

EFFECT OF DIETARY PROTEINS AND AMINO ACIDS ON THE SUSCEPTIBILITY OF MICE TO BACTERIAL INFECTIONS

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In an earlier study, it was found that mice maintained on complete diets in which casein was the sole source of amino acids, except for supplementation with cystine, exhibited a level of natural resistance to experimental bacterial infections which was dependent upon the concentrations of this protein in the diet. For example, resistance was much higher with a dietary content of 15 to 20 per cent casein than with 8 per cent. There is no doubt that the dietary amino acids played an essential role in this phenomenon since resistance to bacterial infection could be increased by supplementing the low casein diet with proper mixtures of synthetic amino acids (1).

The experiments to be described in the present paper constitute supplementary proof of the fact that the *quantitative* aspects of protein and amino acid nutrition are of paramount importance in the response of mice to bacterial disease. In addition, the new findings provide evidence that the *qualitative* aspects of protein nutrition are at least as important as the quantitative aspects in this regard. Specifically, it has been found that mice fed certain diets with an amino acid pattern different from that of casein exhibit a low level of natural resistance to bacterial infections.

Methods

Details concerning the origin and management of the bacterial cultures and mice used in the present study have been provided in a preceding publication from this laboratory (1).

Certain modifications of importance have been introduced in the techniques used for the preparation of the experimental diets. In the preceding study the ingredients of the diet were resuspended in a gelatin solution which was allowed to gel before distribution to the animals. As the presence of gelatin—an incomplete protein—inevitably complicated interpretation of the results with regard to the influence of amino acids on resistance to infection, this constituent was entirely omitted from the diets in the present study.

The basal diet was prepared from ingredients obtained from Nutritional Biochemical Corp. (Cleveland). It had the following composition:—

White dextrin	73.20	per cent
Jones-Foster salt mixture	5.04	“ “
Peanut oil	6.35	“ “
L-cystine	0.30	“ “
Inositol	0.11	“ “
Vitamin diet fortification mixture	2.50	“ “
Alphacel	12.50	“ “

Casein, gluten, soybean α -protein, and the various amino acids used were added to the basal mixture as indicated for each individual experiment. The specified amount of protein and of amino acid was added to 800 gm. of basal diet and the mixture made up to 1,000 gm. with white dextrin. Water was then added in an approximate proportion of 800 cc. per kg. of mixture. The thick slurry was spread on flat aluminum pans (in layers of approximately $\frac{1}{4}$ inch in thickness) and placed in a hot air dryer for 48 hours at 60°–70°C. The mixture was thus converted into a dry brittle mass which could be fragmented and conveniently distributed to the animals.

In one of the experiments the animals were fed a soybean-rice flour mixture, which had been generously supplied to us by Dr. Theodore F. Irmeter of Salada-Shirriff-Horsey, Inc., Little Falls, New York. The amino acid composition of this plant mixture was not determined analytically. Calculations made from available information indicated that it was approximately that of casein.

In many cases, commercial pellets were used in comparison with the experimental diets. During the early parts of the study, these pellets were obtained from Arcady Farms Milling Company, Chicago. Their composition has been described in a preceding paper (1). In more recent experiments the pellets were obtained from Dietrich & Gambrill, Inc., Frederick, Maryland. This diet is processed so as to assure the absence of all organisms known to be pathogenic to rats and mice. According to the manufacturer, it contains an adequate amount of the various vitamins and minerals, and it has the following composition:

Crude protein, minimum	24.0 per cent
Crude fat, minimum	5.0 " "
Crude fiber, maximum	4.5 " "
Ash, maximum	10.0 " "
Carbohydrate	50.9 " "
Calcium	0.9 " "
Phosphorus	0.9 " "

In all cases, food and water were provided *ad lib.* and the animals were maintained until death on the experimental diets which were being fed at the time of infection.

EXPERIMENTAL

It was shown in a preceding paper (1) that mice maintained on a synthetic diet containing 8 per cent casein as sole source of protein and amino acids (except for supplementation with cystine) gained weight rapidly, but nevertheless became more susceptible to experimental bacterial infections than did comparable animals receiving 20 per cent casein in their diet. The following experiment confirms and extends these earlier findings with diets differing in details of their preparation from those used in the earlier study (see under Methods).

Male mice 4 weeks old were fed diets containing either 20, 8, or 5 per cent casein. Twenty-six days later they were infected by the intravenous route with 0.05 ml. of an 18 hour old culture of *Staphylococcus aureus* (strain Giorgio). The cumulative numbers of deaths at indicated times after infection are shown in Table I.

As seen in Table I, the mice fed the 20 per cent casein diet survived longer than those receiving either 8 or 5 per cent casein. There was no detectable

difference in survival time between the two latter groups, even though they differed markedly in weight gain. This fact is worth emphasizing as it has been repeatedly observed that the effect of a diet on resistance to infection cannot be predicted from its ability to permit rapid growth of uninfected animals (1-3).

It is well known that most plant proteins differ markedly from animal proteins with regard to their percentage composition in amino acids. For example, wheat gluten is low in lysine as well as in sulfur amino acids, a fact which accounts in large part for its low nutritional efficiency. It will be shown in the following experiments that mice fed diets containing gluten as sole source of protein became highly susceptible to bacterial infections.

TABLE I
Effect of Dietary Casein on Susceptibility to Infection

Male mice infected intravenously with 0.05 ml. *Staph. aureus* (strain Giorgio).

Diet (26 days before infection)	Average weight change*	No. of mice	Cumulative deaths at indicated days (d.) postinfection				
			2 d.	3 d.	5 d.	7 d.	9 d.
	gm.						
20 per cent casein	+5.1	10	0	1	4	5	8
8 " " "	+3.3	9	3	5	7	7	7
5 " " "	+0.9	9	4	7	7	7	9

* The figures indicate the average weight change per mouse between the time at which the animals were placed on the experimental diet and the time of infection. The animals were kept in boxes of 5 throughout the experiment.

Male mice fed a diet containing 20 per cent gluten as sole source of protein were compared with mice fed diets containing either 20 or 5 per cent casein. In all cases the diets were supplemented with cystine (see under Methods). Fifteen days after having been put on the experimental regimens, the animals were infected by the intravenous route with 0.2 ml. of an 8 day old culture of *Mycobacterium tuberculosis* var. *bovis* (strain Vallée) in tween-albumin medium. From then on they were maintained on the experimental diets until death (Table II).

As was to be expected from earlier findings, the animals on the low casein diet proved extremely susceptible to experimental tuberculosis. This was also the case for the animals fed gluten, even though their diet contained 20 per cent of this protein. Thus gluten is an inadequate protein for mice both in its effect on weight gain and on susceptibility to tuberculous infection (Table II). The inadequacy of gluten from these two points of view is further illustrated by the results of the following experiments with *Klebsiella pneumoniae* (type C).

In this experiment female mice were fed for 2 weeks before infection diets containing either 20 per cent gluten, 20 per cent casein, or 5 per cent casein. Another group of animals received

corn grain as sole source of food. The infective dose was administered by the intravenous route and consisted of 0.02 ml. of an 18 hour old culture of *Klebsiella pneumoniae* (type C) in meat infusion broth (Table III).

It is seen in Table III that mice fed a diet containing 20 per cent casein were more resistant to infection with *Klebsiella pneumoniae* than mice re-

TABLE II

Effect of Dietary Protein on Susceptibility to Infection

Male mice infected intravenously with 0.2 ml. *Myc. tuberculosis* var. *bovis* (strain Vallée),

Diet (15 days before infection)	Average weight change*	No. of mice	Cumulative deaths at indicated days (d.) postinfection		
			17 d.	21 d.	25 d.
	<i>gm.</i>				
20 per cent casein	+3.6	15	3	9	15
5 " " "	-4.6	15	11	15	—
20 " " gluten	-2.8	14	7	14	—

* The figures indicate the average weight change per mouse during the 2 weeks on experimental diets before the animals were infected. The animals were kept in groups of 5 before infection, then placed in individual cages until death.

TABLE III

Effect of Diet on Susceptibility to Kl. pneumoniae Infection

Female mice infected intravenously with 0.02 ml. *Kl. pneumoniae* (type C).

Diet (14 days before infection)	Average weight change*	No. of mice	Cumulative deaths at indicated days (d.) postinfection				
			1 d.	2 d.	3 d.	4 d.	5 d.
	<i>gm.</i>						
20 per cent casein	+1.6	10	0	0	3	8	9
5 " " "	-1.2	10	1	3	6	8	9
20 " " gluten	+2.3	10	2	2	5	7	10
Corn	-2.4	12	8	8	8	11	11

* As in Table I.

ceiving either 20 per cent gluten or 5 per cent casein. Mice fed corn grain as sole source of food were the most susceptible. The results of the following experiment confirm that the gluten diet also increases susceptibility to bovine tubercle bacilli, even when administration of this diet was delayed until 1 week after infection.

Male mice were infected by the intravenous route with 0.05 ml. of an 8 day old culture of *Myc. tuberculosis* var. *bovis* (strain Vallée) in tween-albumin medium and housed two per cage. They were fed pellets for 1 week after infection. Then they were divided into subgroups

and placed on the experimental diets. They were maintained on these diets from then on until death or the end of the experiment (Table IV).

As appears from the results in Table IV, mice fed either 5 per cent casein or 20 per cent gluten, died more rapidly of tuberculous infection than did those on 20 per cent casein or on pellets. The differences observed in this experiment are the more striking in view of the fact that all the animals were fed pellets for the 1st week after infection and were placed on the experimental diets only after that initial period. In agreement with results reported and discussed in a preceding paper, it is seen that animals fed pellets died more rapidly than animals fed 20 per cent casein (1).

Further information concerning the time required for the establishment of

TABLE IV
Effect of Dietary Protein on Susceptibility of Mice to Tuberculosis

Male mice infected intravenously with 0.05 ml. *Myc. tuberculosis* var. *bovis* (strain Vallée).

Diet*	No. of mice	Cumulative deaths at indicated days (d.) postinfection					
		17 d.	18 d.	19 d.	20 d.	21 d.	24 d.
20 per cent casein	20	0	0	2	3	8	11
5 " " "	21	5	7	8	14	15	21
20 " " gluten	21	2	2	6	14	15	20
Pellets	20	0	1	1	7	11	19

* Mice fed pellets for 1 week after infection, then maintained on experimental diets until death.

the dietary effect on susceptibility to infection is provided by the following experiment.

Male mice were placed on three experimental diets 5 days after weaning. Each group was then divided into three subgroups which were infected at different times, namely 1, 2, or 3 weeks after the beginning of the experiment. In all cases the infective inoculum consisted of 0.05 ml. of an 18 hour old culture of *Staphylococcus aureus* (strain Giorgio) (Table V).

In this particular experiment (Table V), the increase in susceptibility to infection could be detected 1 week after the mice had been put on diets containing either 5 per cent casein or 20 per cent gluten and this effect was still apparent after the animals had been maintained for 3 weeks on these diets. However, it must be pointed out that these time intervals were not entirely reproducible from one experiment to another. It was found in a number of experiments that a period of 1 week on the diet was not sufficient to establish susceptibility to infection. On the other hand, it was also observed on other occasions that animals on the gluten diet recovered their normal resistance after being maintained for several weeks on this regimen. It seems possible

that the recovery of resistance was due to the development of an intestinal flora that permitted an escape from the nutritional deficiency, but this hypothesis has not been investigated.

In most of the experiments so far described the animals had been kept in groups of 5 while on the different diets. The weights reported in the tables correspond to averages per mouse in these groups of 5. More recently animals have been housed singly in individual cages with wire grids—all individual cages being placed in a common cabinet with a common aeration system. This has permitted a more careful study of weight curves of individual animals

TABLE V
Effect of Dietary Protein and Time on Diet on Susceptibility to Infection
Male mice infected intravenously with 0.05 ml. *Staph. aureus* (strain Giorgio).

Diet	Days (d.) on diet	Average weight change*	No. of mice	Cumulative deaths at indicated days (d.) postinfection		
				3 d.	5 d.	7 d.
	<i>d.</i>	<i>gm.</i>				
20 per cent casein	7	+3.1	7	2	2	7
5 " " "	7	-2.1	8	6	7	8
20 " " gluten	7	+1.7	8	3	5	8
20 per cent casein	15	+6.1	8	1	3	6
5 " " "	15	-3.4	8	6	7	8
20 " " gluten	15	+3.4	7	4	5	7
20 per cent casein	21	+7.5	7	3	3	5
5 " " "	21	-3.2	7	7	7	7
20 " " gluten	21	+1.8	8	6	6	8

* As in Table I.

during the course of the experiment. Only one experiment will be described in detail to illustrate results obtained by this technique.

Male mice were infected by the intravenous route with 0.05 ml. of an 8 day old culture of *Mycobacterium tuberculosis* var. *bovis* (strain Vallée). They were immediately placed in individual cages and fed either one of the four following diets: (a) pellets; (b) synthetic diet with 15 per cent casein; (c) synthetic diet with 15 per cent gluten; (d) the same diet as (c) but supplemented with 1 per cent L-lysine. These animals were not weighed. The rate of death is recorded in Table VI C.

In a parallel group, the mice were fed the same diets as above (in individual cages), but not infected and their individual weights were recorded at intervals of time. The weight gains and losses are reported in Table VI A.

After being maintained on the experimental diets for 24 days, the mice of this second group were infected by the intravenous route with 0.02 ml. of an 18 hour old culture of *Klebsiella pneumoniae* type C (Table VI B).

As was to be expected, mice fed the gluten diet either failed to gain weight, or actually lost weight after 1 week on this regimen. In contrast, the animals fed the gluten diet supplemented with lysine gained weight at about the same rate as those fed pellets or the casein diet (Table VI A). This greater weight gain, however, was not reflected in any detectable effect on response to infection.

TABLE VI A
Weight Changes of Individual Mice on Four Different Diets

Diet	Days on diet	Cumulative weight change of individual mice (Nos. 1-8)								Average weight change
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
15 per cent casein	3	-2.3	+0.4	-0.4	-1.1	+2.3	-0.9	+0.8		-0.17
	7	+0.3	+1.2	+1.5	+1.0	+2.4	+1.8	+2.0		+1.5
	10	+1.5	+1.0	+2.1	+0.9	+3.0	+3.2	+2.8		+2.1
	17	+2.9	+2.5	+4.7	+2.9	+4.2	+5.0	+4.5		+3.8
	24	+3.8	+3.7	+6.5	+3.3	+5.3	+6.8	+6.7		+5.2
15 per cent gluten	3	-2.7	-0.6	+1.0	+1.7	-0.2	-1.7	-0.7	+1.0	-0.3
	7	+1.3	-1.5	+1.9	-0.3	+1.1	+1.0	-0.6	+1.2	+0.5
	10	-1.6	-1.9	+0.9	-3.0	-1.0	-2.7	-1.3	-0.2	-1.4
	17	-0.5	-2.2	+2.1	-2.2	-0.3	-3.0	+0.1	+0.3	-0.7
	24	+0.5	-2.6	+4.0	-3.4	+1.0	-2.5	+2.0	+1.4	+0.1
15 per cent gluten + 1 per cent L-lysine	3	+0.5	+1.9	+2.6	0	+1.1	-1.0	+0.2	-0.2	+0.6
	7	-0.1	+3.8	+4.6	+0.8	+0.8	-0.5	+1.5	+0.5	+1.4
	10	+0.3	+4.5	+8.5	+0.9	+1.7	-0.9	+2.7	+1.2	+2.4
	17	+2.2	+7.2	+8.5	+1.7	+2.6	+1.8	+7.2	+2.0	+3.8
	24	+4.3	+8.6	+14.0	+2.6	+5.8	+3.6	+6.0	+3.4	+6.0
Pellets	3	+1.1	+1.0	+2.2	-0.2	+2.8	+2.0	+2.4		+1.6
	7	+3.9	+2.2	+3.9	+3.3	+5.1	+3.0	+3.2		+3.5
	10	+4.1	+3.0	+5.1	+0.1	+5.9	+4.0	+3.7		+3.7
	17	+7.0	+6.3	+8.4	-0.4	+4.2	+6.0	+5.2		+5.2
	24	+7.3	+8.5	+10.4	+0.8	+5.4	+4.2	+7.4		+6.3

In the case of both *Myc. tuberculosis* and *Kl. pneumoniae* the animals fed either the gluten diet, or the gluten-lysine diet proved far more susceptible to infection than those fed either pellets or the casein diet (Tables VI B and VI C).

It will be noticed that the animals infected with tuberculosis revealed marked differences in susceptibility even though they were put on the experimental diets only at the time of infection. This finding, which is in agreement with the results presented in Table IV, is due almost certainly to the fact that the tuberculous process evolves slowly, thus permitting the nutritional effect to become manifest during the course of the infection.

In unpublished experiments from this laboratory, it has been repeatedly observed that mice fed various cereal grains, or soybeans, as sole source of food for periods longer than 1 week become increasingly susceptible to bacterial infections. This is illustrated by the results with corn presented in Table III.

TABLE VI B
Effect of Four Diets on Susceptibility of Mice to Klebsiella Pneumoniae
Male mice infected intravenously with 0.02 ml. *Klebs. pneumoniae* (type C).

Diet*	Average weight gain†	No. of mice	Cumulative deaths at indicated times (hrs.) postinfection				
			18 hrs.	26 hrs.	48 hrs.	72 hrs.	96 hrs.
	gm.						
15 per cent casein	+5.2	7	1	1	2	3	3
15 " " gluten	+0.1	8	5	5	6	7	8
15 " " " + 1 per cent L-lysine Pellets	+6.0	8	3	5	8	—	—
	+6.3	7	1	2	4	5	6

* Animals kept in individual cages throughout the experiment. Infected 24 days after being placed on experimental regimens.

† The individual weight changes before infection are presented in Table VI A.

TABLE VI C
Effect of Four Diets on Susceptibility of Mice to Tuberculosis
Male mice infected intravenously with 0.05 ml. of *Myc. tuberculosis* var. *bovis* (strain Vallée).

Diet*	No. of mice	Cumulative deaths at indicated days (d.) postinfection				
		16 d.	18 d.	19 d.	22 d.	24 d.
15 per cent casein	20	0	2	4	11	15
15 " " gluten	20	2	4	8	17	19
15 " " " + 1 per cent L-lysine Pellets	20	1	2	6	18	18
	20	1	1	1	14	18

* Animals infected with tubercle bacilli and immediately placed on experimental diets. (For weight changes of uninfected animals see Table VI A).

(See also following paper.) Although no systematic study has yet been made of the effect of isolated plant proteins on infectious processes, one preliminary experiment carried out with the α -protein of soya bean will be reported at this time.

Female mice fed a diet containing 20 per cent of the α -protein of soya bean were compared with mice receiving either 20 per cent casein or 5 per cent casein. All animals were weighed at weekly intervals and just before infection. Half of them were infected 10 days after being

placed on the experimental diets, and the other half after 20 days. In all cases the inoculum consisted of 0.05 ml. of an 18 hour old culture of *Staph. aureus* (strain Giorgio) injected by the intravenous route (Table VII).

The results presented in Table VII suggest that animals fed the α -protein of soya bean as sole source of protein retained a normal level of resistance to infection longer than did animals fed the same concentration of gluten (Tables II, to VI). Only in the test carried out after the animals had been on the soya bean diet for 20 days did the decrease in resistance become manifest. The trend in the weight curve of the uninfected animals also suggests that the soya bean deficiency did not become established until then.

It has long been claimed on the basis of epidemiological data that human populations living on vegetable diets are usually more susceptible to infection

TABLE VII
Effect of Dietary Protein on Susceptibility to Infection
Female mice infected intravenously with 0.05 ml. *Staph. aureus* (strain Giorgio).

Diet	Days (d.) on diet	Average weight change	No. of mice	Cumulative deaths at indicated days (d.) postinfection			
				2 d.	4 d.	6 d.	8 d.
	<i>d.</i>	<i>gm.</i>					
20 per cent casein	10	+3.0	8	1	6	6	7
5 " " "	10	-2.0	8	5	8	—	—
20 " " α -protein of soybean	10	+3.0	8	1	5	7	7
20 per cent casein	20	+5.0	8	1	2	3	3
5 " " "	20	-1.5	8	3	4	7	8
20 " " α -protein of soybean	20	+3.0	8	0	4	7	7

than populations with diets rich in animal proteins. The decrease in resistance observed in mice fed the gluten and soya bean protein diets would at first sight seem to justify this generalization. However, other experiments have revealed that—at least as far as mice are concerned—it is possible to devise diets consisting only of plant food and yet compatible with an adequate level of resistance to mycobacterial infection. This is illustrated in the following experiment.

Female mice were fed a diet consisting of a mixture of soybean and rice flour with no other source of protein. The two plant products were mixed in suitable proportions to provide 15 per cent total protein with an amino acid pattern similar to that of casein. This mixture was prepared and kindly supplied to us by Dr. Theodore F. Irmiter, Salada-Shirriff-Horsey, Inc., "Junket" Brand Foods, Division of Chr. Hansen's Laboratory, Inc., Little Falls, N. Y. Other animals of the same age received diets containing either 15 per cent casein, 5 per cent casein, or 15 per cent gluten. All animals were kept in individual cages and weighed individually at regular intervals of time. After having been kept on the experimental diets for 10 days, they were all infected by the intravenous route with 0.05 ml. of a 3 day old culture of *Myc. fortuitum* (strain Penso) in tween-albumin medium (Table VIII).

As seen in Table VIII, the mice fed the diet containing 15 per cent protein made up of a mixture of rice flour and soy bean flour, gained weight more rapidly and proved more resistant to infection with *Myc. fortuitum* than did the animals fed 15 per cent gluten. As the rice-soybean mixture contained approximately the same relative proportions of amino acids as casein, the findings presented in Table VIII suggest that the infection-enhancing effect of unmixed plant protein diets is an expression of amino acid unbalance. Additional support for this view can be found in an earlier publication from this laboratory (1), in which it was shown that low protein diets supplemented with the proper mixture of synthetic amino acids were fully as effective as

TABLE VIII

*Effect of Dietary Protein on Susceptibility of Mice to Infection*Female mice infected intravenously with 0.05 ml. *Myc. fortuitum* (strain Penso).

Diet (10 days before infection)	Average weight change*	No. of mice	Cumulative deaths at indicated days (d.) postinfection			
			7 d.	14 d.	21 d.	28 d.
	<i>gm.</i>					
15 per cent casein	+3.0	9	0	0	0	8
5 " " "	+0.1	10	3	4	9	10
15 " " gluten	+0.8	10	1	4	8	9
15 " " mixed plant‡	+3.8	10	0	3	4	5

* The figures are averages of weight changes for mice housed in individual cages and maintained on experimental diets for 10 days before infection.

‡ Mixture of rice and soya bean flour having an amino acid pattern similar to that of 15 per cent casein.

high casein diets in promoting resistance to infection. This fact has been confirmed by the results of other experiments in which exclusively the L forms of amino acids were used to supplement the diet. The studies with amino acid mixtures will be described in detail in a subsequent publication and only a few general remarks will be made at the present time.

In the experiments previously reported (1), the amino acid mixture used to supplement the low protein diet had a composition known to be optimum for the regeneration of blood proteins (4). In more recent experiments, the amino acid mixture had been designed to approximate the composition of casein. Both mixtures proved effective in increasing resistance to infection. In contrast, we have consistently found that the addition of unbalanced amino acid mixtures to the diet increases susceptibility. The findings presented in Tables VI A, VI B and VI C reveal furthermore that whereas supplementation of gluten with lysine rendered the diet much more satisfactory from the point of

view of weight gain, there was no evidence that it increased the resistance of animals to bacterial infection.

In all the experiments so far reported, the infective dose of the various pathogens had been administered by the intravenous route. The following experiment illustrates that the nutritional effect on infection can be brought out just as well by intraperitoneal injection.

Mice were fed either corn grain, or complete diets with 20 per cent casein or 20 per cent gluten as sole source of protein. Three weeks later they were infected with an overnight culture of *Kl. pneumoniae* (type C). One group of animals received 0.02 ml. administered by the intravenous route, a second group received 0.0002 ml. by the intraperitoneal route (Table IX).

TABLE IX
Effect of Route of Infection on Resistance to Kl. pneumoniae of Mice Fed Different Diets

Diet	Average weight change*	Route of infection	No. of mice	Cumulative deaths at indicated days (d.) postinfection				
				1 d.	2 d.	3 d.	4 d.	5 d.
	<i>gm.</i>							
20 per cent casein	+3.1	Intravenous (0.02 ml.)	10	0	2	3	3	8
20 " " gluten	-0.3	" " "	"	1	4	6	6	10
Corn	-5.6	" " "	"	8	9	10	—	—
20 per cent casein	+2.0	Intraperitoneal (0.0002 ml.)	"	2	7	9	10	—
20 " " gluten	-0.2	" " "	"	5	8	10	—	—
Corn	-4.7	" " "	"	8	10	—	—	—

* Male mice maintained on experimental diets for 3 weeks before infection. Weight changes represent averages for 10 mice during these 3 weeks.

The test with *Kl. pneumoniae* shows that the effect of nutrition on resistance could be detected by intraperitoneal infection as well as by intravenous infection (Table IX). The following experiment demonstrates that differences in resistance could be brought out also by aerosol administration of tubercle bacilli.

One group of male mice was infected by the intravenous route with 0.05 ml. of an 8 day old culture of *Mycobacterium tuberculosis* var. *bovis* (strain Vallée) in tween-albumin medium. A similar group was infected by the aerosol route. For this purpose, all animals of the second group were placed in an inoculation chamber so designed as to permit uniform distribution of a mist delivered by air pressure from a nebulizer (Venturi unit). The animals were kept in the chamber for 1 hour during which they were exposed to the spray from 8 ml. of the same culture as above, but filtered through filter paper before nebulization.

Immediately after infection, both groups of mice were distributed in individual cages, placed on the experimental diets as shown in Table B, and kept on these diets until death.

Similar groups of animals on the same diets were left uninfected in individual cages and were weighed at regular intervals of time. The weight records of these uninfected mice are presented in Table X A, the times of death of infected animals in Table X B.

As found in earlier experiments, animals fed the gluten diet supplemented with lysine gained weight much faster than those fed the diet in which gluten

TABLE X A
Average Weight Changes of Uninfected Mice on Three Different Diets

Diet	Cumulative average weight changes* (gm./mouse) after indicated days on the diet				
	1 day	7 days	13 days	20 days	25 days
15 per cent casein	-1.0	+1.2	+3.1	+5.1	+6.0
15 " " gluten	-1.2	-0.4	-0.4	+1.4	+1.5
15 " " " + 1 per cent L-lysine	-1.8	+0.6	+1.9	+3.6	+5.0

* These figures are averages for 9 mice housed in individual cages.

TABLE X B
Effect of Route of Infection on Resistance to Tuberculosis of Mice Fed Three Different Diets

Male mice infected intravenously with 0.05 ml. of *Myc. tuberculosis* var. *bovis* (strain Vallée) or exposed to a spray of filtered culture for 1 hour.

Diet*	Route of infection	No. of mice	Cumulative deaths at indicated days (d.) postinfection					
			8 d.	15 d.	22 d.	29 d.	31 d.	40 d.
15 per cent casein	Intravenous	24	0	0	2	8	15	24
15 " " gluten	"	24	0	1	6	16	22	24
15 " " " + 1 per cent L-lysine	"	24	1	2	6	13	21	24
15 per cent casein	Air-borne	23	0	0	0	0	1	—
15 " " gluten	" "	23	1	2	2	2	4	—
15 " " " + 1 per cent L-lysine	" "	23	1	3	3	3	4	—

* Animals were placed on diets and housed in individual cages immediately after infection.

was the only source of amino acids (except for cystine). But lysine supplementation did not increase resistance to infection in a manner detectable by the techniques used in these experiments. In contrast, mice fed the 15 per cent casein diet proved much more resistant to tuberculosis than did those fed the gluten or gluten-lysine diet, irrespective of whether the infective dose of tubercle bacilli was administered by aerosol or by the intravenous route.

It will be noticed in Table X B that only a small number of animals died within the period of observation when the infective dose was administered by the air-borne route. This has been the general experience with the strain of mice used in the present experiments. However, air-borne infection has proven to be more uniformly and rapidly fatal in another strain of albino mice (RFVL strain) to be described in a later publication. Table XI presents the results of one experiment which illustrates that mice of the RFVL strain fed a diet containing 20 per cent casein proved more resistant to air-borne infection with tubercle bacilli than did mice receiving only 5 per cent casein. Many other

TABLE XI

Effect of Casein Content of the Diet on Resistance of RFVL Mice to Air-Borne Infection with Tubercle Bacilli

Mice of RFVL strain (5 weeks old) exposed for 1 hour to a spray of filtered culture of *Mycobacterium tuberculosis* var. *bovis* (strain Vallée).

Diet*	No. of mice	Cumulative deaths at indicated days (d.) postinfection						
		30 d.	35 d.	45 d.	55 d.	65 d.	75 d.	85 d.
20 per cent casein	14	0	2	3	3	8	10	11
5 " " "	14	3	8	9	11	11	11	13

* Animals were placed on experimental diets and housed in individual cages immediately after infection.

experiments, to be reported later, have revealed that the dietary effects described in this and the following paper could be readily confirmed with the RFVL strain of mice.

SUMMARY

Young mice were maintained for periods of 1 to 6 weeks on experimental diets containing all known growth factors, but differing in their protein and amino acid contents. All diets were supplemented with L-cystine.

The effect of the nutritional regimen on infection was tested by inoculating the animals with either one of four pathogens (*Mycobacterium tuberculosis* var. *bovis*, *Mycobacterium fortuitum*, *Staphylococcus aureus*, *Klebsiella pneumoniae* type C), and by observing the survival time. The infective dose was administered by either one of three routes: intravenous, intraperitoneal, or air-borne (aerosol).

In some experiments, the animals were maintained in groups of five throughout the tests. In other experiments they were housed in individual cages. This difference in housing did not affect the results in a detectable manner.

Mice fed diets containing 5 or 8 per cent casein as sole source of amino acid

(except for cystine supplementation) proved more susceptible to the experimental diseases than did mice fed diets containing 15 or 20 per cent of the same protein.

Susceptibility to infection developed when wheat gluten, or soybean α -protein, was substituted for casein—even in high concentrations (15 or 20 per cent).

In one experiment, mice were fed a diet containing as sole source of amino acids a mixture of soybean and rice flour, so designed as to provide a protein concentration of 15 per cent, with an amino acid pattern similar to that of casein. These animals gained weight at the same rate as those fed a diet containing 15 per cent casein and they exhibited a satisfactory level of resistance to bacterial infection.

The infection-enhancing effect of low casein concentration (5 and 8 per cent) could be corrected by supplementing the diet with the proper mixture of amino acids. This could be done using either synthetic or natural amino acids. In contrast, susceptibility to infection developed when low casein diets were supplemented with unbalanced mixtures of amino acids.

The infection-enhancing effect of gluten diets could not be corrected by supplementing the latter with lysine even though this supplementation markedly improved weight gains in uninfected animals.

It appears in conclusion that the relative proportion of the various amino acids in the diet is as important a factor as their total amount in conditioning resistance to bacterial infections. This effect of nutrition on resistance can be detected irrespective of the route of infection: intravenous, intraperitoneal, or air-borne. Moreover, the effect has been observed with two strains of mice differing markedly in their natural resistance to bacterial infection.

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