STUDIES ON THE INFLUENCE OF DIET ON RESISTANCE TO INFECTION

II. THE EFFECT OF VARIOUS DIETS ON THE RESISTANCE OF MICE TO BACTERIAL INFECTION

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(With 5 Figures in the Text)

The preceding 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 paper 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 & Howe (1925), Goldblatt & Benischek (1927), Green & Mellanby (1928, 1930), Hotta (1928), Reiter (1929), Lassen (1930, 1931, 1932), Gudjónsson (1930), McClung & 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 concentration, 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 & 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 & 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 & 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 & 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 tested 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 & 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 $4–5 \times 10^6$ *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) (see p. 424).

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 & 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 & 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 vitamin A-containing diets as compared with those 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 & 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 & 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 & 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 & 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 & 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 & 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 paper, 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 I and II. 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 I

	Diets ...	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 II

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 I, 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 paper, are repeated for convenience in Table III.

Table III

Diets ...	N_2	N_4	N_5	N_8
Coarse oatmeal	92	87	40	40
Dried separated milk	—	—	25	25
Dextrine	—	—	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_5. 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 IV–VI and in Figs. 1–5.

Table IV. *Bred mice.* Per os *infection*

Date of infection	No. of *Bact. typhi-murium* injected	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_5	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_5	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_5	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_5	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_5 in which the oatmeal,

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

	Diets compared	Difference	S.E. of difference	Difference/S.E. of difference	χ^2 test of group difference*
Exp. 1. 10. vii. 35	N_2 and N_4	−22	9·7	2·3	$\chi^2=11{\cdot}36$
	N_2 and N_5	−30	9·4	3·2	$n=2$
	N_4 and N_5	− 8	8·3	0·9	$P<0{\cdot}01$
Exp. 2. 13. ix. 35	N_2 and N_4	+ 4	9·7	0·4	$\chi^2=28{\cdot}50$
	N_2 and N_5	−44	9·7	4·5	$n=2$
	N_4 and N_5	−48	9·8	4·9	$P<0{\cdot}01$
Exp. 3. 31. i. 36	N_2 and N_4	−26	9·5	2·7	$\chi^2=8{\cdot}83$
	N_2 and N_5	−24	9·5	2·5	$n=2$
	N_4 and N_5	+ 2	10·0	0·2	P nearly 0·01
Exp. 4. 27. iii. 36	N_2 and N_5	+10	9·9	1·0	—
Exp. 5. 26. vi. 36	N_2 and N_5	−40	10·0	4·0	$\chi^2=27{\cdot}04$
	N_2 and N_8	−48	10·0	4·8	$n=2$
	N_5 and N_8	− 8	9·0	0·9	$P<0{\cdot}01$

* 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 χ^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 VI. *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. vii. 35	N_2 and N_4	−3·70	1·43	2·59
	N_2 and N_5	−4·30	1·43	3·01
	N_4 and N_5	−0·60	1·24	0·48
Exp. 2. 13. ix. 35	N_2 and N_4	+1·06	1·66	0·64
	N_2 and N_5	−6·22	1·46	4·26
	N_4 and N_5	−7·28	1·51	4·82
Exp. 3. 31. i. 36	N_2 and N_4	−1·66	1·72	0·96
	N_2 and N_5	−1·42	1·75	0·81
	N_4 and N_5	+0·24	1·89	0·13
Exp. 4. 27. iii. 36	N_2 and N_5	+0·12	1·74	0·07
Exp. 5. 26. vi. 36	N_2 and N_5	−6·14	1·54	3·99
	N_2 and N_8	−7·34	1·50	4·89
	N_5 and N_8	−1·20	1·42	0·85

forming 92 per cent of the N_2 diet, was reduced to 40 per cent, the remainder being replaced by dried separated milk (25 per cent), dextrine (23 per cent) and coconut oil (4 per cent). Taking Table IV 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 V and VI, 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

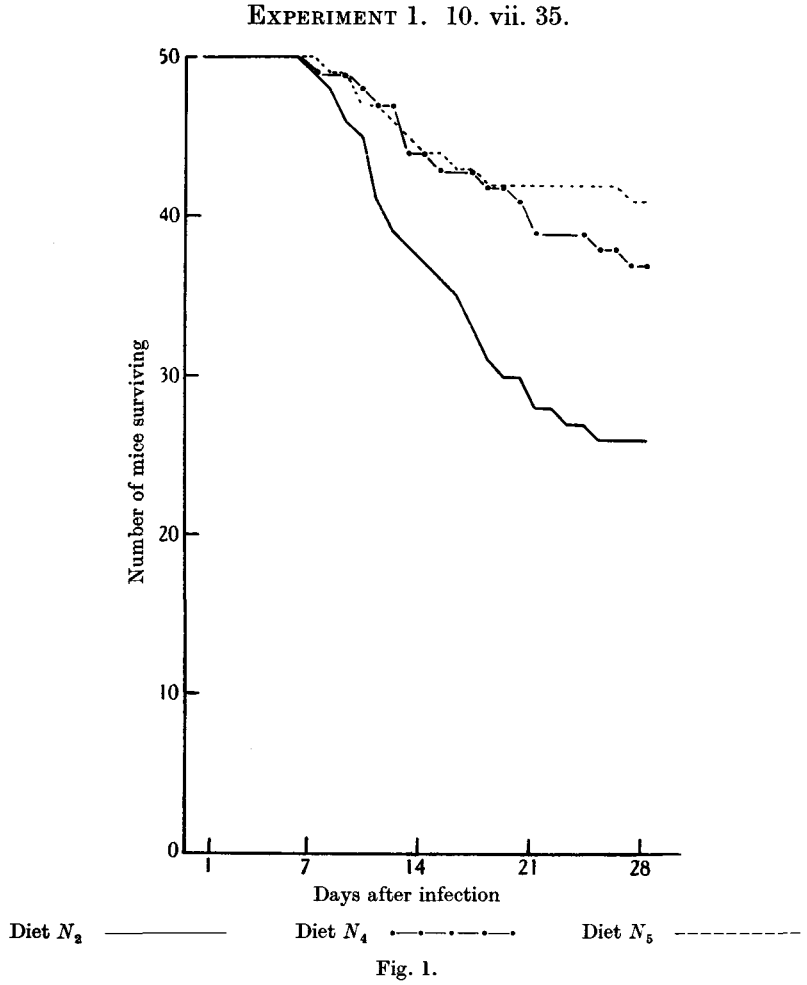

Fig. 1.

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.

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_5, 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_5 diet.

EXPERIMENT 2. 13. ix. 35.

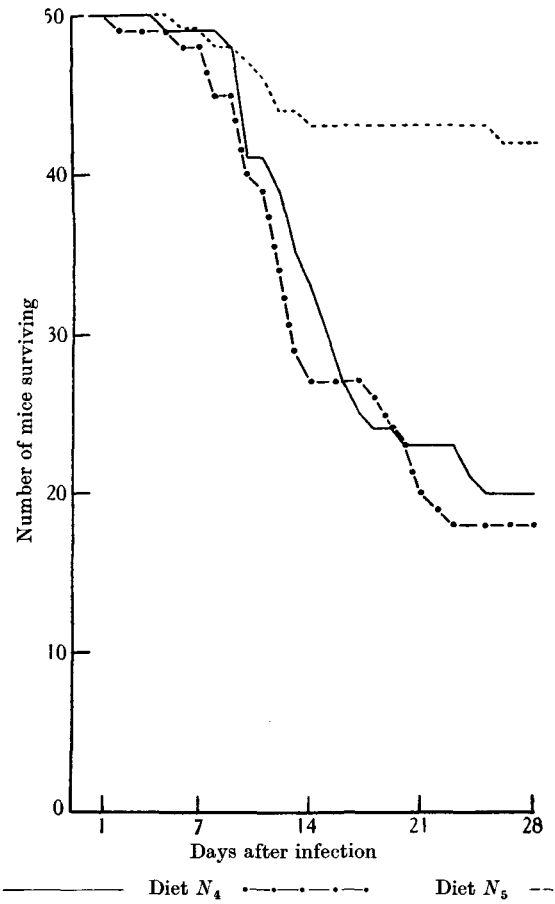

Fig. 2.

We believe, therefore, that some factor intervened to neutralize the effect of the N_5 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_5 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.

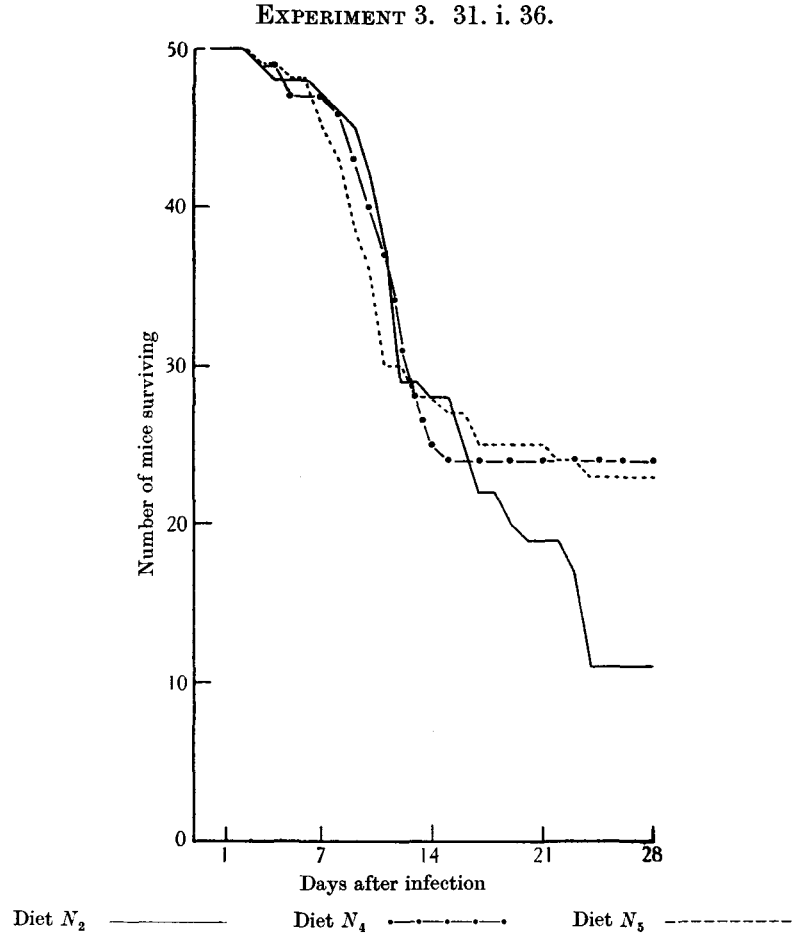

Fig. 3.

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

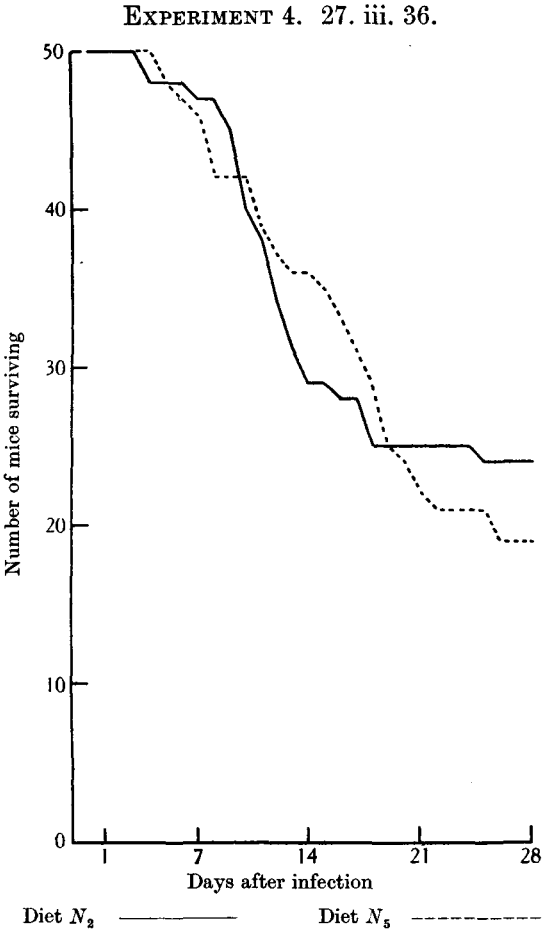

Fig. 4.

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

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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

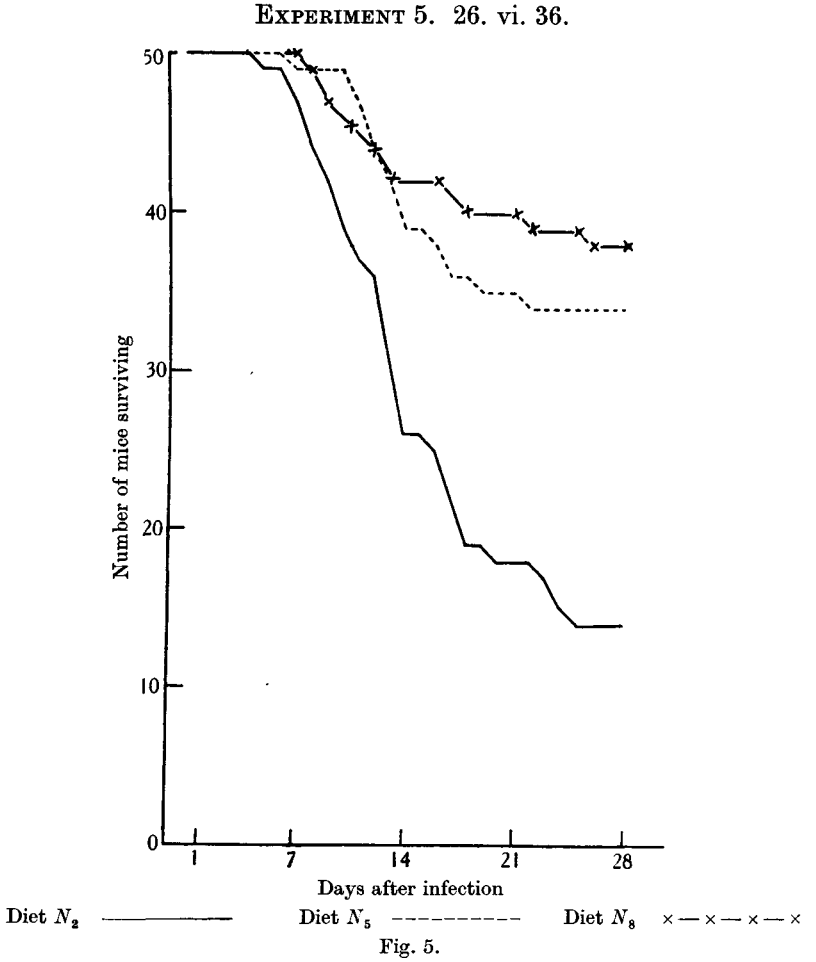

Fig. 5.

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

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days. Only two tests of this type were carried out. The results are recorded in Tables VII–IX 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.

Table VII. *Bred mice. Intraperitoneal infection with* Bact. typhi-murium

Date of infection	No. of *Bact. typhi-murium* injected	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	7·88 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 VIII. *Bred mice compared using percentage survivors on 28th day*

	Diets compared	Difference	S.E. of difference	Difference/S.E. of difference	χ^2 test of group differences
Exp. 1. 2. x. 35	N_2 and N_4	0	5·4	—	$\chi^2 = 4·55$
	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	$\chi^2 = 3·61$
	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 IX. *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

(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 & 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 isolated independently by Boivin and his colleagues, using a somewhat different method (Boivin & Mesrobeanu, 1933, 1934; Boivin *et al.* 1933*a*, *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 & 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.

Table X. *Bred 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 % from N_2	S.E. of difference	χ^2 test of group differences
5. xi. 35	1	N_2	50	50	0·0	—	—	$\chi^2=34·43$
		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	84·0	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. ix. 36	1	N_2	50	38	24·0	—	—	$\chi^2=60·56$
		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	—	—	$\chi^2=34·73$
		N_5	50	5	90·0	44·0	9·3	$n=2$
		N_8	50	5	90·0	44·0	9·3	$P<0·01$
28. ix. 36	1	N_2*	50	24	52·0	—	—	$\chi^2=19·84$
		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.

The results are set out in Table X, 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.

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 used 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 XI, XII and XIII. In the first experiment the mice on diet N_5 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_5 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 XIV, XV and XVI. In each case the mice fed on diet N_5 show an advantage

Table XI. *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 XII. *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 XIII. *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

Table XIV. *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 XV. *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 XVI. *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

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 & 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 biscuit. 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 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 & Pritchett with the McCollum diet were due to the same factor that rendered our diets N_5 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 XVII. In each case the mice fed on diet N_5 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 XVII. *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 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 that organism 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.

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

CONCLUSIONS

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 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.

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