THE EFFECT OF DIET ON EPIDEMICS OF MOUSE-TYPHOID

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(With 1 Chart in the Text)

In previous reports (Watson 1937*a*, *b*) one of us has described a series of experiments in which various diets were tested in regard to their effect on the growth, fertility, survival and resistance of mice. It was found that, when part of the oatmeal in a particular diet was replaced by dried skimmed milk, the does were more fertile, and the young mice gained weight more rapidly and showed a lower mortality during the first 8 weeks of life. Mice bred and reared on the latter diet were found to show a significantly increased resistance to *per os* infection with living cultures of *Bact. typhi-murium*, and to the intraperitoneal injection of a toxic fraction derived from that organism. The results obtained in a few tests suggested that these mice were also more resistant to the intraperitoneal injection of living *Bact. typhi-murium*; but the differences observed were smaller, and of doubtful significance. In the present paper we record the effect of these two diets on the epidemic spread of mouse-typhoid.

The extensive, and confusing, literature on the effect of diet on resistance to experimental infection has already been reviewed at some length (Watson, 1937*b*), and it is not necessary to refer to it here, except to note that Webster (1930) has reported a significant decrease in mortality following a change in the diet given to an infected herd of mice. The possible relation between our own observations and those recorded by Webster & Pritchett (1924) and by Pritchett (1927) has been considered in the earlier paper referred to above.

Experiments

The experimental method employed has been that of the "closed epidemic" (see Greenwood *et al.* 1936). In the initiation of each epidemic twenty-five mice were infected with *Bact. typhi-murium* by the intraperitoneal injection of a uniform dose of bacilli, and were placed in a single cage of the type used in all our experiments, together with 100 normal mice. The course of events was followed over the succeeding 60 days. All mice dying during this period were examined post-mortem, unless they had been partially eaten by their companions, and cultures were taken from the heart and spleen. Survivors were killed, and cultures were taken from their spleens. The records which follow refer only to the 100 mice in each group submitted to contact infection. The twenty-five mice infected by inoculation at the start of each experiment almost all died within 21–28 days.

The two diets under comparison, N_2 and N_5, were composed as follows:

	N_2	N_5
Coarse oatmeal	92	40
Dried skimmed milk	—	25
Dextrin	—	23
Coconut oil	—	4
Cod-liver oil	1	1
Yeastrel (dry weight)	2	2
Wheat bran	5	5
	100	100

The ration of each diet was 6 g. per mouse per day. In addition, each cage of mice on diet N_2 was supplied with a drinking vessel containing equal parts of sterilized milk and water, the amount allowed being approximately 2 c.c. per mouse per day. At night these vessels were replaced by others containing water. Mice on diet N_5 were given water only.

The mice on diet N_5 had been bred and reared on that diet, since earlier experiments (Watson, 1937*b*) had shown that its full effect in increasing resistance was not attained by feeding it to mice, bred on other diets, for 3 weeks before infection. The mice on diet N_2 were stock mice that had been on that diet for at least 3 weeks before the epidemic started. It had been shown in previous experiments (Watson, 1937*b*), that breeding and rearing on this diet led to no increase in resistance as compared with the various other diets examined. Neither group was genetically pure; but the does used for breeding on the N_5 diet were taken from the normal stock, so that the genetic character of the two groups, though not necessarily identical, was the result of random matings from the same mixed stocks.

In all, three experiments were made. In the first there were six groups, each of 100 mice, all males. In this, and all other experiments, the twenty-five infected mice, added to provide the source of contact infection, were fed on diet N_2 until the day on which they were infected, so that the development of the disease among these mice, and hence the risk to which the 100 contacts were exposed, was approximately the same in all groups. In this first experiment, four groups, A, B, C and D, were fed on diet N_5, and two groups, E and F, on diet N_2.

In the second experiment there were also six groups. Groups G and H, each consisting of 100 male mice, and groups J and K, each consisting of 100 female mice, were fed on diet N_5, while group L (100 male mice) and group M (100 female mice) were fed on diet N_2.

In the third experiment there were four groups. Group N (100 male mice) and group O (100 female mice) were fed on diet N_5, while group P (100 male mice) and group Q (100 female mice) were fed on diet N_2.

The relevant data for all three experiments are set out in Table I, and the course of the four epidemics in the third experiment is shown graphically in Chart I, in which the numbers of surviving mice are plotted against time.

Few comments are necessary, so far as the effect of the diets is concerned. Taking each of the three experiments, the epidemic mortality was less among the groups fed on N_5 than among the groups fed on N_2. Judged on the percentage of mice surviving for 60 days, the difference is in each case statistically significant. Judged on the mean expectation of life limited to 60 days [60 E_x], it is insignificant in the first experiment, but clearly significant in the second and third. The differences in the second experiment (56·3% survivors

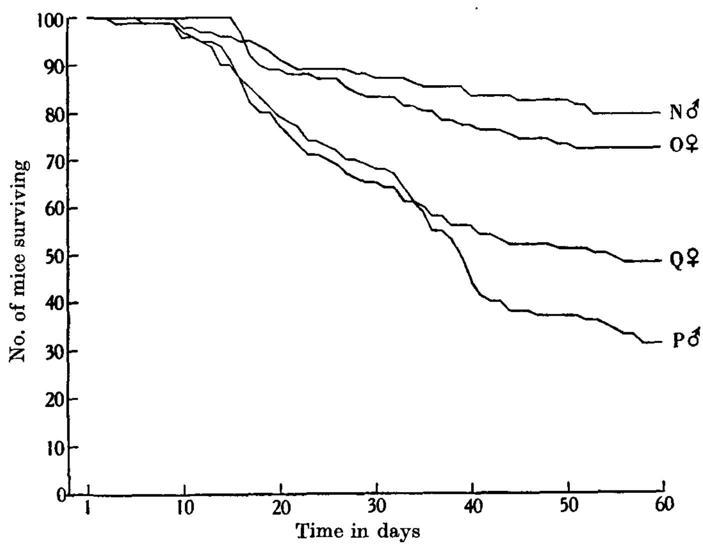

Chart I.

on diet N_5 as compared with 18·5% on diet N_2), and in the third (75·5% survivors on diet N_5 as compared with 39·5% on diet N_2), are, in respect of the absolute difference, much greater than in the first (18·5% survivors on diet N_5 as compared with 9·0% on diet N_2). Moreover, the ratio of the differences to their standard errors, particularly in respect of the 60 E_x values, are much higher in the second and third experiments than in the first.

On the main problem at issue there would, however, seem to be no doubt. Mice fed on diet N_5 are more resistant to natural contact infection, as well as to artificial infection, than mice fed on diet N_2; and, in consequence, if herds of mice fed on diets N_5 or N_2 are subjected to epidemics of equal potential severity, the herds fed on diet N_5 will suffer a significantly lower mortality.

One other point may be noted. Among the survivors from the four groups in the first experiment that were fed on diet N_5, the infection rate, as judged from spleen cultures, varied from 66·7 to 85·7% (mean 75·6%). Among the survivors from the two groups fed on diet N_2 the infection rate

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Table I

Experiment	Diet	Group*	No. of mice dying	% survivors on 60th day		S.E.	Difference	S.E. of difference	$60\ E_x$		S.E.	Difference	S.E. of difference
I	N_5	A ♂	82	18	18·5	1·94	9·5	2·80	25·16	28·26	0·88	0·54	1·29
		B ♂	76	24					34·89				
		C ♂	86	14					24·14				
		D ♂	82	18					28·87				
	N_2	E ♂	89	11	9·0	2·02			28·26	27·72	0·93		
		F ♂	93	7					27·17				
II	N_5	G ♂	58	42	56·3	2·48	37·8	3·70	40·68	46·18	0·87	12·70	1·44
		H ♂	59	41					42·65				
		J ♀	23	77					52·70				
		K ♀	35	65					48·70				
	N_2	L ♂	90	10	18·5	2·75			29·19	33·48	1·15		
		M ♀	73	27					37·77				
III	N_5	N ♂	21	79	75·5	3·04	36	4·61	53·31	52·20	1·06	11·30	1·68
		O ♀	28	72					51·08				
	N_2	P ♂	69	31	39·5	3·46			39·61	40·90	1·30		
		Q ♀	52	48					42·19				

* Each group consisted of 100 mice.

was 90·9 and 100% (mean 95·5%). In the second experiment, in the four groups fed on diet N_5, the infection rate among the survivors varied from 73·8 to 100% (mean 84·9%), while in the two groups fed on diet N_2 it was 96·3 and 100% (mean 98·2%). In the third experiment the infection rates were 31·9 and 32·9% (mean 32·4%) among the survivors from the two groups fed on diet N_5, and 64·6 and 77·4% (mean 71·0%), among the survivors from the two groups fed on diet N_2. This suggests that N_5 has an effect on the risk of developing a latent infection, as well as on the risk of dying, though the differences, except in the third experiment, are less striking than those relating to mortality.

The relative resistance of male and female mice

The figures set out in Table I show a curious difference in the mortalities experienced by male and female mice. Exp. I yields no information on this point, since all six groups under test were composed exclusively of males. In Exp. II the differences were very striking. In the two herds of male mice fed on diet N_5 the total mortality was 58 and 59% respectively, while in the two herds of female mice on the same diet the mortality was 23 and 35%. In the single male herd fed on diet N_2 the mortality was 90%, while in the female herd fed on this diet it was 73%. It semed very unlikely that so large and consistent a difference could be due to chance; but the results of Exp. III showed no clear sex difference. The male herd fed on diet N_5 suffered a slightly lower mortality (21%) than the female herd (28%), though the mortality in the male herd on diet N_2 was higher (69%) than that in the female herd on the same diet (52%).

Since fighting is commoner among the males than among the females, it seemed possible that the differences observed in mortality rates observed in Exp. II might be the result of fighting and cannibalism among the males, with a consequent increase in the risk of infection from the consumption of infected mice. The detailed records of the experiments, however, showed that this was not the case. In Exp. II the numbers of dead mice partially eaten by their companions, including the twenty-five infected mice added to each herd, were, in the three male herds, 0, 1 and 0 respectively, as compared with 8, 0 and 2 in the three female herds. In Exp. III the numbers eaten in the two male herds were 5 and 4, as compared with 22 and 10 in the two female herds. So far as these few records go, the habit of cannibalism would seem to have at least as many addicts among female mice as among males; and, if this factor played any part in these particular experiments, it would presumably have increased the relative mortality among the females in Exp. III, and so perhaps have obscured a naturally higher resistance, rather than have induced the increased relative mortality in males in Exp. II.

Since we had in our possession card records of numerous experiments in which mice on different diets had been injected with toxic fractions from

Bact. typhi-murium, or fed with living suspensions of this organism, we turned to them to see whether there was any evidence of a sex difference in resistance. Our colleagues Dr Bradford Hill and Miss J. M. Hatswell had a large number of similar records, obtained during the course of an investigation that is still proceeding, and they have kindly allowed us to include these with our own.

The relevant figures are set out in Table II. In each case the totals and averages given are for groups of different tests of the same kind. It will be

Table II

Inoculum	Mode of inoculation	Dose	No. of mice inoculated	Sex of mice inoculated	% mortality	Difference	S.E. of difference	χ^2
			Diet N_5					
Toxic fraction isolated from *Bact. typhi-murium*	Intra-peritoneal	1–4 mg.	1913 1857	♂ ♀	57·1 49·9	7·2	1·62	
Bact. typhi-murium	*Per os*	100,000,000 bacteria	134 116	♂ ♀	40·3 31·9	8·4	6·06	
Bact. typhi-murium	Intra-peritoneal	100,000 bacteria	66 34	♂ ♀	92·4 76·5	15·9	7·97	$\chi^2=6.38$ $n=3$ *P* slightly less than 0·10
Str. haemolyticus	Intra-peritoneal	3,200 bacteria	50 50	♂ ♀	70·0 58·0	12·0	9·52	
			Diet N_2					
Toxic fraction isolated from *Bact. typhi-murium*	Intra-peritoneal	1–4 mg.	197 203	♂ ♀	74·1 67·5	6·6	4·53	
Bact. typhi-murium	*Per os*	100,000,000 bacteria	133 117	♂ ♀	63·2 60·7	2·5	6·15	$\chi^2=8.26$ $n=4$ *P* slightly less than 0·10
Bact. typhi-murium	Intra-peritoneal	100,000 bacteria	64 36	♂ ♀	100·0 88·9	11·1	5·24	
Str. haemolyticus	Intra-peritoneal	3,200 bacteria	45 55	♂ ♀	86·7 74·5	12·2	7·76	

noted that, in each case, the mortality among the males was higher than among the females, though the difference was seldom great. In the group of tests in which a toxic fraction was injected into mice fed on diet N_5, the difference between the male and female mortality, though not large, is clearly significant on any statistical test. In the other groups, on either diet, the difference sometimes approaches statistical significance, but is often below it. When the χ^2 test is applied to these groups it gives a figure which, in spite of the consistency, does not satisfy the usual criterion of significance. When these large groups are broken up into the individual tests of which they are composed, some of which were very small, the males are no longer consistently less resistant than the females. In a total of thirty tests made on mice fed on diet N_5, males were less resistant than females in twenty-four tests, more resistant in five, and equally resistant in one. In a total of seventeen tests made on mice fed on diet N_2, males were less resistant than females in eight tests, more resistant in five, and equally resistant in four.

The only reference to this problem that we have been able to find in the literature is in a recent paper by Schutze *et al.* (1936). These workers were comparing the resistance of different strains of mice to various bacterial infections. In a series of tests in which mice were injected intraperitoneally with living cultures of *Bact. typhi-murium*, they observed a consistently higher mortality among the males. In a second series of tests in which mice were injected with *Bact. enteritidis* there was much less difference in resistance, and the differences were not consistent, though the balance suggested a slightly greater resistance among the females. In a third series of tests, in which mice were injected with a living culture of *Past. muriseptica*, the males appeared to be more resistant than the females, though the results were far less consistent than in the first series of tests with *Bact. typhi-murium.*

Taking the evidence as a whole, we think there is a strong suggestion, falling short of adequate demonstration, that there are significant sex differences in resistance to bacteria, or to their toxins, and that females tend to be more resistant than males. The differences are, however, curiously inconstant; and this inconsistency perhaps suggests that the factors concerned may be of a fluctuating rather than of a permanent kind. We should, at least, agree with Schutze and his colleagues that, in any test on resistance, it is important to ensure that the groups under comparison are either composed of a single sex, or contain equal numbers of each sex.

Conclusions

1. Mice fed on a diet containing dried skimmed milk, oatmeal and other constituents are more resistant to natural contact infection with *Bact. typhi-murium* than mice fed on a diet from which the dried skimmed milk is omitted. In consequence, an epidemic of mouse-typhoid produces a lower mortality among mice fed on the former diet than among mice fed on the latter.

2. Female mice appear, on the average, to be more resistant than males to *Bact. typhi-murium*, and perhaps to other bacteria; but the difference in sex resistance is not a constant one.

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