days
					†	†
10.vii.35	100×10^6	N_2	50	24	52 S.E. = 7·07	21·04 S.E.= 1·13
		N_4	50	13	74 S.E. = 6·20	24·74 S.E.= 0·88
		N_8	50	9	82 S.E. = 5·43	25·34 S.E.= 0·87
13.ix.35	100×10^6	N_2	50	30	40 S.E. = 6·93	19·12 S.E.= 1·14
		N_4	50	32	36 S.E. = 6·79	18·06 S.E.= 1·20
		N_8	50	8	84 S.E. = 5·18	25·34 S.E.= 0·92
31.i.36	100×10^6	N_2	50	39	22 S.E. = 5·86	16·84 S.E.= 1·11
		N_4	50	26	48 S.E. = 7·06	18·50 S.E.= 1·32
		N_8	50	27	46 S.E. = 7·05	18·26 S.E.= 1·35
27.iii.36	100×10^6	N_2	50	26	48 S.E. = 7·06	19·22 S.E.= 1·27
		N_8	50	31	38 S.E. = 6·86	19·10 S.E.= 1·19
26.vi.36	100×10^6	N_2	50	36	28 S.E. = 6·35	17·06 S.E.= 1·14
		N_5	50	16	68 S.E. = 6·60	23·20 S.E.= 1·03
		N_8	50	12	76 S.E. = 6·04	24·40 S.E.= 0·98

† Standard errors of the proportions and means respectively.

It will be convenient to take first the comparison between the mice fed on the control diet (N_2) containing oatmeal, cod-liver oil, yeastrel and bran, with milk and water to drink, with those fed on diet N_8 in which the oatmeal, forming 92 per cent of the N_2 diet, was reduced to 40 per cent, the

Table XVIII. Bred mice compared using percentage survivors on 28th day

	Diets compared	Difference	S.E. of Difference	Difference/ S.E. of difference	X^2 test of group difference
Exp. 1. 10.7.35	N_2 and N_4	- 22	9·7	2·3	X^2 = 11·36
	N_2 and N_6	- 30	9·4	3·2	n = 2
	N_4 and N_8	- 8	8·3	0·9	P < 0·01
Exp. 2. 13.9.35	N_2 and N_4	+ 4	9·7	0·4	X^2 = 28·50
	N_2 and N_6	- 44	9·7	4·5	n = 2
	N_4 and N_6	- 48	9·8	4·9	P < 0·01
Exp. 3. 31.1.36	N_2 and N_4	- 26	9·5	2·7	X^2 = 8·83
	N_2 and N_8	- 24	9·5	2·5	n = 2
	N_4 and N_6	+ 2	10·0	0·2	P nearly 0·01
Exp. 4. 27.3.36	N_2 and N_6	+ 10	9·9	1·0	-
Exp. 5. 26.6.36	N_2 and N_6	- 40	10·0	4·0	X^2 = 27·04
	N_2 and N_8	- 48	10·0	4·8	n = 2
	N_6 and N_8	- 8	9·0	0·9	P < 0·01

† In addition to comparing the separate pairs by means of their standard errors, the differences between the group of diets in each experiment have been tested by the X^2 method. Where the probability P is less than 0·02 the observed differences may be regarded as unlikely to have arisen by chance.

Table XIX. Bred mice compared using mean survival time of mice infected.

	Diets compared	Difference	S.E. of difference	Difference/S.E. of difference
Exp. 1 10.7.35	N_2 and N_4	- 3·70	1·43	2·59
	N_2 and N_8	- 4·30	1·43	3·01
	N_4 and N_6	- 0·60	1·24	0·48
Exp. 2 13.9.35	N_2 and N_4	+ 1·06	1·66	0·64
	N_2 and N_8	- 6·22	1·46	4·26
	N_4 and N_8	- 7·28	1·51	4·82
Exp. 3 31.1.36	N_2 and N_4	- 1·66	1·72	0·96
	N_2 and N_6	- 1·42	1·75	0·81
	N_4 and N_6	+ 0·24	1·89	0·13
Exp. 4. 27.3.36	N_2 and N_6	+ 0·12	1·74	0·07
Exp. 5 26.6.36	N_2 and N_6	- 6·14	1·54	3·99
	N_2 and N_8	- 7·34	1·50	4·89
	N_6 and N_8	- 1·20	1·42	0·85

remainder being replaced by dried separated milk (25 per cent), dextrine (23 per cent) and coconut oil (4 per cent). Taking Table XVII it will be seen that the mice fed on diet N_5 proved much more resistant than mice fed on diet N_2 in four out of the five trials. In the one exception, the experiment started on 27.iii.36, the mice fed on diet N_2 proved slightly more resistant than the mice fed on diet N_5.

Turning to Tables XVIII and XIX, it will be seen that the observed difference between the percentage survivorship of the N_2 and N_5 groups is statistically significant in each of the four instances in which the N_5 group proved the more resistant, but is insignificant in the single instance in which the N_2 mice appeared more resistant than the N_5 mice. The difference in mean survival time is significant in three of four trials in which the N_5 mice were more resistant. It is insignificant in the trial of 31.i.36, in which the percentage survivorship of the N_5 mice was significantly greater than that of the N_2 mice, and in the trial of 27.iii.36, in which the N_2 mice appeared slightly more resistant. This suggests the possibility that the factors, whatever they may have been, that were responsible for the anomalous results obtained in the trial of 27.iii.36 were beginning to be operative in January of that year, or possibly somewhat earlier.

Omitting the tests of 31.i.36 and 27.iii.36, the results of the remaining three experiments are rather striking. In each of them the mice fed on diet N_5 proved much more resistant to per os infection with Bact. typhi-murium than the mice fed on diet N_2, the mortalities being 18, 16 and 24 per cent in the N_5 groups, as compared with 48, 60 and 72 per cent in the N_2 groups.

Fig. 4.

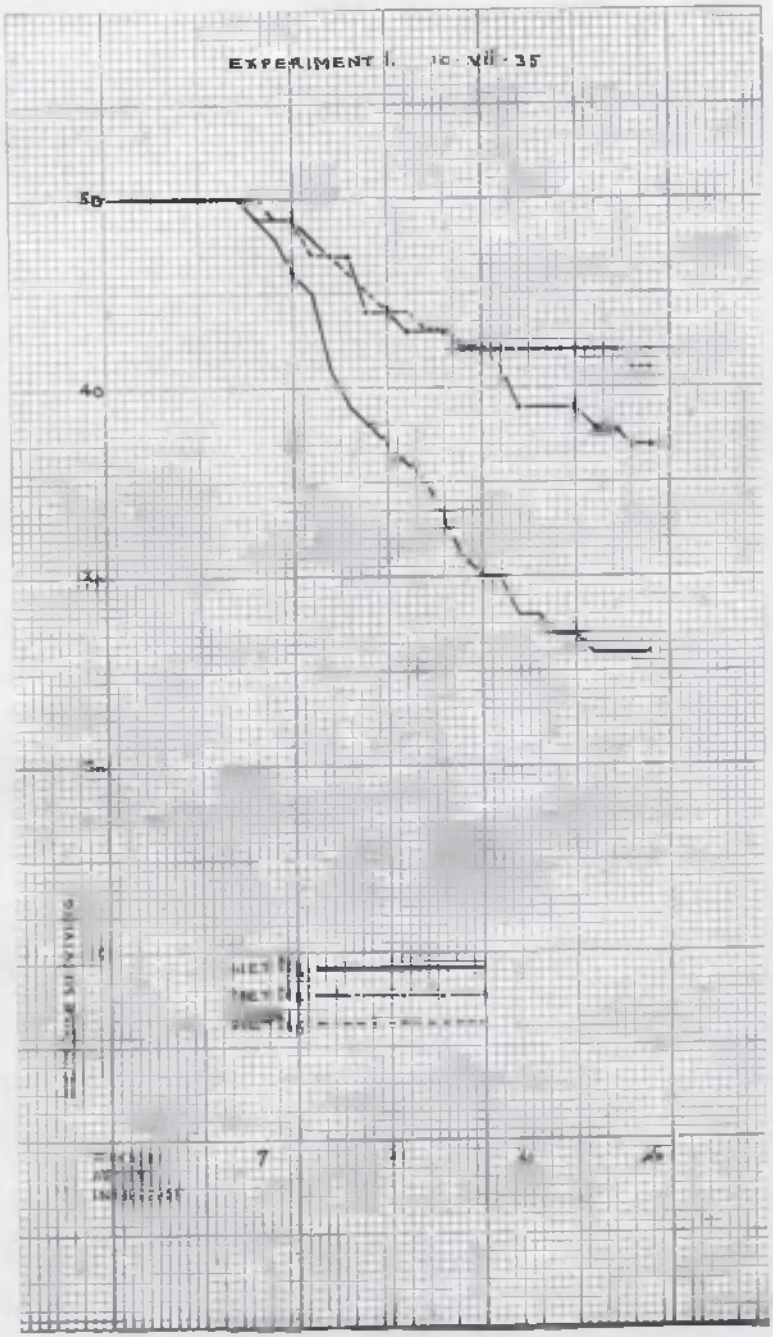


Fig. 5

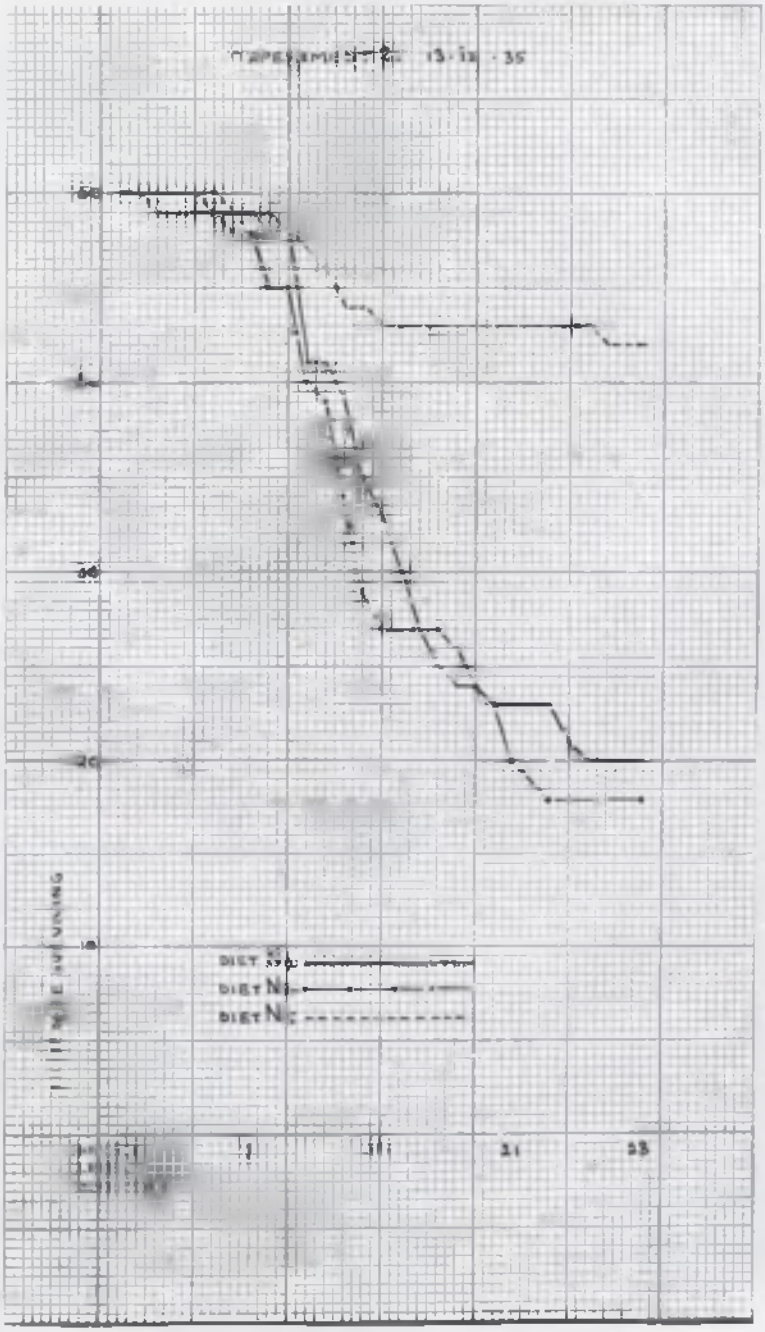


Fig. 6

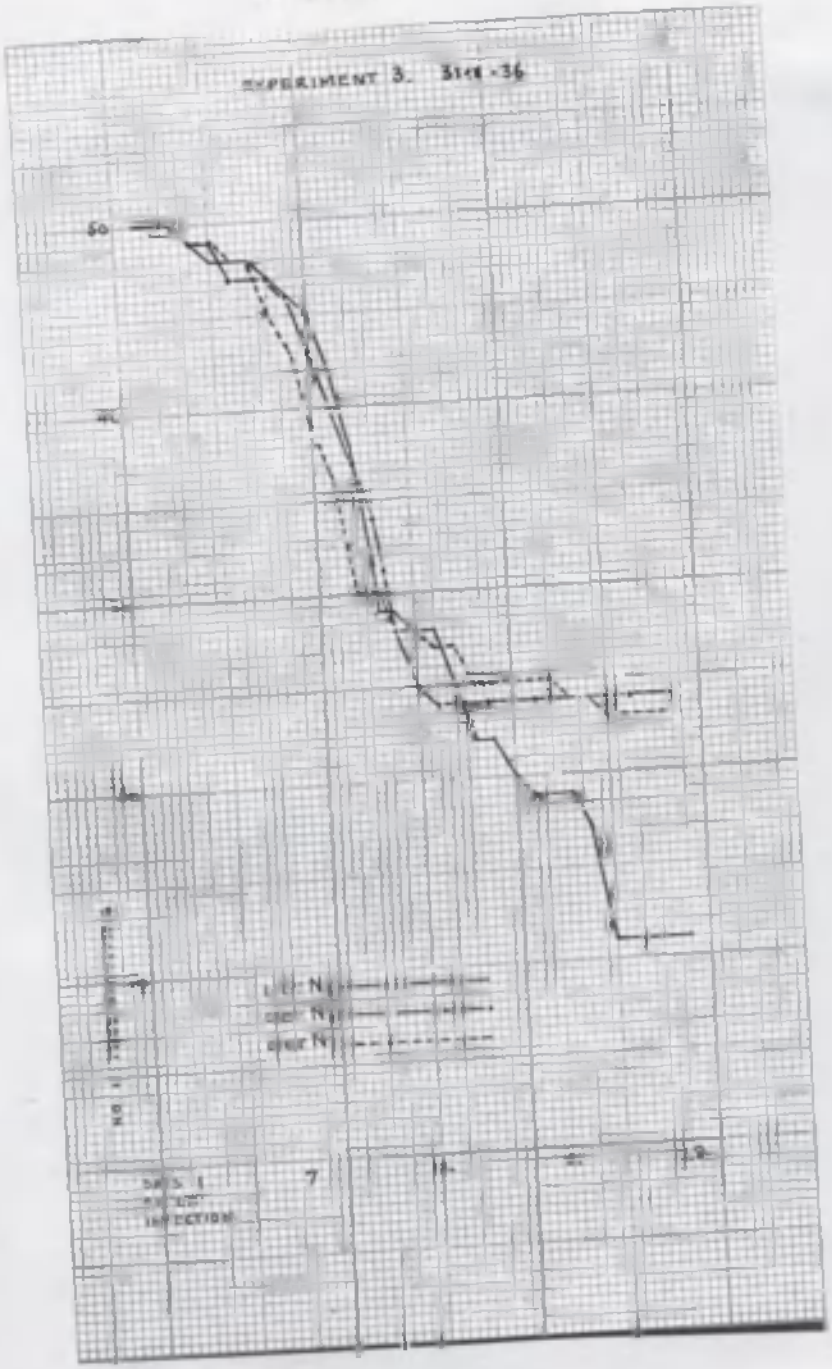


Fig. 7

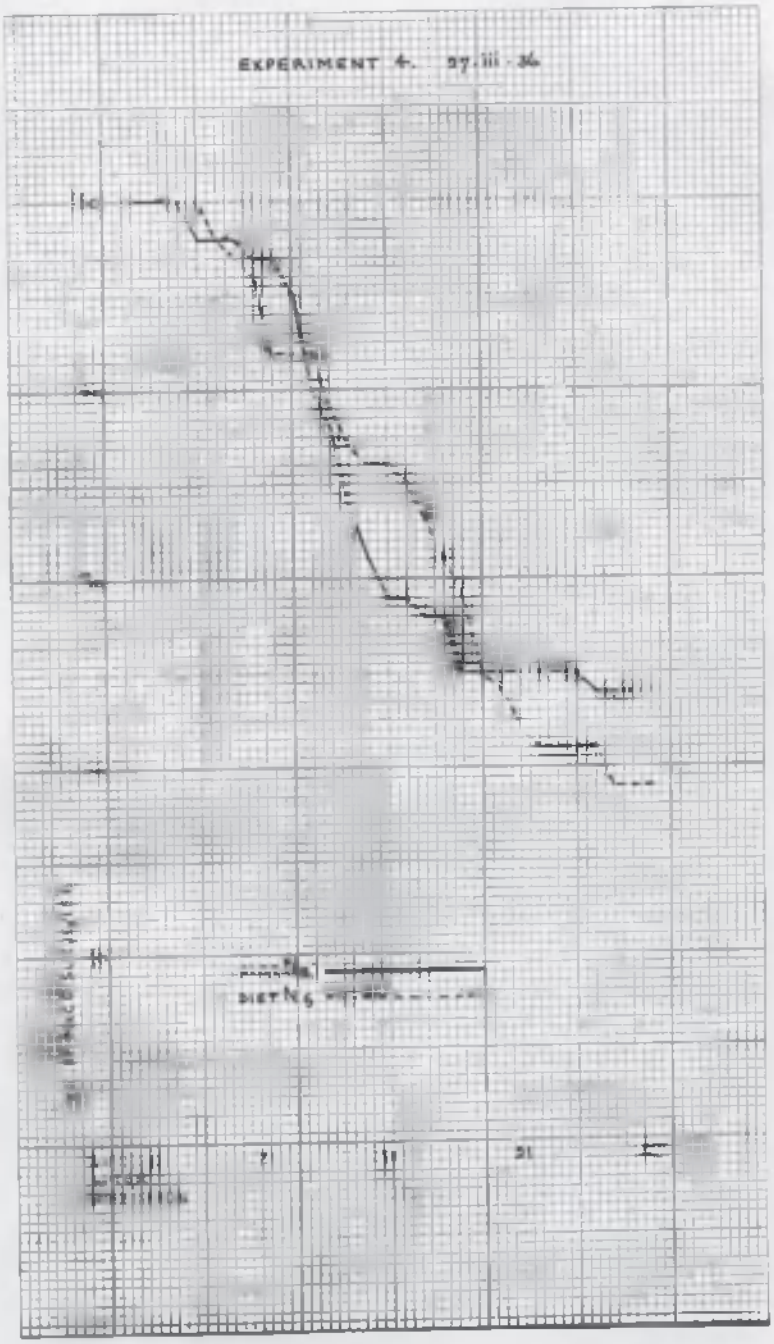
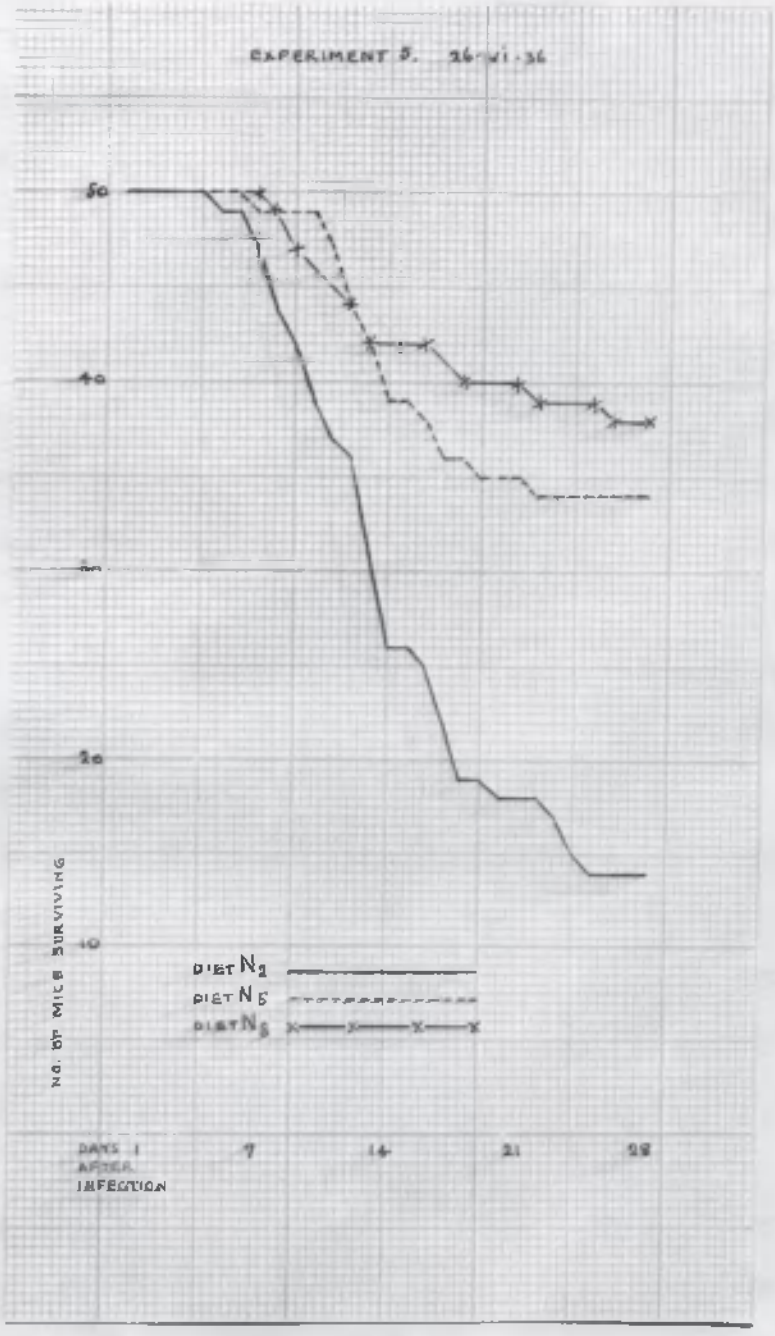


Fig. 8


EXPERIMENT 5. 26-VI-36
50
40
30
20
10
NO. OF MICE SURVIVING
DIET N_1
DIET N_5
DIET N_8
DAYS AFTER INFECTION
7
14
21
28

We cannot, however, disregard the discrepant result obtained in the test of 27.iii.36. We think it very unlikely that this discrepancy was due to chance. As will be seen in later sections, similar discrepancies were observed in other series of tests, and they occurred during approximately the same period. Moreover, it was noted in the preceding paper that, of the two series of breeding experiments with diet N_6, the young mice in the first series recorded throve considerably better than the young mice in the second series. It was the young mice of the second series that failed to show an increased resistance in this, and in other, series of tests. Subsequent resistance tests, such as that of 26.vi.36, were carried out on mice, not referred to in the preceding paper, which had shown optimal development on the N_6 diet.

We believe, therefore, that some factor intervened to neutralize the effect of the N_6 diet in one particular series of mice.

What this factor may have been we cannot tell. It had become clear, by the time this discrepancy was observed, that the N_6 diet probably owed its efficacy in improving fertility, growth, survival and resistance to the dried milk which it contained; an obvious possibility was that some difference existed in the quality of the milk used during the two periods in question, and, in particular, that this difference might be traceable to periods of stall and pasture feeding of the cattle.

Kohler *et al.* (1936) found that rats given milk from stall-fed cows grew more slowly than rats given milk from pasture-fed cows, and that the addition of fresh grass juice to winter milk increased the weight of the rats fed on it almost to the level attained by rats fed on summer milk.

The dried separated milk used in our experiments was obtained from Wilts United Dairies (London) Limited, who have very kindly supplied us with information concerning the time of preparation of the various batches, and their probable relation to stall and pasture feeding. The correlation of these data with the experimental results obtained by us yields no support to the view that the difference between stall and pasture feeding, or any other discoverable factor relating to the quality of the milk, can be made to account for the discrepancies observed by us. For the moment, then, we must be content to leave these discrepancies unexplained, merely noting that their time relations, and their occurrences in mice that have shown a relatively poor development on the N_5 diet, make it unlikely that they were due to simple sampling errors. No one who has had much experience of animal experiments of this kind will be surprised to encounter discrepant results in any such series of tests. We are very far as yet from being able to control all our variables.

Turning briefly to diets N_4 and N_8, it will be noted that N_4, containing no dried separated milk, but containing an alkaline salt mixture, gave very anomalous results. It was tested on three occasions; on the first the mice reared on it appeared to have a resistance intermediate between those reared on diet N_2 and those reared on diet N_5, on the second the N_4 mice proved as susceptible as the N_2 mice, while the N_5 mice were highly resistant, on the third the N_4 mice were as resistant as the N_5 mice, and considerably more resistant than the N_2 mice. It will be more convenient to discuss the probable significance of these findings at a later stage.

In the single test with diet N_8, which resembled diet N_5 in containing

dried separated milk, but differed from it in containing flour-and-water biscuit in place of dextrine, the N_8 mice were slightly more resistant than the N_5 mice, which in their turn were greatly more resistant than the N_2 mice. This experiment therefore strengthens the view that a diet containing dried separated milk increases resistance to per os infection with Bact. typhi-murium.

(2) Intraperitoneal infection with Bact. typhi-murium

In these experiments young mice that had been bred and reared on the various diets under test were injected intraperitoneally with 100,000 Bact. typhi-murium, and thereafter housed in separate cages, and observed for 28 days. Only two tests of this type were carried out. The results are recorded in Tables XX-XXII and Figs. 9 and 10 and need little comment. Mice reared on diet N_5 show a slightly greater resistance than mice bred on diet N_2; but the difference bears no comparison with the difference to infection per os. Mice reared on diet N_4, containing the alkaline salt mixture but no dried milk, are no more resistant to intraperitoneal infection than mice reared on diet N_2.

(3) Resistance to the intraperitoneal injection of a toxic fraction isolated from Bact. typhi-murium

The toxic fraction used in these experiments was that prepared by Raistrick, Topley and their colleagues by tryptic digestion of the bacterial bodies followed by alcohol precipitation (Raistrick and Topley, 1934). It consists of a complex polysaccharide, united to a component containing nitrogen, phosphorus, sulphur and fatty acids, that may be a phosphatide. It is identical with, or very closely similar to, the toxic fraction

Table XX. Bred mice. Intraperitoneal infection with Bact. typhi-murium

Date of infection	No. of Bact. typhi-murium infected	Diet	No. of mice infected	No. of mice dying	% survivors on 28th day	Mean survival time limited to 28 days
2.x.35	100,000	N_2	50	46	8 S.E.= 3·84	9·06 S.E.= 0·98
		N_4	50	46	8 S.E.= 3·84	7·38 S.E.= 0·93
		N_5	50	40	20 S.E.= 5·66	13·08 S.E.= 1·20
21.i.36	100,000	N_2	50	50	0 S.E.= -	5·3 S.E.= 0·34
		N_4	50	49	2 S.E.= 1·98	5·9 S.E.= 0·60
		N_5	50	47	6 S.E.= 3·36	8·42 S.E.= 0·88

Table XXI. Bred mice compared using percentage survivors on 28th day

	Diets compared	Difference	S.E. of difference	Difference/ S.E. of difference	X^2 test of group differences
Exp. 1. 2.x.35	N_2 and N_4	0	5·4	-	X^2 = 4·55
	N_2 and N_5	- 12	6·9	1·7	n = 2
	N_4 and N_5	- 12	6·9	1·7	P > 0·1
Exp. 2. 21.i.36	N_2 and N_4	- 2	2·0	1·0	X^2 = 3·61
	N_2 and N_5	- 6	3·4	1·8	n = 2
	N_4 and N_5	- 4	3·9	1·0	P > 0·1

Table XXII. Bred mice compared using mean survival time of mice infected

	Diets compared	Difference	S.E. of difference	Difference/S.E. of difference
Exp. 1. 2.x.35	N_2 and N_4	+ 1·18	1·35	0·87
	N_2 and N_5	- 4·02	1·55	2·59
	N_4 and N_5	- 5·20	1·52	3·42
Exp. 2. 21.i.36	N_2 and N_4	- 0·6	0·69	0·87
	N_2 and N_5	- 3·12	0·94	3·32
	N_4 and N_5	- 2·52	1·07	2·36

Fig. 9

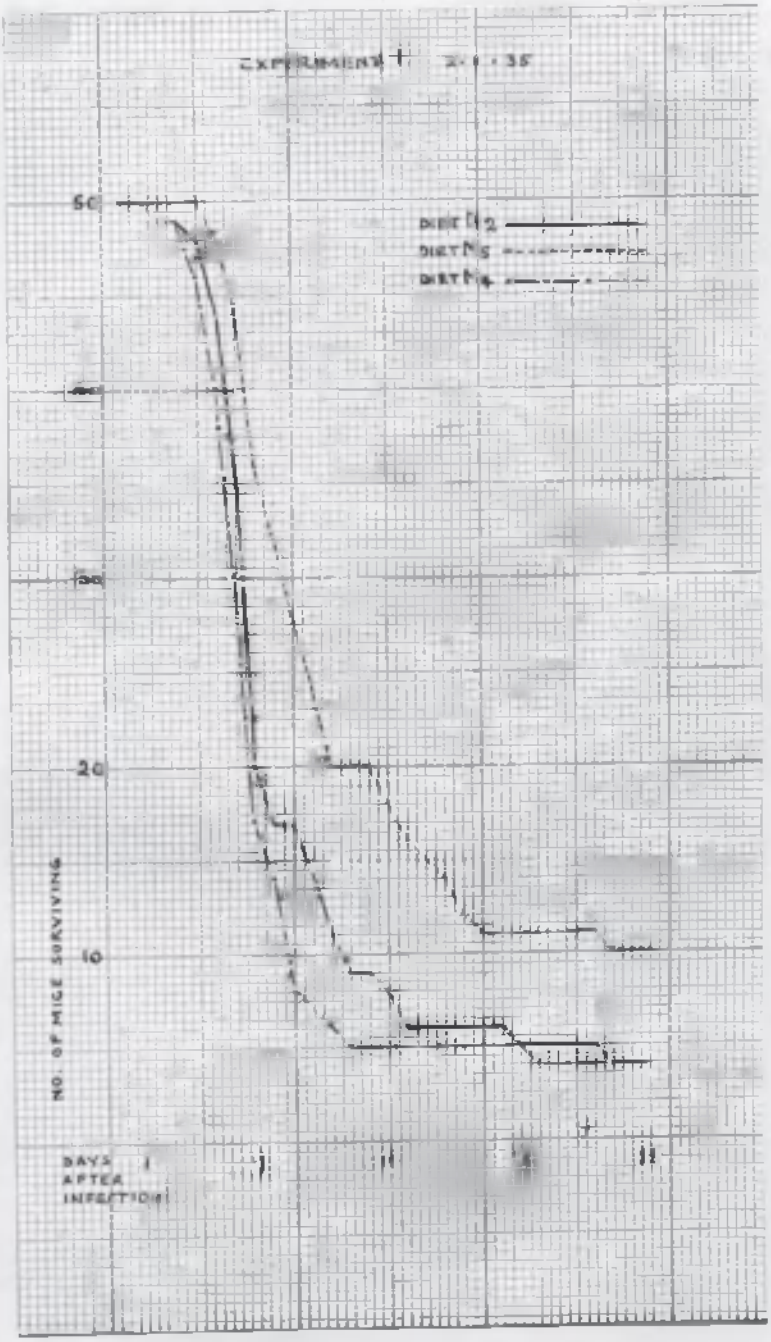
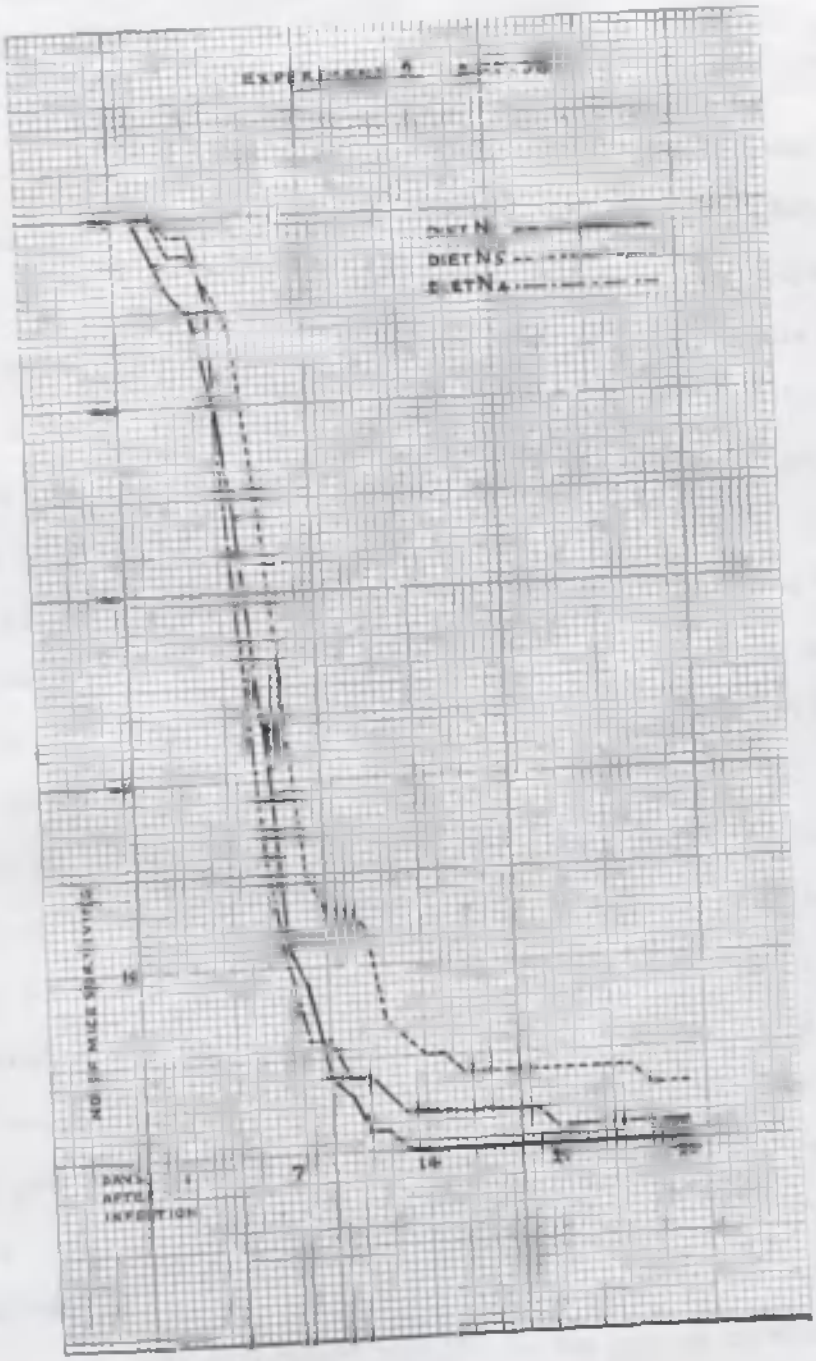


Fig. 10



isolated independently by Boivin and his colleagues, using a somewhat different method (Boivin and Mesrobeanu, 1933; Boivin et al. 1933a, b, 1934). It is highly toxic for mice (Martin, 1934), and for rabbits (Delafield, 1934). As Delafield has shown it produces in rabbits a hyperglycaemia, followed by a hypoglycaemia. More recently Delafield and Smith (1936) have been able to show that it has a characteristic influence on the oxygen uptake of certain toxic extracts in the presence of certain substrates. This substance usually produces a 50-75 per cent mortality when injected intraperitoneally into mice in a dose of 0·5 mg. In the present series of experiments it was injected in a dose of 1·0 mg. The mice were subsequently observed for 5 days. Almost all deaths from this toxic fraction occurred within 72 hours, most of them within 24–48 hours. The methods of breeding and rearing the mice, and the times at which the resistance tests were carried out, were the same as in the preceding experiments.

The results are set out in Table XXIII and in Figs. 11-16, and again need little comment. In five of the six tests the mice bred and reared on diet N_5 were much more resistant to the action of the toxin than mice bred and reared on diet N_2. The differences are large - mortalities of 50, 16, 24, 10 and 28 per cent among the N_5 mice, as compared with 100, 70, 76, 54 and 48 per cent among the corresponding N_2 mice. There is one divergent result. In the test carried out on 5.v.36 the N_5 mice proved almost as susceptible as the N_2 mice. This test was carried out within 2 months of the test that gave discrepant results in the per os infection series, and was made on mice from the same breeding group. There can, we think, be no doubt that the same factor, whatever it may have been, was responsible for the failure of the diet to exert its usual effect in those two instances.

Table XXIII. Bred mice. Intraperitoneal injection with Bact.typhi-murium toxin.

Date of injection	Dose of toxin	Diet	No. of mice injected	No. of mice dying	% survivors	Difference % from N_2	S.E. of difference	X^2 test of group differences
5.xi.35	1	N_2	50	50	0·0	-	-	$X^2 = 34·43$
		N_4	50	39	22·0	22·0	5·8	$n = 2$
		N_5	50	25	50·0	50·0	8·7	$P < 0·01$
18.ii.36	1	N_2	50	35	30·0	-	-	-
		N_5	50	8	34·0	54·0	9·8	
5.v.36	1	N_2	50	35	30·0	-	-	
		N_5	50	33	34·0	4·0	9·3	-
10.vi.36	1	N_2	50	38	24·0	-	-	$X^2 = 60·56$
		N_5	50	12	76·0	52·0	10·0	$n = 2$
		N_8	40	0	100·0	76·0	10·5	$P < 0·01$
28.ix.36	1	N_2†	50	27	46·0	-	-	$X^2 = 34·73$
		N_5	50	5	90·0	44·0	9·3	$n = 2$
		N_8	40	5	90·0	44·0	9·3	$P < 0·01$
28.ix.36	1	N_2†	50	24	52·0	-	-	$X^2 = 19·84$
		N_5	50	14	72·0	20·0	9·6	$n = 2$
		N_8	50	4	92·0	40·0	9·0	$P < 0·01$

† Stock mice fed on diet N_2.

Fig. 11

Fig. 12

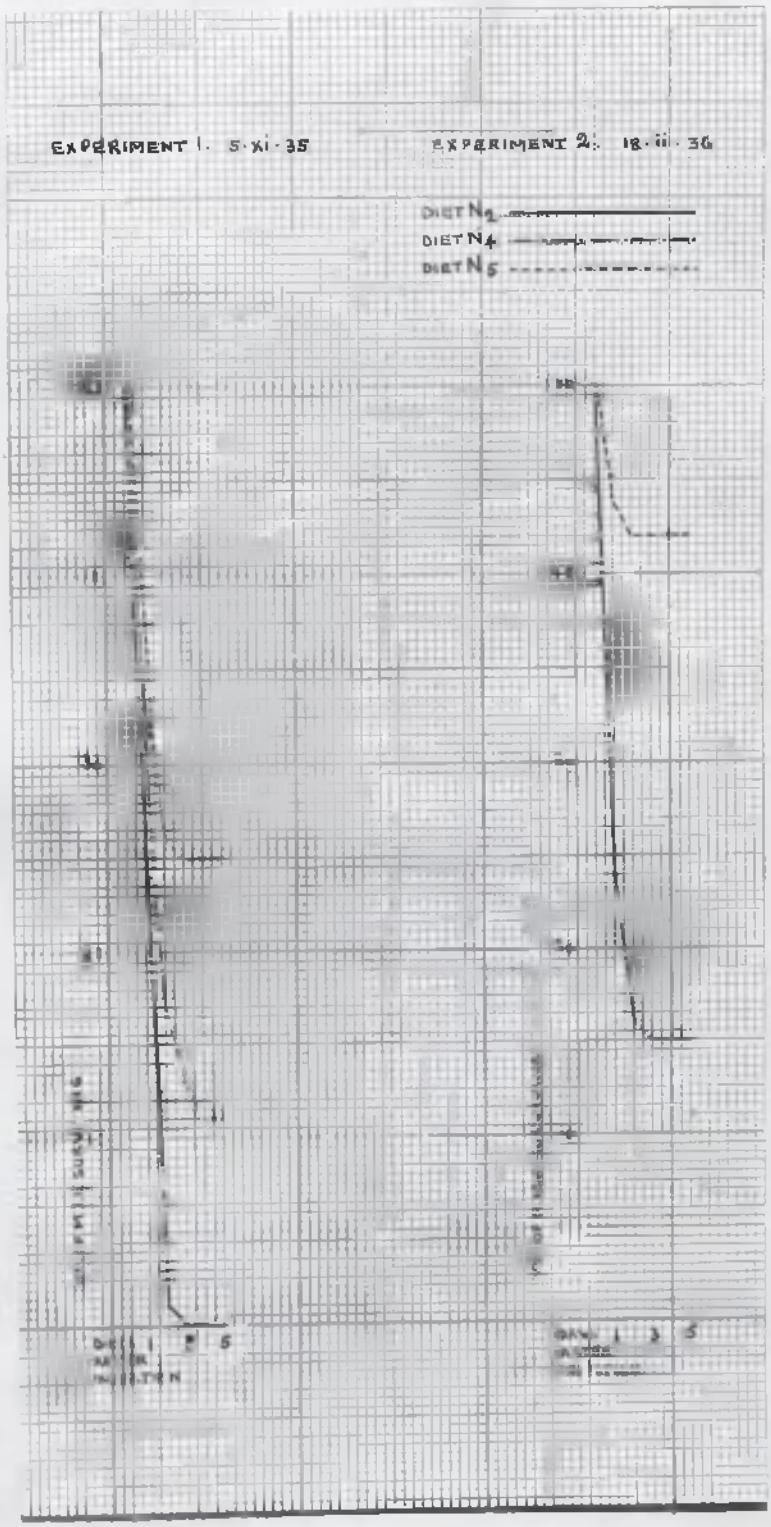

EXPERIMENT 1. 5·XI·35
EXPERIMENT 2. 18·III·36
DIET N1
DIET N4
DIET N5

Fig. 13

Fig. 14

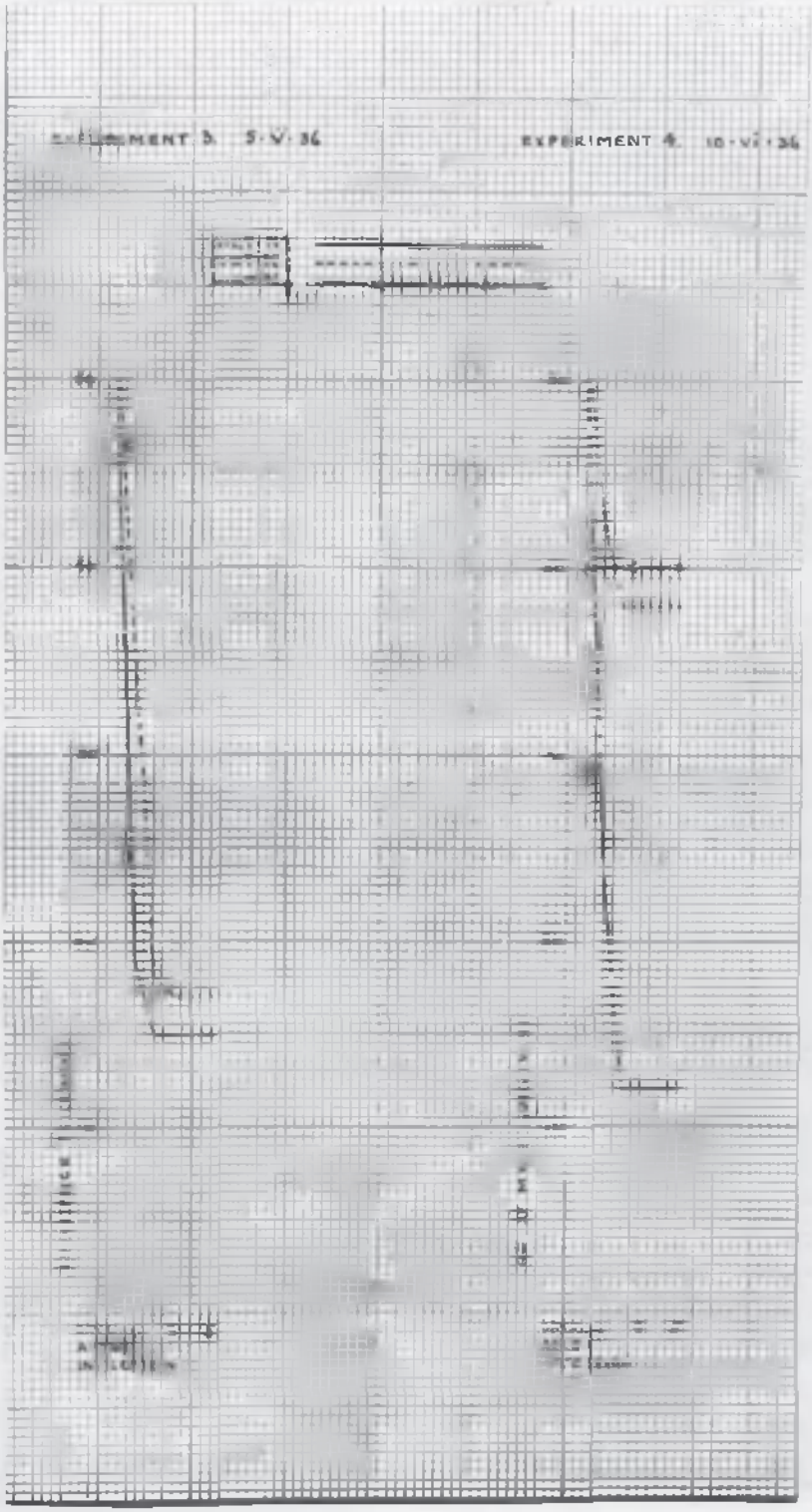


Fig. 15

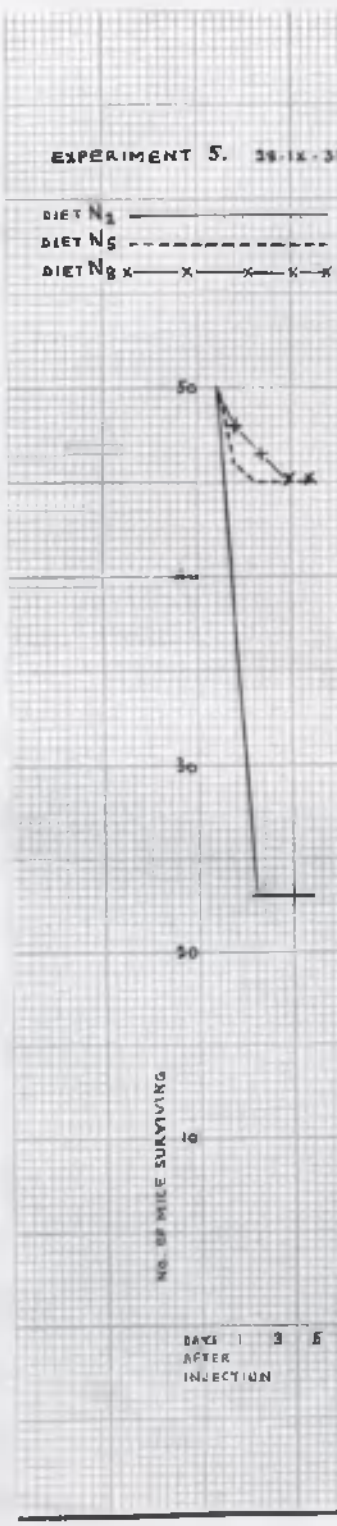
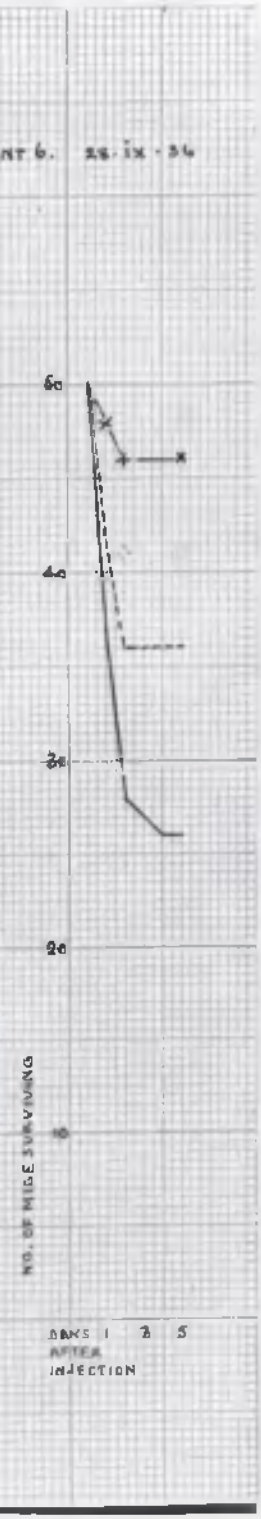

EXPERIMENT 5. 28-IX-34
DIET N_1
DIET N_5
DIET N_8
50
30
10
NO. OF MICE SURVIVING
DAYS 1 3 5
AFTER
INJECTION

Fig. 16


EXPERIMENT 6. 28-IX-34
60
40
20
0
NO. OF MICE SURVIVING
DAYS 1 2 5
AFTER
INJECTION

In two of the three trials in which it was included, mice on diet N_8 proved even more resistant than mice on diet N_5; in the third trial the N_8 and N_5 mice were equally resistant. In all three trials the N_8 mice were much more resistant than the N_2 mice, thus affording additional evidence that the presence of dried separated milk in a diet greatly increases the resistance of the mice receiving it.

Diet N_4 was tested once only in this series of experiments. The mice receiving it proved significantly more resistant to the toxin than mice receiving diet N_2, but significantly less resistant than mice fed on diet N_5.

It should perhaps be noted that the N_2 mice of the last two experiments in this series differed from the N_2 mice in the first four trials, in that they had not been bred and reared on this diet, but were imported mice that had been fed on diet N_2 for several weeks before being tested. Since, however, we have several times compared the resistance of mice bred and reared on diet N_2 with the resistance of our ordinary stock mice which are fed on this diet after importation, and have never noted any significant difference, it is unlikely that this departure from the routine method of experiment had any effect on the result. In fact, as will be noted from the table, the control mice in those last two tests showed a rather lower mortality than those used in the earlier tests, so that the advantage shown by the N_5 and N_8 mice was certainly not increased by comparing them with unduly susceptible controls.

Experiments on mice fed for short periods on various "natural" diets.

In view of the results recorded above, in which mice bred and reared on certain diets (N_5 and N_8) had shown a considerable increase in resistance to

the per os administration of Bact.typhi-murium, and to the intraperitoneal injection of a toxic fraction derived from it, it was clearly of interest to determine whether these diets, if fed for a few weeks to mice reared on less favourable diets from birth, would induce any similar increase in resistance.

In these experiments young stock mice, of approximately the same age and weight as those used in the experiments on specially bred mice, were placed on each of the diets under test for 3 weeks before the administration of living Bact.typhi-murium, or its toxin, and were maintained on this diet after the test injection until the termination of the experiment. In all other relevant particulars these experiments were similar to those carried out with the specially bred mice, and the results obtained may be considered without further description.

(1) Resistance to per os infection with living Bact.typhi-murium.

Only two experiments were carried out. The results are summarized in Tables XXIV, XXV and XXVI and in Figs. 17 and 18. In the first experiment the mice on diet N_6 were slightly less resistant than the mice on diet N_2, in the second experiment they were significantly more resistant. This discrepancy was probably not due to the same factor as that which induced the discrepancies in the tests on bred mice. The mice in the first experiment were tested 5 months before the discrepant results occurred in the other series. It seems likely, though it is, of course, by no means certain, that the shorter period of feeding on diet N_6 induces a slighter, and less constant, increase in resistance than is induced in mice bred and reared on it.

(2) Resistance to intraperitoneal infection with living Bact.typhi-murium.

Two experiments were carried out. The results are summarized in Tables

Table XXIV. Stock mice. Per os infection with Bact.typhi-murium.

Fifty mice in each diet group.

	Diets	% survivors on 28th day	Mean survival time limited to 28 days
Exp. 1. 20.x.35	N_2	40 S.E.= 6·93	17·16 S.E.= 1·31
	N_5	32 S.E.= 6·60	16·20 S.E.= 1·28
Exp. 2. 17.ii.36	N_2	18 S.E.= 5·43	13·88 S.E.= 1·08
	N_5	44 S.E.= 7·02	18·66 S.E.= 1·33

Table XXV. Stock mice compared using percentage survivors on 28th day.

	Diets compared	Difference	S.E. of Difference	Difference/S.E. of difference
Exp. 1. 20.x.35	N_2 and N_5	+ 8	9·60	0·8
Exp. 2. 17.ii.36	N_2 and N_5	- 26	9·25	2·8

Table XXVI. Stock mice using mean survival time of mice infected

	Diets compared	Difference	S.E. of Difference	Difference/S.E. of difference
Exp. 1. 20.x.35	N_2 and N_5	+ 0·96	1·83	0·5
Exp. 2. 17.ii.36	N_2 and N_5	- 4·78	1·71	2·8

Fig. 17

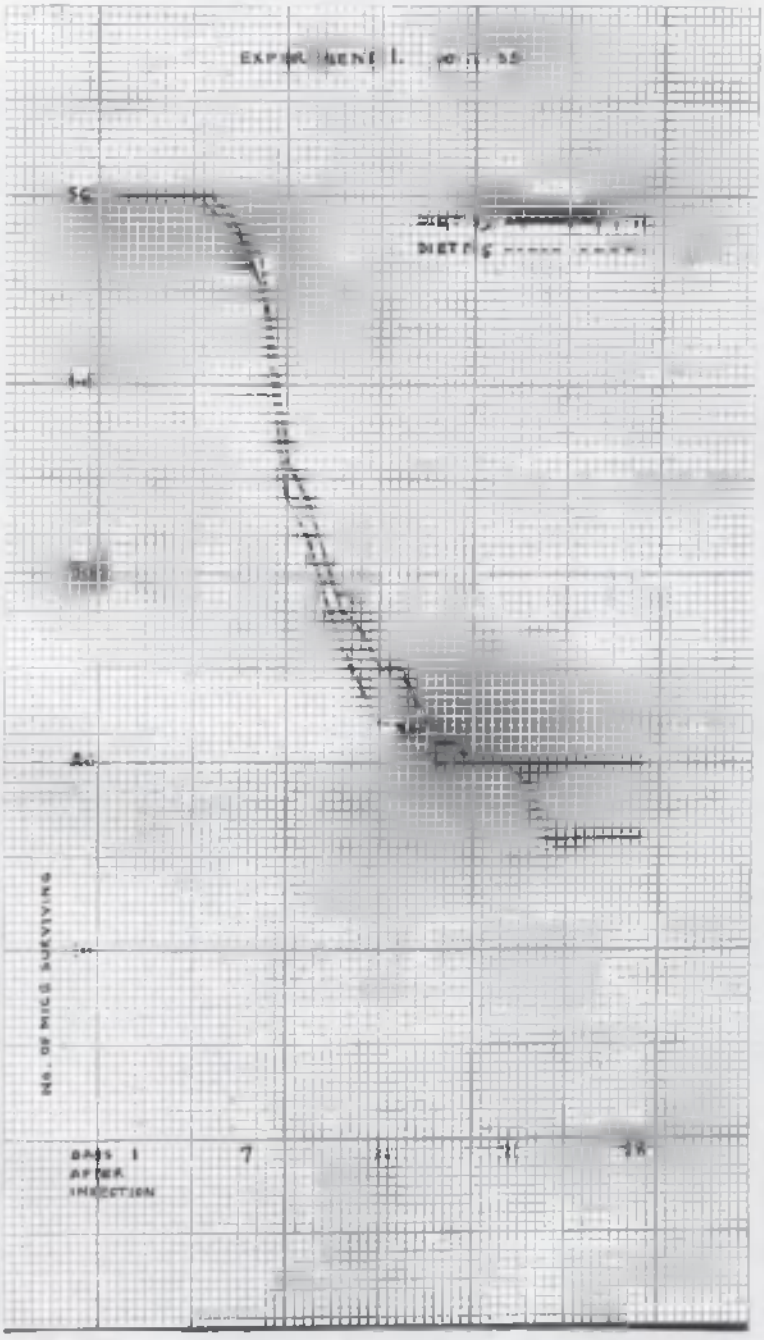
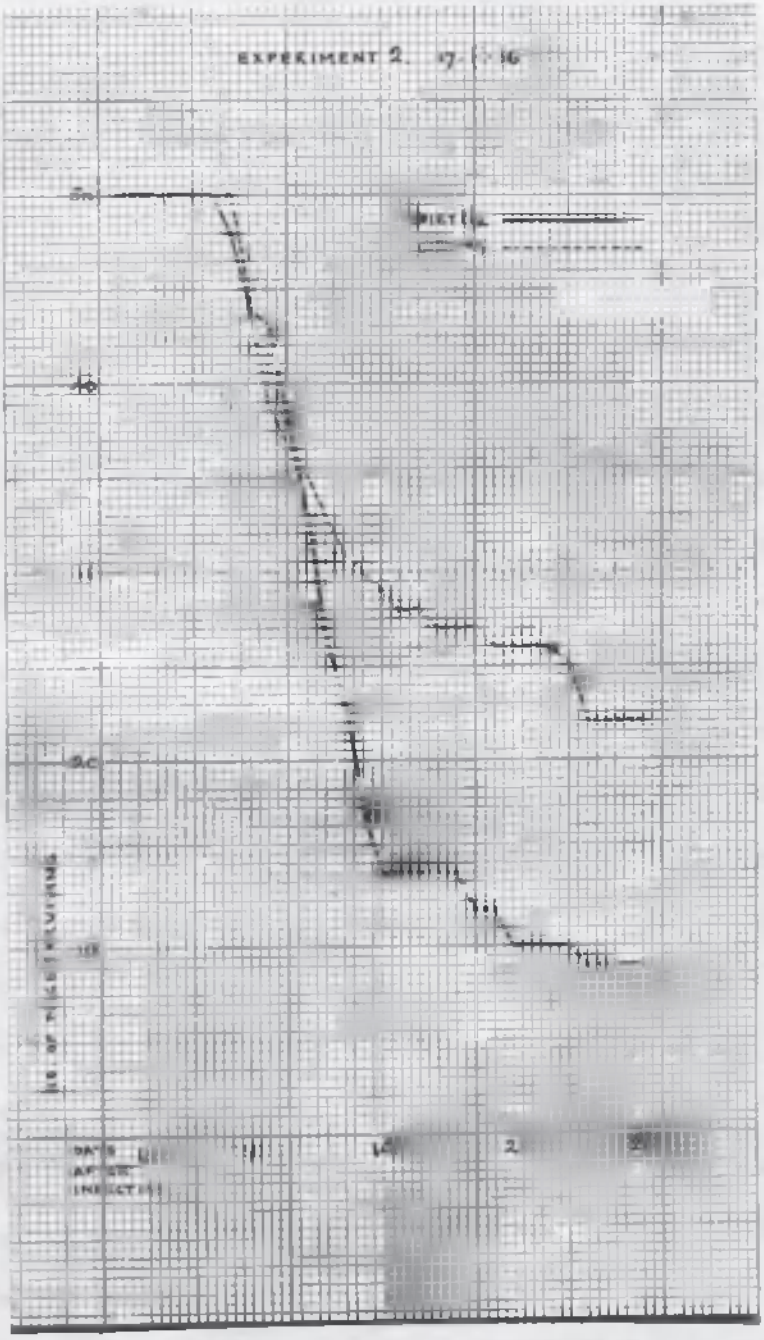


Fig. 18



XXVII, XXVIII and XXIX and in Figs. 19 and 20. In each case the mice fed on diet N_5 show an advantage over the mice fed on diet N_2; but the advantage is relatively slight, and is doubtfully significant. It is of much the same order as the difference observed in similar tests on mice bred and reared on these diets.

We may recall here the results recorded by Webster and Pritchett (1924) and Pritchett (1927). By far the most striking results recorded by them were obtained in the comparison of mice bred and reared on the modified McCollum diet with mice bred and reared on the Institute diet. The modified McCollum diet contained 10 per cent dried milk powder and 15 per cent casein. The Institute diet consisted of a daily ration of bread soaked in pasteurized milk with two weekly feedings of an oatmeal and buckwheat mixture and one weekly feeding of dog biscuits. The average difference in mortality recorded in this comparison was large, 15·9 per cent among the mice fed on the McCollum diet against 77·8 per cent among the mice on the Institute diet.

No differences of this order are recorded by Pritchett in her later experiments, in which the mice were fed on the test diets for 2 weeks before infection; but, in considering the various factors that might account for the increased resistance conferred by the McCollum diet, no consideration was given to the dried milk powder. The conclusion that fat, particularly fat rich in vitamin A, was the main factor concerned, was based on differences in mortality of quite a small order, in tests in which various constituents were added to the Institute diet - 53·3 per cent as against 69·4 per cent, and 49·3 per cent as against 61·2 per cent for cod-liver oil. There was also, as has been noted, the anomalous result that "Crisco", a

Table XXVII. Stock mice. Intraperitoneal infection with Bact.typhi-murium. Thirty mice in each diet group

	Diets	% survivors on 28th day	Mean survival time limited to 28 days
Exp. 1. 20.iii.35	N_2	6·67 S.E.= 4·56	6·67 S.E.= 1·21
	N_5	16·67 S.E.= 6·80	10·17 S.E.= 1·62
Exp. 2. 5.iv.35	N_2	13·33 S.E.= 6·21	7·40 S.E.= 1·57
	N_5	16·67 S.E.= 6·80	11·03 S.E.= 1·47

Table XXVIII. Stock mice compared using percentage survivors on 28th day

	Diets compared	Difference	S.E. of difference	Difference/S.E. of difference
Exp. 1. 20.iii.35	N_2 and N_5	- 10	8·3	1·20
Exp. 2. 5.iv.35	N_2 and N_5	- 3·34	9·2	0·36

Table XXIX. Stock mice compared using mean survival time of mice infected.

	Diets compared	Difference	S.E. of difference	Difference/S.E. of difference
Exp. 1. 20.iii.35	N_2 and N_5	- 3·50	2·02	1·73
Exp. 2. 5.iv.35	N_2 and N_5	- 3·63	2·15	1·69

Fig. 19

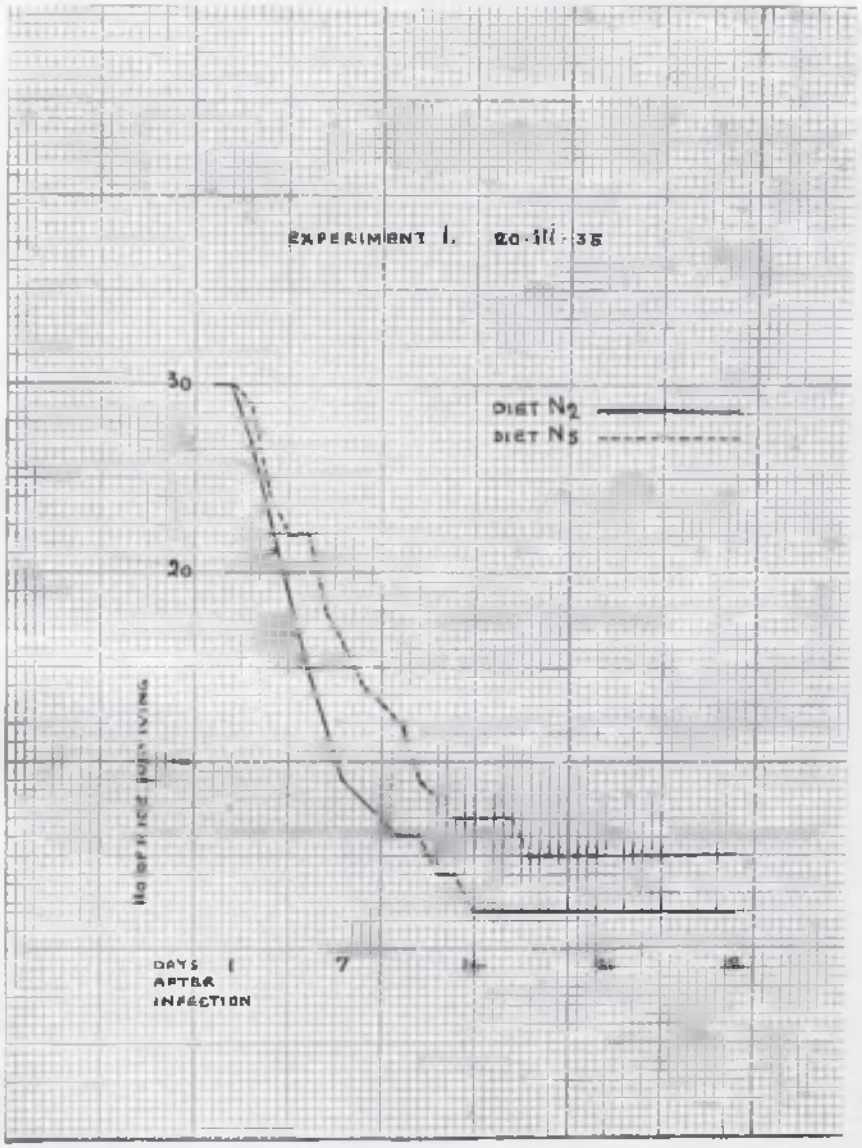
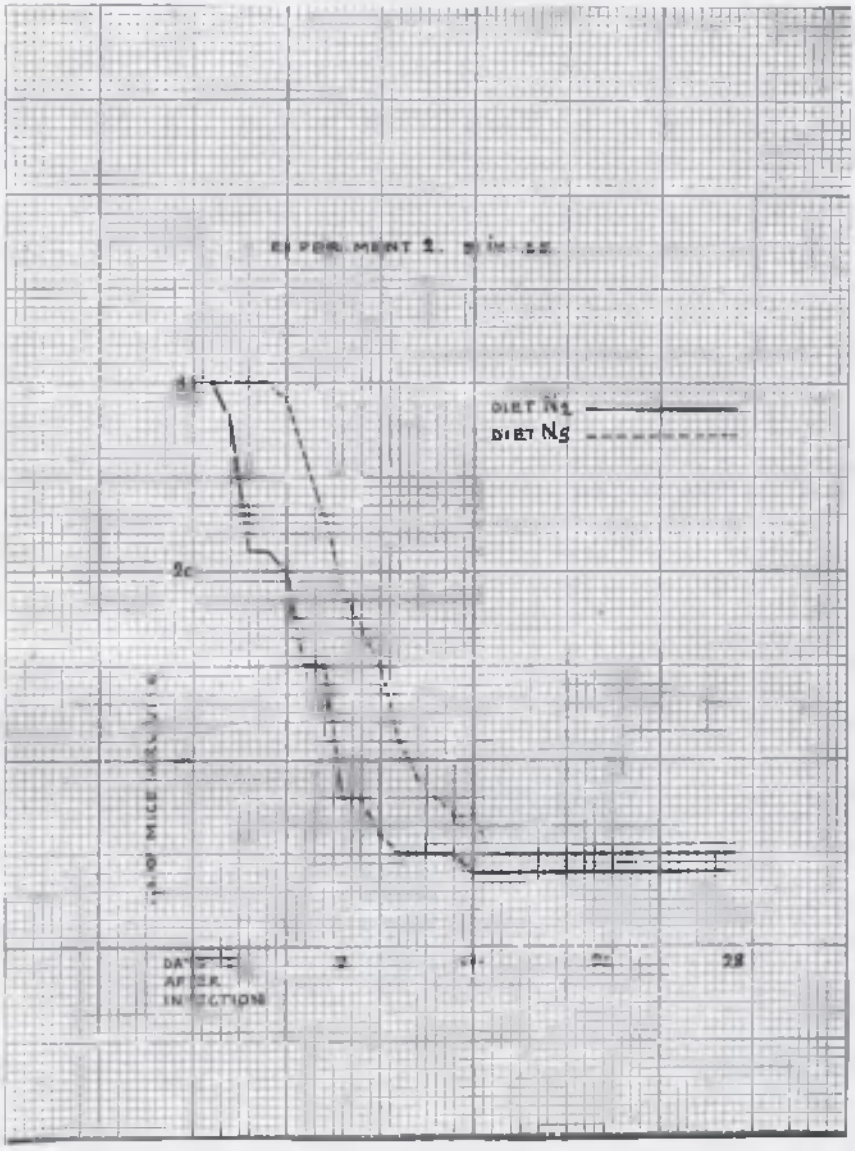


Fig. 20



vegetable fat devoid of vitamin A, gave very similar results to those obtained with butter or cod-liver oil - a mortality of 53·3 per cent among the "Crisco" fed mice as against a mortality of 61·2 per cent among the controls. In particular the mice fed on the McCollum diet minus fat proved superior, when compared to the mice on the control Institute diet, to any of the above diets to which fat of one kind or another had been added - a mortality of 47·3 per cent for the McCollum diet minus fat as compared with 81·5 per cent for the Institute diet. It seems to be highly probable that the favourable results obtained by Webster and Pritchett with the McCollum diet were due to the same factor that rendered our diets N_6 and N_8 so effective in inducing an increased resistance, and in particular to the incorporation in the McCollum diet of the dried milk powder.

We may also note that this view is entirely compatible with the results recorded by Topley et al. (1931). The diets they employed contained no milk powder, and the various fats tested failed to induce any increase in resistance.

(3) Resistance to the intraperitoneal injection of the toxic fraction derived from Bact. typhi-murium.

Two experiments were carried out, the results of which are summarized in Table XXX and in Figs. 21 and 22. In each case the mice fed on diet N_6 proved more resistant than the mice fed on diet N_2. In each case the difference is statistically significant, and in the case of the second experiment it is large.

Table XXX. Stock mice. Intraperitoneal injection with Bact.typhi-murium toxin

Date of injection	Dose of toxin mg.	Diet	No. of mice injected	No. of mice dying	% survivors	Difference %	S.E. of difference
3.xii.35	1	N_2	50	43	14·0	-	-
		N_5	50	33	34·0	20·0	8·5
26.ii.36	1	N_2	50	31	38·0	-	-
		N_5	50	9	82·0	44·0	9·5

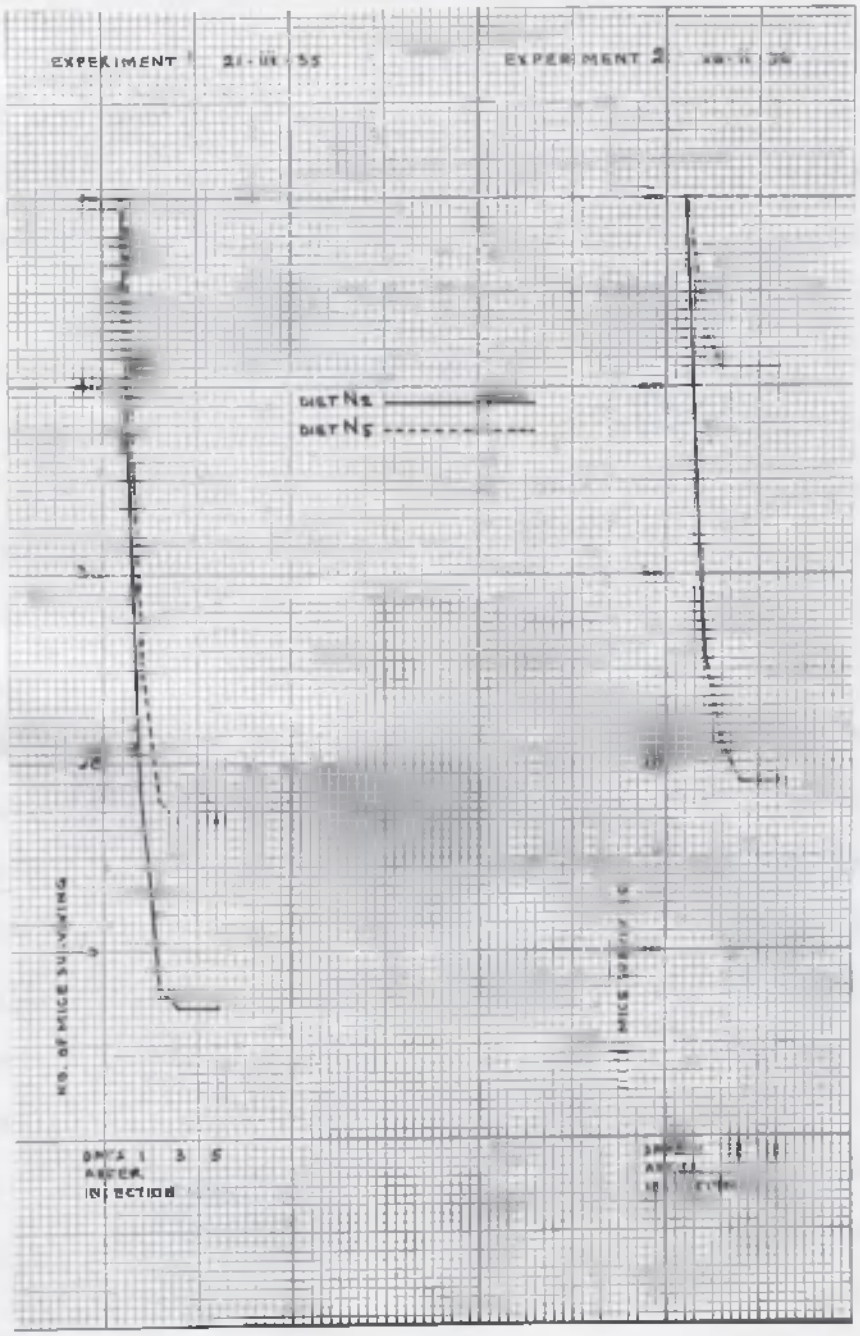
Discussion

Taking these results as a whole, certain conclusions of some interest and importance seem to be rendered highly probable.

The number of comparisons in which mice receiving a diet containing dried separated milk proved more resistant than mice fed on a diet from which this constituent was absent is too large, and many of the observed differences are too great, to make tenable the view that these differences are due to the errors of random sampling. As to the way in which this dietetic factor produces its effects no opinion can yet be given. Three points may however be noted. Firstly the mice on the control diets, from which the dried separated milk was absent, were given milk and water to drink. The difference between the test and control groups was in the far greater proportion of milk proteins, and other milk constituents excluding fat, present in diets N_5 and N_8. Secondly, it will be recalled that, in the experiments with the "synthetic" diets, the mice receiving their protein, other than that present in the bran, in the form of caseinogen, or a mixture

Fig. 21

Fig. 22


EXPERIMENT
DIET N2
DIET N5
NO. OF MICE SURVIVING
DAYS AFTER INFECTION

of caseinogen and gluten, proved slightly more resistant than mice receiving their protein as gluten alone. Thirdly, whatever the factor, or factors, at work, we are not dealing with any dietetic constituent that increases resistance to a bacterium, or its toxin, without influencing the general well-being of the mice in other ways - there is no evidence of the presence in the milk of any "anti-infective" factor in a restricted sense. The mice on diets N_5 and N_8 throve better in all observable ways than the mice on diet N_2, or on most of the other diets.

In regard to the infection-resisting body mechanisms that are improved by feeding on the separated-milk-containing diets it is equally impossible to hazard any opinion at this stage. It is, however, clear that the factor involved is not simply a change that renders more difficult the passage of bacteria from the mouth or intestine to the tissues. It is true that the increased resistance of the mice on diets N_5 and N_8 appears to be far greater against per os than against intraperitoneal infection with living Bact.typhi-murium; but the increased resistance of the mice on these diets to the intraperitoneal injection of the toxic fraction isolated from the organisms is, if anything, greater still. It is of some interest that a dietetic factor should have been found to increase resistance to a bacterial toxin that is, so far as we are aware, the only toxic bacterial product that can be isolated in a state approaching chemical purity, and the effect of which on the metabolic processes of the host has been studied in some detail.

Other suggestions are offered by the results recorded. It seems likely, for instance, that feeding on the diets N_5 and N_8 for relatively short periods induces a resistance of the same kind as that induced by

breeding and rearing on these diets, but of a lesser degree. This suggestion accords with a priori probabilities; but the experiments carried out by the short-period feeding method are too few to justify replacing a suggestion by a conclusion.

Another suggestion is that diet N_4, containing no dried milk, but containing an alkaline salt mixture, induces a resistance greater than that of mice fed on an oatmeal diet from which this component is absent, but less than that of mice fed on a separated-milk-containing diet. Experiments on this point were, however, few, and their results were irregular. Further work is necessary before any definite significance can be attached to them.

Finally, we may note that we have as yet no evidence as to whether the increased resistance is specific in any immunological sense, whether, as seems unlikely, it is confined to Bact.typhi-murium and its toxin, whether it extends to other bacteria that produce toxic products of the same general kind, or whether it covers a far wider range of bacteria, bacterial toxins, and perhaps other poisonous agents. The enquiry, even in regard to the particular dietetic factor or factors involved in these experiments, is only in its earliest stages.

Conclusions

I. 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.

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.

"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.

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.

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.

II. We would limit our conclusions to those that seem to be established with a high degree of probability, and these are as follows.

Young mice, bred and reared on a diet containing oatmeal, dried separated milk, dextrine or flour-and-water biscuit, coconut oil, cod-liver oil, yeastrel, bran, and milk and water to drink, are more resistant to *per os* infection with *Bact. typhi-murium*, and to the intraperitoneal injection of a toxic substance isolated from that organism, than young mice bred and

bred and reared on a diet in which the amount of oatmeal is increased, and the dried separated milk, dextrine or flour-and-water biscuit, and coconut oil are omitted.

It is probable that the factor responsible for this increase in resistance is the dried separated milk.

It is also probable that the feeding on this diet for shorter periods induces a similar resistance of slighter degree.

References.

Alexander, J. and Bullowa, J.G.M. (1910). J.Amer.med.Ass. 55, 1196.

Batchelor, R.P., Fehnel, J.W., Thomson, R.M. and Drinker, K.R. (1926). J.industr.Hyg. 8, 322.

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

Bertrand, G. and Bhattacherjie, R.C. (1934). C.R.Acad.Sci., Paris, 198, 1823.

Boivin, A. and Mesrobeanu, L. (1933). C.R.Soc.Biol., Paris, 112, 76.

" " " " " (1934). Ibid. 115, 304, 309.

Boivin, A., Mesrobeanu, I. and Mesrobeanu, L. (1933 a). Ibid. 113, 490.

" " " " " " " (1933 b). Ibid. 114, 307.

" " " " " " " (1934). Ibid. 115, 306.

Clausen, S.W. (1934). Physiol.Rev. 14, 309.

Cramer, W. (1923). Lancet, ii, 1046.

" " (1924). Ibid. i, 633.

" " (1927). Ibid. ii, 774.

Daniel, J.F. (1912). Amer.Nat. 46, 591.

Delafield, M.E. (1934). Brit.J.exp.Path. 15, 130.

Delafield, M.E. and Smith, H.A. (1936). Ibid. 17, 379.

Drummond, J.C. (1919). Biochem.J., 13, 95.

Evans, H.E. and Burr, G.O. (1917). Mem.Univ.Calif. Vol. 8.

Goldblatt, H. and Benischek, M. (1927). J.exp.Med. 46, 699.

Green, H.N. and Mellanby, E. (1928). Brit.med.J. ii, 691.

" " " " " (1930). Brit.J.exp.Path. 5, 11.

Greene, M.R. (1933). Amer.J.Hyg. 17, 60.

Greenwood, M., Hill, A.B., Topley, W.W.C. and Wilson, J. (1936). Sp.Rep. Ser.Med.Res.Counc. Lond., No. 209.

Gross, L. (1924). J.Path.Bact. 27, 27.

Gudjónsson, S.V. (1930). Experiments on Vitamin A Deficiency in Rats and the Quantitative Determination of Vitamin A. (Copenhagen).

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

Hess, A., McCann, G.F. and Pappenheimer, A.M. (1921). J.biolChem. 47, 395.

Hotta, Y. (1928). Zbl.Bakt. 108, 413.

Hubbell, R.B. and Mendel, L.B. (1927). J.biol.Chem. 75, 567.

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

Kligler, I.J. and Geiger, A. (1928). Proc.Soc.exp.Biol., N.Y., 25, 385.

Kohler, G.O., Elvehjem, C.A. and Hart, E.B. (1936). Science, 83, No. 2158, 445.

Langa, L.B. and Simmonds, N. (1923). Amer.Rev.Tuberc. 7, 49.

Lassen, H.C.A. (1930). J.Hygiene, 30, 30.

" " (1931). Experimental Studies on the Course of Paratyphoid Infections in Avitaminotic Rats with Special Reference to Vitamin A Deficiency. (Copenhagen).

" " (1932). Z.Immunitätsforsch. 73, 221.

Martin, A.R. (1934). Brit.J.exp.Path. 15, 137.

McClung, L.S. and Winters, J.C. (1932). J.infect.Dis. 51, 469.

McCollum, E.V., Simmonds, N. and Pitz, W. (1916). J.biol.Chem. 28, 483.

" " " " " " " (1917). Ibid. 29, 342.

McHargue, J.S. (1926). Amer.J.Physiol. 77, 245.

Osborne, T.B. and 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.

Pritchett, I.W. (1927). J.exp.Med. 46, 557.

Raistrick, H. and Topley, W.W.C. (1934). Brit.J.exp.Path. 15, 113.

Reiter, H. (1929). Z.Immunitätsforsch. 61, 433.

Robertson, E.C. (1934). Medicine, 13, No. 2, 123.

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

Robertson, T.B. and 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. and Drinker, K.R. (1927). Ibid. 80, 65.

Topley, W.W.C. (1923). J.Hygiene, 21, 226.

Topley, W.W.C., Greenwood, M. and Wilson, J. (1931). J.Path.Bact. 34, 163.

Webster, L.T. (1930). J.exp.Med. 52, 931.

Webster, L.T. and Pritchett, I.W. (1924). Ibid, 40, 397.

Workman, C.H. (1923). J.infect.Dis. 32, 247, 255.

Wheeler, R. (1913). J.exp.Zool. 15, 209.

Wolbach, S.B. and Howe, P.R. (1925). J.exp.Med. 42, 753.