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THE RELATION OF DIET TO DISEASE

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Nutrition and Susceptibility to Infection

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The guess that diet might exert an important influence on susceptibility to infection was well worth making. Reviews of the literature (Clausen, 1934; Robertson, 1934; Watson, 1937*a*, *b*; Watson, Wilson & Topley, 1938; Perla & Marmorston, 1941; Aycock & Lutman, 1944; Berry, Davis & Spies, 1945; Leitch, 1945; Schneider, 1946*a*; Wilson & Miles, 1946; Gell, Parry, Leitner, Howie & Hartley, 1948) reveal many suggestive correlations between the food of men and animals, and the incidence of infection in the community or herd; but the same reviews make it clear that promotion of the guess to an accepted place in scientific knowledge involves a struggle that is not yet won. I think it worth discussing why the evidence for a relation between diet and infection is not better accepted, and what must be done to secure for this problem the critical attention and disciplined study it requires.

Evidence from man

The first difficulty is that data from the human field, which make a good prima facie case for a dietary influence on host resistance or susceptibility to infection, and are naturally of greater interest, are none the less inconclusive. Poorly fed communities commonly experience a high incidence of infection and the diet is often named as the cause. But a poorly fed community is as a rule poorly organized in other respects also. It lacks hospital beds and public health organization to isolate infectious diseases; its members suffer fatigue, anxiety, and low morale; they crowd into uncomfortable, often infested, dwellings; their personal hygiene is poor; and they do not know how to look after themselves in sickness or in health. These influences, as well as those of age, sex, and heredity, can help to increase or decrease both the weight of infection to which the community is exposed and the resistance of its individual members. Attempts to separate and measure the dietary influence on infection in such difficult circumstances cannot yield satisfying results without direct experiments on the diet.

In the last resort, the human subject must be his own experimental animal and it will be useful if more investigators in the human field will follow the example of Green, Pindar, Davis & Mellanby (1931). In order to test their hypothesis that a supplement

of vitamins A and D might reduce the incidence of puerperal infection, a much graver problem in 1931 than to-day, these workers made a direct experiment in the following way. They administered a suitable vitamin concentrate during the 4 weeks before labour to 275 women attending the antenatal clinics of the Jessop and Nether Edge Hospitals, Sheffield, and observed as controls an equal number of alternate women attending the same clinics who did not receive the vitamin supplement. All the women in the experiment were delivered in hospital. In the group given the vitamin supplement the incidence of puerperal pyrexia was 19·2% and of severe sepsis 1·5%, whereas in the untreated groups the figures were 30·9 and 3·6%. Even if we do not now believe that vitamin A has specific anti-infective properties this evidence is worth notice, and the method is one that should be applied to other groups of people, other variations of diet, and other risks of infection. Evidence collected by this kind of experiment is the proper climax to reasoning based on careful study and analysis of natural events. In the human field such experiments present real but not insuperable difficulties, and we need more of them. Even if the results are negative, as in the trial of a multiple vitamin supplement given to children by Bransby, Burn, Magee & MacKecknie (1946), they are of great value. Unsupported by such crucial experiments even the closest and most skilful analysis of suggestive associations between diet and infection in the community or herd can only help to define the problems most suitable for attack; it cannot tell us if our beliefs are right or wrong.

Animal experiments

Unhappily, when we turn to the animal experiments that have been done to test the effect of diet on infection we are confronted with so many inadequate experiments and so many apparent contradictions that the weight and force of bad evidence may drive out the good. The most useful thing I can do at this stage is to connect the results of selected experiments which are consistent with one another and furnish coherent evidence of the relation between diet and infection and a starting-point for new work.

Webster & Pritchett (1924) at the Rockefeller Institute showed that mice born and reared on one of two different diets had a marked difference in susceptibility to experimental stomach-tube infection with one of the mouse-typhoid organisms, *Salmonella typhi-murium* (*'Bacillus pestis caviae'*). The differences were substantial and consistent in three separate experiments. On the diet more favourable to resistance eleven of sixty-nine mice died (15·9%) against fifty-six of seventy-two (77·8%) on the diet less favourable. The diet more favourable to resistance was a modified McCollum diet consisting of whole wheat 67·5, casein 15, milk powder 10, sodium chloride 1, calcium carbonate 1·5 and butterfat 5%; the less favourable, the stock mouse-diet of the Rockefeller Institute at that time, consisted of a daily ration of baker's bread soaked in fresh pasteurized milk supplemented by two feedings a week of an oatmeal and buckwheat mixture and one feeding a week of dog biscuit. Webster & Pritchett (1924) did not give actual figures for breeding performance and growth, but stated that on both diets the mice grew and bred well and presented all the appearances of good health. I describe this work in detail because I cannot see how in face of it the point may still be debated, as it often is debated: 'Is there really anything in this story about diet and

infection?' Some of the doubts may arise from a study of Pritchett's later unhappy attempts to establish vitamin A as the constituent of the McCollum diet responsible for the superior resistance (Pritchett, 1927). These were criticized in detail by Watson (1937*b*, p. 422), a worker at the London School of Hygiene; and in a recent review, Schneider (1946*a*, p. 59) of the Rockefeller Institute upheld Watson's criticisms of Pritchett's conclusions. It is enough to say here that in a complicated series of comparisons Pritchett failed to make the crucial test of her hypothesis: to compare in one experiment McCollum diet with butterfat versus McCollum diet without butterfat. This was particularly disappointing since Pritchett's (1927) data showed that the McCollum diet without butterfat actually conferred high resistance, and the addition of butterfat or cod-liver oil to the Rockefeller Institute stock diet did not lead to significantly greater resistance than resulted from addition of 'Crisco'—a hydrogenated vegetable oil regarded as free of vitamins. In spite of the criticism brought against Pritchett's interpretations, the work firmly established a relation between diet and infection. Besides offering a still unanswered question and showing how profitable an empirical approach can be, it warns that our task may lie in the characterization of still unknown nutrients or in clarifying the relation between physiological state and the pathology of infection and not in deciding to which known nutrient we may most fittingly award the epithet 'anti-infective'.

Doubt about the interpretation of these important experiments was deepened by work carried out at the London School of Hygiene, in which Topley, Greenwood & Wilson (1931) demonstrated that the addition of various foods containing vitamin A to the diet of their mice not only failed to decrease susceptibility to *Salm. typhi-murium* infection in experimental epidemics but actually increased it in some of the experiments. Rational judgement should have concluded only that vitamin A was not established as a specific anti-infective vitamin; but in this country at least many bacteriologists seemed to go a stage beyond the evidence and came to feel that the whole idea of a correlation between diet and infection had been disproved by the careful experiments of a group of workers of great authority.

That this was not the conclusion of the workers themselves was shown by later experiments at the London School of Hygiene by Watson (1937*a*, *b*) and Watson *et al.* (1938). From these experiments it emerged once again that diet could exert a decisive influence on the outcome of experimental salmonella infection in mice. The salient fact was that in repeated trials mice on a poor diet (N_2) with coarse oatmeal as its principal constituent (92% of the diet) were significantly and consistently more susceptible to experimental salmonella infection than mice on an improved modification of the diet (N_5) which contained dried skim milk 25, dextrin 23, and coconut oil 4% in place of an equivalent amount of coarse oatmeal, which was thus reduced from 92 to 40% in the improved diet. As Watson (1937*a*, *b*) observed, and her protocols clearly confirm, the differences in susceptibility to infection were not the only evidence that the two diets were of different biological value. In particular, the breeding performance of the mice was much superior on the improved diet. For example, the averages of seven tests (Watson, 1937*a*, p. 402) with the poor diet (N_2) showed 12·5% of sixty-four mated does without litters in contrast to the averages of two tests with the improved diet (N_5)

which showed only 3·5% of fifty-four mated does without litters. Further (Watson, 1937*a*, p. 403), the data for 6 months for fifty does first mated in January 1935 showed 11·5% of seventy-seven litters entirely eaten by does on the poor diet against only 0·7% of 128 litters on the improved diet. For the same period, on the poor diet, 370 mice were born alive in litters not entirely eaten at birth or later; at 4 weeks 76·0% and at 8 weeks 54·5% survived, whereas on the improved diet of 892 mice born 84·5% were alive at 4 weeks and 82·5% at 8 weeks. On the poor diet the average weights at 1, 4, and 8 weeks were 3·5, 8·5, and 15·0 g. against 4·0, 13·5, and 23·0 g. for the improved diet. In Watson's (1937*b*) view the dried skim milk was probably responsible for the increased resistance, although in her discussion she discounted the likelihood of there being any specific anti-infective factor in the milk, and noted that it was impossible to say what infection-resisting body mechanism had been improved. Wilson & Miles (1946, p. 1200) thought that 'the effect on metabolism of a well-balanced diet' was the likely explanation of the superior resistance.

At this stage it is worth emphasizing that for breeding and rearing young mice reduction of the oatmeal from 92 to 40% would have been of benefit even if a nutrient other than dried skim milk had been used for the replacement. In unpublished experiments at the Rowett Institute, for example, we reduced the ill effects of what proved an excessive proportion (66%) of whole oats in the diet of breeding mice by offering the animals a supplement of maize starch, and no doubt there are other methods of improving such a diet. Our unsatisfactory oat diet was the same as Schneider & Webster's (1945) Rockefeller Institute diet no. 123: whole oats 66, dried whole milk 33, sodium chloride 1%, and our findings confirm their conclusion that the diet was a poor one for breeding mice as judged by the number of survivors to maturity, the growth rate, and the resistance to salmonella infection of the young mice. This is true, it should be noted, although the diet contains 33% of dried whole milk. Watson's (1937*b*) conclusion about the importance of the milk might therefore be re-stated in more general terms by saying that improved resistance to infection was brought about by adjusting a manifest imbalance—too high a proportion of oatmeal—in a poor diet.

Recent experiments

Schneider & Webster's (1945) important contribution to problems of diet and infection introduced whole wheat as a foodstuff which must now be considered as possibly having anti-infective properties, at least for mice exposed to salmonella infection. Slightly modifying the formula for diet B of Sherman (Sherman & Campbell, 1924), Schneider & Webster (1945) compared the Rockefeller Institute diet 100 (whole wheat 66, dried whole milk 33, sodium chloride 1%) with other similar diets in which the whole wheat was replaced by an equal proportion of one or other of the following: dried cooked potato meal, whole corn (maize), whole rye, whole oats, whole rice. Mice bred and reared on the diets with wheat, maize, rye, and rice had approximately equal susceptibility to salmonella infection; the undersized mice reared on oats and on potato were extremely susceptible to the infection. Although the maize, rice, and rye diets were inferior to the wheat diet for the reproduction, growth, and survival of the

mice to the age of 5 weeks, those that survived had almost the same resistance to the infection as the more numerous survivors on the wheat diet.

As a result of these trials Schneider & Webster (1945) chose the wheat diet (diet 100) as a standard for comparison with a 'synthetic' diet (diet 191). At weaning (3 weeks of age) the mice, bred and suckled by mothers on a modified Steenbock stock diet, were transferred to wheat diet or synthetic diet. Male mice grew only a little better on the wheat diet than on the synthetic diet, but female mice on the wheat diet showed an advantage over females on the synthetic diet of about 1·6 g. at 8 weeks old, 3·0 g. at 12 weeks, 4·0 g. at 16 weeks, and 6·0 g. at 20 weeks. The performance on the synthetic diet could not be styled failure of growth, for on it females averaged 20·4 g. at 8 weeks and 24·8 g. at 20 weeks—quite good weights for mice—and all the animals remained in good health. After being fed for 3 weeks on either the wheat diet or the synthetic diet the mice were infected with salmonella organisms by mouth. Mice maintained on the wheat diet showed striking and consistent superiority in resistance to experimental salmonella infection over those on the synthetic diet. If 66 of the 72·5 g. of glucose in 100 g. of synthetic diet were replaced by whole wheat, the resistance of the mice was increased, but not if the substitute was whole dried milk. With this as his starting-point Schneider (1946*b*, 1948) has begun an attempt to identify a resistance-enhancing agent in whole wheat, an effort in which it is a pleasure to wish him success. Whether or not he succeeds in this objective, students of diet and infection are much in his debt for his stimulating review (Schneider, 1946*a*) of the subject. This, together with his new technical and intellectual approaches, has greatly freshened the atmosphere surrounding the subject of our discussion.

It seems necessary to note, however, that great difficulties attend the breeding and rearing of mice on synthetic diets (Rogers, McElroy & Cowgill, 1942; Fenton & Cowgill, 1947), and this was true of diet 191 (Schneider, personal communication) although it allowed good growth from weaning to maturity of mice bred and suckled by mothers on a modified Steenbock stock diet. This clearly establishes that there is some important deficiency in synthetic diet 191, and comparison with it and the other diets mentioned by Schneider & Webster (1945) is not perhaps the most satisfactory test of the anti-infective properties of the slightly modified diet B of Sherman (diet no. 100), if that diet is to be generally adopted as a reference point. Indeed, my colleague Dr S. R. Sengupta and I have now prepared for publication our evidence that mice bred and reared on Schneider & Webster's (1945) diet 100 are more susceptible to experimental tuberculosis than mice bred and reared on a Rowett Institute stock cube with 14% skim milk powder, and a supplement of 5 ml. of fresh whole milk daily to the pregnant and nursing does (Sengupta & Howie, 1948–9). In our paper we have indicated some of the differences between the two diets: compared with the Rowett Institute cubed diet, the modified diet B of Sherman has a low calcium content, an unfavourable calcium : phosphorus ratio, a little less protein, more fat, and a number of other differences which we did not measure. In conjunction with Dr J. M. Naftalin we are now trying to discover which of the differences are important and how they operate.

It is of interest that experimental salmonella infection by mouth fails to reveal any difference in susceptibility between mice bred, reared, and infected on the two diets

used for the tuberculosis experiment. This is worth mentioning to emphasize that 'infection' is not a homogeneous entity. There is a wide array of infecting agents and we are only now beginning to learn about their different nutritional needs. Viruses pathogenic for man and animals, for example, can be cultivated artificially only in the presence of actively growing animal cells. In many experiments poorly nourished animals have actually proved less susceptible to virus infections than those that were better fed (see, e.g. Hulse & Edwards, 1937; Sprunt, 1942; Foster, Jones, Henle & Dorfman, 1944*a*, *b*; Rasmussen, Waisman, Elvehjem & Clark, 1944). I mention this important work not to advocate malnutrition as a prophylactic against virus infections, but to emphasize that the nutritional state will not necessarily have the same influence on all infections.

In an experimental survey of this problem at the Rowett Institute events have made us face what nutritionists accept as fundamental but bacteriologists often forget: that the effects of a dietary change may not appear quickly, or in the absence of some stress, or indeed until the second or even later generations. Accordingly we propose to do most of our experiments on animals whose parents have been on the test diets from mating. Moreover, we propose to follow Bruce's (1947) mouse-breeding technique in which monogamous pairs of mice are allowed to breed continuously. This will secure not only a steady supply of test animals for repetition of promising experiments at short intervals, but also the stress that may be required to reveal dietary imperfections (Bruce & Emmens, 1948).

We shall use salmonella organisms and tubercle bacilli as our test infections, and we should like to add a virus when our experimental arrangements are complete. As an infection on which to test the effect of diet, tuberculosis is indicated by many suggestive correlations, well summarized by Leitch (1945). That an abrupt change to a very poor diet at the time of inoculation increased the susceptibility of mice to experimental tuberculosis was recently noted by Dubos & Pierce (1948), although they emphasize that theirs was a study of diagnostic technique and not of the part played by nutrition. In view of the abruptness of the change to the poor diet and the nature of the diet the animals probably ate very little food for some days after infection.

Perhaps I ought to make it plain that the mouse is the central figure of our discussion and of our work in the immediate future, because an experimental study of diet and infection demands in its first stages—and we should be clear that we are in our first stages—an animal which breeds rapidly, needs little space, tolerates important changes in its diet without becoming moribund, and responds to a great variety of infecting agents in a fairly regular and predictable fashion. With an adequate community of mice under controlled conditions of breeding and housing it is possible to examine two test populations whose only important difference from each other is their diet, and it is easy to arrange numbers so that our measurement of susceptibility to a test infection is made by counting the dead and the living, as well as by any other measurements that may suit the particular infection under study. Survival rate is the only measurement that is an unequivocal criterion of resistance or susceptibility to infection, a point much overlooked in some of the plausible discussions of experimental results. Later, if mouse experiments clarify our understanding of diet and infection, I shall wish to test our findings on animals of economic importance and I may then have to pay more

attention to indirect measurements of susceptibility. But until these have been correlated with death or survival they must not be made to bear too heavy an argument. Belief in the relationship between diet and infection will grow only if we can point to an increasing number of precisely defined relationships between particular diets and specific infections and offer some attempt at explaining the mechanism. Experiments on mice have given the relationship of diet and infection a place in science, and further experiments on the same animal offer the best hope of giving shape to the present vague mass of information.

Conclusions

1. The relation between diet and susceptibility to infection is not widely accepted among bacteriologists, but this review surveys a selected sample of the experimental evidence to establish that the relationship is real and well worth defining in terms of greater precision than can yet be used.

2. The results of some important experiments by different workers, if closely analysed, are seen to be consistent with one another, and not contradictory, as insufficient study might suggest.

3. Future work on diet and infection at the Rowett Institute will measure the suitability of the test diets for growth and reproduction of mice under the stress of continuous mating and rearing as well as the influence of the diets on susceptibility to infection.

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