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Diet and Resistance to Experimental Tuberculosis in Mice

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On clinical and epidemiological grounds it is generally accepted that nutrition has an important influence on resistance or susceptibility to tuberculosis (see, e.g. Long, 1941*a*, *b*; Orr, 1941–2; Day, 1942, 1948; Keers, 1943, 1945, 1948; Leitch, 1945; Rich, 1946; Daniels & Hart, 1948). But in field studies it is difficult to isolate diet from the other agents known to determine resistance or susceptibility to infection—for example, housing and ventilation, physical and mental strain, age, sex, heredity, previous contact with the infection, and the number of infecting organisms encountered. Cuthbertson (1940–1, p. 11) stated: 'It is difficult to distinguish the many separate factors that may be concerned. There certainly appears to be an inverse relationship between the incidence of tuberculosis and social prosperity in which diet plays a part.' In view of this, laboratory studies on diet and tuberculosis are clearly required, keeping all other conditions as uniform as possible in order to bring out the effect of differences in diet. In Rich's (1946) words: 'Considering the importance of the matter, there has been surprisingly little pointed experimental study of the relation of nutrition to resistance in tuberculosis. The little that has been done in this direction has not shed any significant or conclusive light upon the problem.' In the past few years, interest in the mouse as an experimental animal for tuberculosis (Browning & Gulbransen, 1926; Schwabacher & Wilson, 1936–7) has been revived by the work of Glover (1944), Youmans & McCarter (1945), Martin (1946), and Dubos (1947). Dubos (1947) indeed studied the effect of various diets of natural food and reported (p. 51) that: 'Animals kept on a poor diet (comprising a very large proportion of starch and gelatin) developed more numerous and extensive pulmonary lesions than animals maintained on a more

complete diet.' This work was reported in more detail by Dubos & Pierce (1948). Koerner, Getz & Long (1949) also reported that survival time increased in experimental tuberculosis in rats as the dietary casein was increased from 15 to 40%. In April 1947 we decided to begin work on the effect of diet on resistance to experimental tuberculosis in mice; the first results are given here.

METHODS

Mice. The mice employed were albinos of the W-Swiss strain brought from the Rockefeller Institute to England in 1938. The strain was inbred by brother-sister mating for twelve consecutive generations before being brought to this country. Thereafter it underwent a further uncertain number of brother-sister matings before being outbred. The colony at this Institute was reared from fifty breeding females supplied by the Agricultural Research Council's Field Station at Compton; it is maintained as a closed population without brother-sister inbreeding. Our breeding methods follow the technique of Schneider & Webster (1945), who claim that dietary effects on resistance to infection may be obscured if the population of test animals is deprived of some degree of genetic heterogeneity in respect of resistance to infection.

Management of animals. The mice were housed in rooms with a temperature that ranged from 70 to 80°. The breeding and storage cages measured 12 in. × 12 in. × 8 in. and were constructed of galvanized iron wire with a mesh of $\frac{1}{4}$ in. For bedding, wood wool was allowed before infection; after infection no bedding was given. The cages rested above trays filled with sawdust; the trays were changed for cleaning twice weekly. For breeding, groups of five females were mated with one male. When pregnant, each female was separated and left to rear as many of her litter as she could. The young were weaned at 23 days of age, when they were divided according to sex and caged in groups of twelve to thirteen. At 8–9 weeks of age they were removed for infection from the breeding colony to a separate room, also at 70–80°, in a different building. In the experiments described in this paper the animals were caged in groups of twelve to thirteen after infection; but in later work, as more equipment became available, they were caged individually.

Diets. For these experiments two diets were studied: diet 1, a stock cube diet and diet 2, the slightly modified diet B of Sherman which Schneider & Webster (1945) and Schneider (1946, 1948) referred to as diet 100 and chose as a 'reference point' in comparing the resistance of mice on various diets to salmonella infection.

Since this paper was drafted we have noted with interest Dubos & Pierce's (1948) report that the same diet conferred on mice greater resistance to experimental tuberculosis than a diet of corn (maize) meal, butter and gelatin.

Diet 1 is based on the stock cube described by Thomson (1936), but its dried skim milk has been increased from 7 to 14% and certain changes have been made in its cereal components. Table 1 gives the composition of the two diets and Table 2 the results of the chemical analysis of samples used in our experiments. We did not attempt vitamin assays at this stage of our work. The appropriate diet was fed to the parents of the test animals from at least 7 days before mating and to the test animals from weaning; all the test animals were from first litters.

On diet 1, breeding females were allowed 5 ml. daily of fresh liquid whole milk from the time of mating until their young were weaned at 23 days of age. Green food was not supplied. From weaning the young received only stock cubes.

Diet 2 was first fed as a dry powder, but later as a moist dough prepared by addition of 30 ml. of water to 100 g. of diet. The change in feeding procedure did not influence the results but prevented loss of food from scattering. With both diets tap water was given *ad lib.* from drinking bottles.

Table 1. *Composition of experimental diets*

Diet 1				Diet 2	
Constituent	Amount (%)	Constituent	Amount (%)	Constituent	Amount (%)
Wheat offal (bran)	17·7	White-fish meal	4·5	Wheat, whole ground	66
Wheat, whole ground	17·7	Meat-and-bone meal	8·8	Milk, whole dried	33
Oats, Sussex-ground	17·7	Dried skim milk	14·0	Sodium chloride	1
Maize, ground	8·8	Dried yeast	1·2		
Barley, ground	8·8	Sodium chloride	0·4		
		Cod-liver oil	0·4		

Diet 1 = Rowett Institute cubes modified from Thomson (1936).
Diet 2 = Diet B of Sherman as modified by Schneider (1946).

Breeding females on diet 1 received 5 ml. daily of fresh liquid whole milk from mating until their young were weaned at 23 days of age. Green food was not given. From weaning the young received only cubes.

The original diet B of Sherman (Sherman & Campbell, 1924) is composed of two-thirds whole ground wheat, one-third whole milk powder, and sodium chloride equal to 2% of the weight of the wheat.

Table 2. *Chemical composition of experimental diets as determined by direct analysis of samples*

Constituent	Amount	
	Diet 1 (%)	Diet 2 (%)
Dry matter	87·6	88·3
Protein	19·2	14·7
Fat	4·9	9·5
Fibre	4·8	1·3
Carbohydrate	52·7	59·9
Ash	6·0	2·9
Calcium	1·28	0·35
Phosphorus	0·99	0·51
Calcium : phosphorus ratio	1·3 : 1	0·69 : 1

The daily consumption of food by the test animals during the 2–3 weeks before infection—that is, at 6–9 weeks of age, was in the region of 5 g. for both diets. The average weaning weight of mice was 10 g. on diet 1 and 9·8 g. on diet 2; the weights at 8 weeks were 21·5 g. for diet 1 and 20·8 g. for diet 2.

Infection procedure. Infection procedure closely followed that described by Martin (1946). The human strain of *Mycobacterium tuberculosis* H905 was grown on slopes of Löwenstein-Jensen medium (Mackie & McCartney, 1948, p. 172) in screw-cap containers for from 14 to 21 days. To maintain an adequate supply of air, the screw caps were loosened every 2nd day, care being taken to avoid entry of contaminants. The surface growth was respread once a week. Under these conditions growth was

abundant. The infecting suspension was prepared by gently removing moist surface growth, without any of the underlying medium. The growth was weighed and transferred to a sterile conical 50 ml. flask with $\frac{3}{16}$ in. diameter steel balls and milled for a total of 20 min. For the first 10 min. the growth was milled without addition of fluid until a uniform thin film spread over the interior surface of the flask. Thereafter sterile distilled water was added as follows: 11 min. from the start of milling, 1 ml.; 15 min. from the start of milling, half the remaining volume required; and 20 min. from the start of milling, the remainder of the distilled water necessary to give a suspension of the strength for inoculation—in these experiments 0·75 mg. of culture in 0·1 ml.

The infecting suspension was inoculated intravenously into the tail vein. Any mice that died within 24 hr. or did not receive the whole inoculum intravenously were discarded from the experiment.

RESULTS

Preliminary observations. A pilot experiment (Exp. 1) was made by inoculating intravenously with 0·75 mg. of moist culture in 0·1 ml. sterile distilled water, six male mice on each of diets 1 and 2. All six mice on diet 2 died between 14 and 21 days after inoculation, whereas only two of the mice on diet 1 died within this period. The experiment was ended at 21 days by killing the four survivors on diet 1 and comparing their lungs with those of the mice that had died on diet 2. The lungs of the mice on diet 2 were more voluminous and had more tuberculous lesions than those of mice on diet 1. The difference in appearance was striking and, in conjunction with that in the number of survivors at 21 days, seemed to warrant a larger experiment to test the possibility that there might be a consistent difference in resistance of mice on the two diets.

The pilot experiment suggested three criteria for measuring resistance: (i) the number of survivors, (ii) the survival time and (iii) the extent and appearance of the lung lesions in animals that survived the experiment. Accordingly two larger experiments were made.

Exp. 2. In this experiment twenty-three males and twenty-two females fed on diet 1 and twenty-five males and twenty-five females on diet 2 were inoculated intravenously, as already described, at 8–9 weeks of age with 0·75 mg. of moist culture in 0·1 ml. of sterile distilled water. The first animal died on the 17th day after inoculation. At 30 days after inoculation only two of forty-five animals on diet 1 were dead: one male and one female, whereas nine of fifty on diet 2 were dead, two males and seven females. This experiment was allowed to continue for 112 days after the animals were infected and at the end of the experiment the survival rates on the two diets were alike. The details are summarized in Table 3. The median survival times, however, were 86 days for mice on diet 1 and 57 days for mice on diet 2. Mr M. H. Quenouille, Lecturer in Statistics at Aberdeen University, kindly examined the figures and reported that the difference in median survival time between diets 1 and 2 (Fig. 1), 29 ± 8 days, was 'obviously significant' ($P < 0{\cdot}001$).

Exp. 3. This experiment was a repetition of Exp. 2. As far as possible all details of procedure were the same, except that the infecting suspension of tubercle bacilli was

less vigorously milled, with the idea of shortening the experiment by using a more rapidly lethal culture. In this experiment we inoculated twenty-five males and twenty-five females on diet 1, and the same numbers on diet 2. The survival data after inoculation (Table 4) again showed that the mice on diet 1, especially the females, had

Table 3. *Exp.* 2. *Survival differences in mice on diets* 1 *and* 2 *after experimental tuberculous infection*

		Mice				
			No. dead Days after inoculation			
Diet no.	Sex	No. inoculated	30	60	90	112
1	M	23	1	7	13	20
	F	22	1	7	11	15
	Total	45	2	14	24	35
2	M	25	2	15	20	21
	F	25	7	11	16	18
	Total	50	9	26	36	39

Table 4. *Exp.* 3. *Survival differences in mice on diets* 1 *and* 2 *after experimental tuberculous infection*

		Mice				
			No. dead Days after inoculation			
Diet no.	Sex	No. inoculated	14	28	42	63
1	M	25	4	10	10	15
	F	25	1	2	2	12
	Total	50	5	12	12	27
2	M	25	4	7	10	21
	F	25	7	8	9	20
	Total	50	11	15	19	41

Table 5. *Exp.* 3. *Lung lesions of surviving mice killed* 63 *days after tuberculous infection*

		Survivors		Lung lesions of survivors*				
Group	No. inoculated	Number	Percentage	+ + + +	+ + +	+ +	+	−
Diet 1	50	23	46	0	2	4	8	9
Diet 2	50	9	18	8	1	0	0	0

* + + + + = Extensive confluent lesions.
+ + + = Lesions separate but too numerous to count.
+ + = Lesions separate and countable, usually ten to twenty.
+ = Lesions small and fewer than ten.
− = No lesion observed.

greater resistance to the infection than those on diet 2. At the 63rd day the survivors were killed: twenty-three of fifty on diet 1 and nine of fifty on diet 2. The difference in survival rate is significant ($P = 0{\cdot}05$). Throughout the experiment the difference of the means in the groups showed a significantly higher survival rate for females on diet 1

than for any other group. At the end of the experiment the males on diet 1 had a significantly higher survival rate than those on diet 2. Details are given in Fig. 2.

When the survivors were killed, their lungs were dissected out, fixed in 5% mercuric chloride in 4% (w/v) formol saline (ten parts commercial formalin to ninety parts of

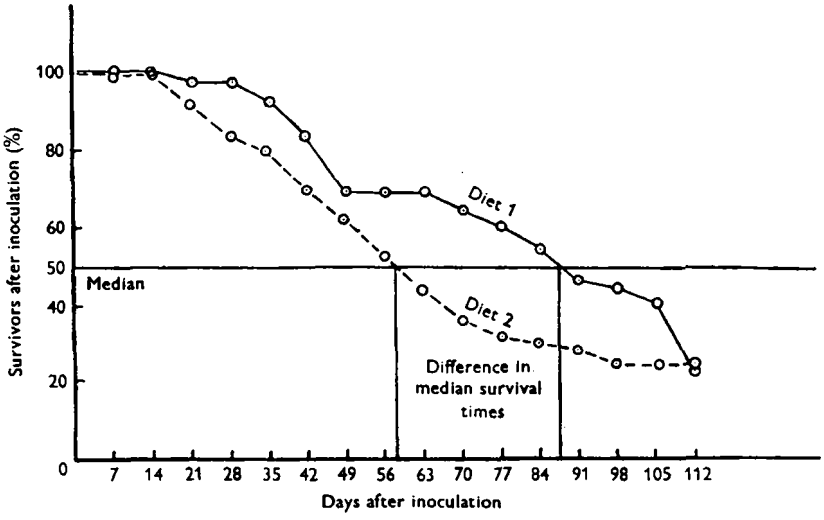

Fig. 1. Exp. 2. Difference in median survival time of mice on two experimental diets after experimental tuberculous infection.

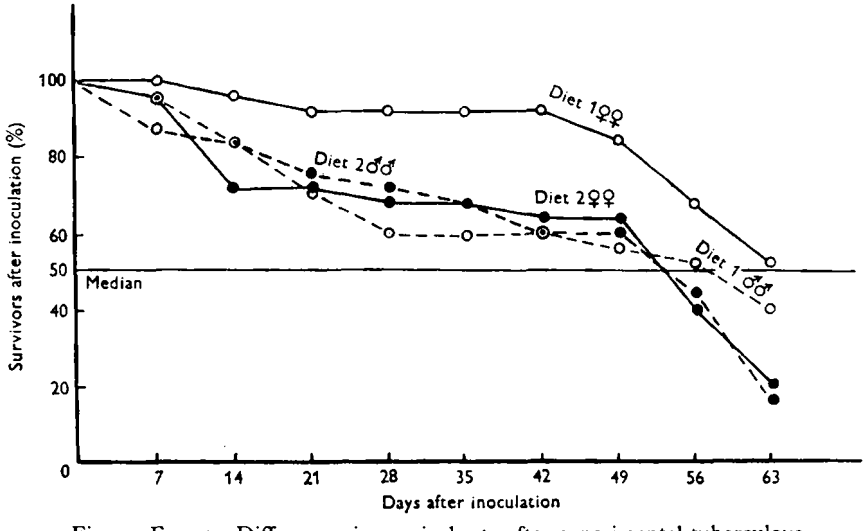

Fig. 2. Exp. 3. Differences in survival rate after experimental tuberculous infection of male and female mice on two experimental diets.

normal saline). This brought into prominence the tuberculous lesions in the lungs which were then examined, by the naked eye, by five independent observers. Values were assigned ranging from + + + + to − according to the degree of lung involvement with the results shown in Table 5; these indicate that the survivors on diet 1 had less extensive lesions than those on diet 2.

Exp. 4. To follow the development of lesions after the experimental infection, additional mice, all females 8–9 weeks old on diets 1 and 2, were inoculated at the same time as those in Exp. 3 and with the same infective suspension. Two animals from each dietary group were killed by a blow on the head at 4, 7, 14, and 21 days after infection. After the 25th day, if an animal died in one dietary group, another was killed to compare with it in the other dietary group. The first tubercles were observed by the naked eye in the lungs of mice killed on the 14th day on diet 2. It soon became evident that, for this procedure to yield satisfying results, it would be necessary to kill larger numbers, say five or ten from each group, and to choose the time when lesions were near their maximum development—about 14–25 days. In future experiments of this kind, which are planned in conjunction with our colleague Dr J. M. Naftalin, the pathology and histology of the lesions will be studied. Since our work was begun, Raleigh & Youmans (1948*a*, *b*) and Youmans & Raleigh (1948) have published a review of the literature on the use of mice as experimental animals for the study of tuberculosis; they emphasize the value of assessing the pathology and histology of the lung lesions.

DISCUSSION

This work shows that the mouse can be used as an experimental animal for testing the effect of diet on resistance to tuberculosis. It also shows that there was a significant increase in resistance, as judged by survival time and survival rate in mice fed our diet 1 compared with that in mice fed our diet 2—a modified diet B of Sherman. Both diets were satisfactory for reproduction and growth, but direct chemical analysis (Table 2) revealed that diet 2 was low in calcium, 0·35% compared with the value of 1·28% for diet 1. The calcium value of 0·35% for our modified diet B of Sherman was in good agreement with Campbell & Sherman's (1945) figure of 0·34% for their original diet. The protein in our diet 2 was only 14·7% compared with Campbell & Sherman's (1945) figure of 16% and the value of 19·2% in diet 1. Other possibly important differences between diets 1 and 2 are revealed by analysis (Table 2). Diet 2 has a higher value for fat, 9·5%, against the 4·9% in diet 1. Diet 1 has more fibre than diet 2, 4·8% compared with 1·3%; a higher value for ash, 6·0% against 2·9%; and a calcium:phosphorus ratio of 1·3:1 against 0·69:1. Diet 1 also differs from diet 2 in containing 1·2% of dried yeast and 0·4% of cod-liver oil. We did not think it necessary to embark at this stage upon the analyses that would be required if a serious attempt were to be made to measure differences between the vitamin content of diets 1 and 2.

At this stage it is not useful to speculate on what feature of diet 2 renders it less suitable than diet 1 for the development of resistance to experimental tuberculosis. The point is that a diet able to support good growth and reproduction, and already accepted as a standard for comparing other diets in respect of the resistance to infection they confer (Schneider & Webster, 1945; Schneider, 1946, 1948; Dubos & Pierce, 1948), does not ensure the development of maximum resistance to experimental tuberculosis in mice. The evidence in this paper is a good starting-point for further exploration of what dietary influences are involved and how they operate.

SUMMARY

1. The influence of diet on resistance to tuberculosis was studied in W-Swiss mice inoculated intravenously with a human strain of the tubercle bacillus.

2. Mice fed on a diet composed of a mixture of cereals, fish meal, meat-and-bone meal, dried skim milk, dried yeast, cod-liver oil, and sodium chloride, showed higher resistance to tuberculosis than those fed on a modified diet B of Sherman (33% whole dried milk, 66% whole ground wheat and 1% sodium chloride), although on both these diets the mice grew and reproduced satisfactorily.

We have much pleasure in thanking Mr W. Godden and the staff of the Biochemistry Department for analysing our diets; Mr M. H. Quenouille, Lecturer in Statistics at Aberdeen University, for statistical analyses; Mr G. Porter and his staff for care of the animals; and Mr W. G. MacLeod, Mr E. B. Reid, and Mr R. Cook for technical assistance.

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