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

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Protein deficiency is the most common and most important form of malnutrition in the world today. The physiological disturbances which are the direct consequences of quantitative and qualitative inadequacies in protein nutrition are well recognized clinically and can be reproduced in the laboratory. There is also much epidemiological and clinical evidence that, in addition to its direct effects, protein malnutrition can indirectly become a contributory factor of disease by increasing susceptibility to infection. Surprisingly enough, however, it has not been easy to demonstrate in laboratory animals a causal relationship between protein intake and the course of experimental infections. The most striking effects on record have been obtained when the protein intake was so inadequate as to result in profound deficiency. In contrast, different groups of investigators who have used less drastic test conditions have reported such conflicting results that a review of their findings does not seem useful at this time. The lack of agreement with regard to the effect of protein intake on experimental tuberculosis is particularly surprising in view of epidemiological and clinical observations in human tuberculosis. Recent experimental studies of this subject, with conflicting results and relevant bibliographies are presented in references 1–12.

The present paper describes experimental models which illustrate that the susceptibility of mice to certain bacterial infections can be influenced by the nutritional intake of proteins and amino acids.

The experimental diets used in this study contained adequate amounts of all the known growth factors. Their protein content—although differing quantitatively and qualitatively from one diet to the other—was sufficient in all cases to permit rapid weight gain of non-infected animals. It can be assumed, therefore, that the high susceptibility to infection observed with some of the diets could not be the result of a grossly defective physiological state. Worthy of special emphasis is one aspect of the experimental procedure which may have contributed to the consistency of the results obtained. All the animals used in this study were young at the time when they were placed on the experimental diets, and furthermore, they were still in a phase of active growth when they were exposed to the infective agents. It is not unlikely that

the response to protein and to amino acid intake was sharpened by the greater needs of tissues at this physiological stage of life.

## *Methods*

*Bacterial Cultures:*

All infection tests were carried out by intravenous injection (caudal vein) of adequate amounts of one of the following cultures diluted to a final volume of 0.2 ml. with physiological saline:—

*Staphylococcus aureus.*—strain Giorgio (described in reference 13).

*Mycobacterium fortuitum.*—strain Penso (described in reference 14).

*Mycobacterium tuberculosis.*—strain Vallée. (This is a virulent culture of bovine type which has been maintained in our laboratory for many years by monthly passage in Tween-albumin medium.)

*Animals:*

All mice were litter mates of the so called Rockefeller Institute Swiss strain. They were raised at the Rockefeller Institute, weaned at 3 to 4 weeks of age, and taken to our laboratory immediately after weaning. Males and females were randomized separately, and the animals were distributed in groups of 5 or 8.

They were housed in metal cages on grids, without sawdust or other bedding. For 1 to 2 weeks after receipt, they were given as drinking fluid a solution of 3 per cent veterinary terramycin (obtained from Chas. Pfizer & Co., Inc. through the courtesy of Dr. Gladys Hobby). In all cases, administration of the drug was discontinued at least 3 days before infection.

In general, the animals were placed on the experimental diets within 1 week after weaning, and maintained on these diets until the end of the experiment. Food and water (or terramycin solution) were provided *ad lib.* Food consumption was not measured.

The animals were weighed in groups of 5 or 8 at the beginning of the experiment, and at regular intervals thereafter, until the time of infection.

*Diets:*

*Pellets.*—(Rockland mouse diet, distributed by Arcady Farms Milling Company, Chicago). According to the manufacturer, these pellets have the following guaranteed composition:—

| | |
|---|---|
| Crude protein (minimum) | 21 per cent |
| Crude fat (minimum) | 4 " " |
| Crude fiber (maximum) | 6 " " |

They provide adequate coverage of mineral elements and contain the following concentrations of vitamins (per 100 gm. of diet):—

*By Rat Bioassay Determination*

| | |
|---|---|
| Vitamin A | 610–680 int'l units |
| Thiamine | 228–465 μg. |
| Riboflavin | 538–652 " |
| Pyridoxine | 209–275 " |
| Pantothenic acid | 1989–2568 " |
| Choline equivalent | 480–492 mg. |
| Inositol (free) | 61 " |
| Vitamin D | 148 U.S.P. units |
| Alpha tocopherol | 7 mg. |
| PABA | 78 μg. |

*By Chemical Determination*

| | |
|---|---|
| Niacin | 14·4 mg. |
| Vitamin C | 360 " |
| Carotene | 42–45 " |
| Choline | 152 " |

According to the label, they are made up of the following ingredients: Soybean oil meal, cane molasses, fish meal, condensed buttermilk, corn gluten meal, irradiated brewers' type yeast, 4 oz. per ton wheat germ oil, O. P. linseed oil meal, corn oil meal, ground oats, wheat bran, wheat flour middlings, ground yellow corn, ground hulled barley, ground hulled oats, ground whole wheat, whole milk powder, alfalfa leaf meal, vitamin A oil, ½ per cent steamed bone meal, 1 per cent calcium carbonate from limestone, 2 per cent salt.

*Special Diets*

20 C —20 per cent casein
8 C — 8 " " "
8 C + AA— 8 " " " + 12 per cent amino acid mixture (AA)
Pellets —21 " " protein, 4 per cent fat (according to distributor)

*Diet 20 C.*—The composition of this diet is indicated in Table I. It corresponds essentially to the formula described in reference 15, except that diet 20 C contains 20 per cent casein. The casein used was "Vitamin free casein," a product distributed by Nutritional Biochemical Corporation (Cleveland). The vitamins and minerals were added in the forms of mixtures as indicated in Table I.

TABLE I

*Basal Diet (per 1000 Gm.)*

| | |
|---|---|
| Casein and amino acid mixture (AA)—as indicated below for each special diet | |
| Cystine HCl | 2 gm. |
| Inositol | 1 " |
| Potato starch | 477 " |
| Peanut oil | 50 " |
| Vitamin diet fortification mixture in dextrose | 20 " |
| Salt mixture (Wesson modification of Osborne and Mendel) | 40 " |
| Alphacel | 70 " |
| Cerelose—to 1000 | |

*Diet 8 C.*—The composition of this diet (Table I) was identical with that of 20 C, except that it contained only 8 per cent casein, the balance being made up with cerelose.

*Diet 8 C + AA.*—This is diet 8 C supplemented with 12 per cent amino acid mixture to render its content in organic nitrogen similar to that of 20 C. The composition of the amino acid mixture is presented in Table II. This mixture has been shown to allow rapid regeneration of blood constituents in rats subjected to repeated bleedings (16).

Diets 20 C, 8 C, and 8 C + AA were resuspended in equal weights of 7.5 per cent gelatin in tap water at approximately 40°C. The mixtures were spread in flat pans and allowed to harden in the refrigerator. They were then cut in sections approximately 10 gm. each for distribution to the animals. As already mentioned, food and water were provided *ad lib.* daily (except for week-ends and holidays when an excess was provided to last for 2 to 3 days).

## RESULTS AND COMMENTS

*1. Effect of Dietary Proteins on Weight Gains of Uninfected Animals.*—Non-infected mice fed pellets, diet 20 C, or diet 8 C shortly after weaning be-

gan to eat avidly within 1 to 2 days and gained weight regularly from then on.

Figs. 1 and 2 illustrate typical growth curves for males or females (averages for groups of 8 animals) maintained for 1 month or longer on pellets or on diets 20 C or 8 C. As will be noticed, the animals commonly lost weight at the beginning of the experiment, until they had become adjusted to their new regimen. Following this initial loss, mice fed diets 20 C and 8 C gained weight at approximately the same rate, somewhat more rapidly than did those fed pellets. The weight gains to be reported in the various experiments (see accompanying tables) are in agreement with the general trend indicated in Figs. 1 and 2.

TABLE II

*Amino Acid Mixture* (AA)

(Sebrell and McDaniel, 1952)

| | |
|---|---|
| L-arginine-HCl | 1.14 |
| L-histidine-HCl | 0.56 |
| L-lysine-HCl·$H_2O$ | 1.91 |
| DL-methionine | 0.63 |
| DL-phenylalanine | 1.40 |
| DL-isoleucine | 1.44 |
| DL-leucine | 3.24 |
| DL-threonine | 1.40 |
| DL-tryptophan | 0.81 |
| DL-valine | 2.88 |

*2. Effect of Dietary Protein on Susceptibility to Infection.—*

In a large number of independent experiments, carried out over several months (from December, 1956 to July, 1957), mice were fed diet 20 C, diet 8 C, or pellets for various lengths of time as indicated in the various tables. They were then infected by the intravenous route with *Staph. aureus* (Giorgio), *Myco. fortuitum* (Penso), or *Myco. tuberculosis* (Vallée). Following infection the animals were maintained on the same diet that they had received during the experimental period prior to infection. The cumulative numbers of deaths at various periods of time after infection are recorded in the respective tables.

As seen in Tables III to VIII and XI, mice fed diet 8 C died of infection much faster than did mice fed diet 20 C. The resistance of mice fed pellets was in general intermediate between that of the other two groups. The effect of diet on the outcome of infection was qualitatively the same whatever the species of pathogen used, the size of the infective dose, and the duration of the disease. For example, the differences between the diets could be readily recognized whether death was caused by an acute staphylococcal infection causing death within a few days (Table III), or by tuberculosis with a protracted course of several months (Table VI and VII).

Resistance was obviously independent of the weight of the animal at the time of infection or of the weight gained before infection.

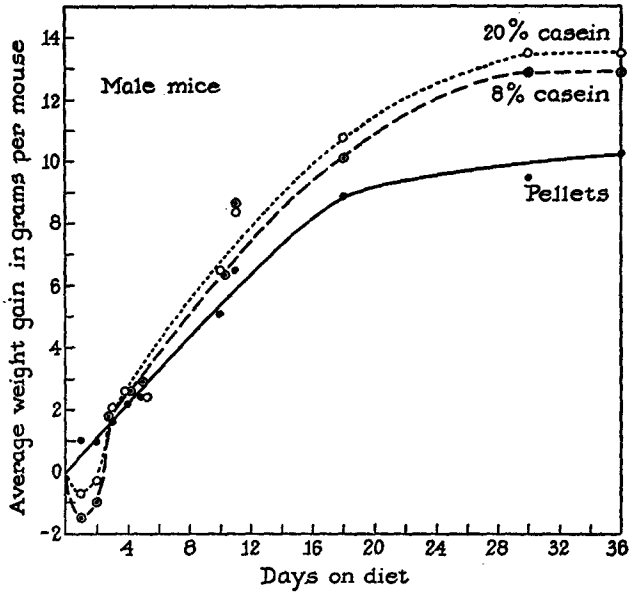


FIG. 1. Effect of dietary proteins on weight gains of uninfected male mice.

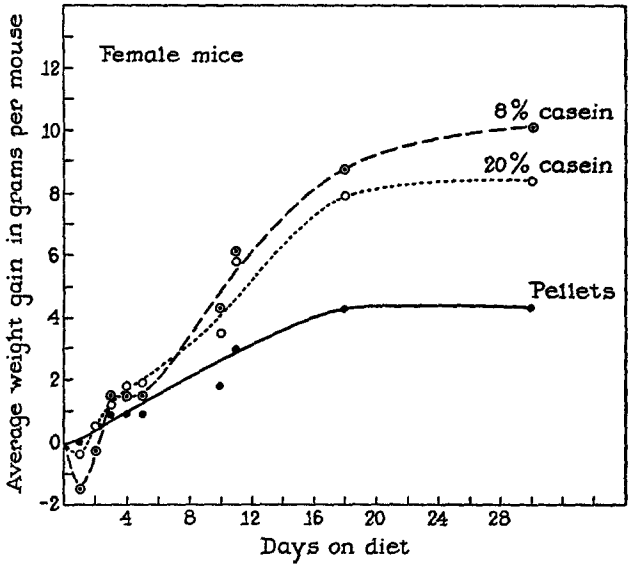


FIG. 2. Effect of dietary proteins on weight gains of uninfected female mice.

TABLE III

| Diet (14 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) post infection | | | |
|---|---|---|---|---|---|---|---|
| | Original | At infection time | (Gain) | 3 d. | 5 d. | 7 d. | 11 d. |
| | *gm.* | *gm.* | *gm.* | (out of 8 mice) | | | |
| 20 C | 25.1 | 30.1 | (5.0) | 0 | 2 | 2 | 4 |
| 8 C | 25.1 | 28.9 | (3.8) | 3 | 5 | 6 | 8 |
| Pellets | 22.3 | 31.2 | (8.9) | 0 | 3 | 3 | 7 |

Male mice; fed pellets for 2 weeks (after weaning) before being placed on experimental diets.

Infected i.v. with 0.05 ml. *Staph. aureus* (Giorgio).

TABLE IV

| Diet (14 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | | |
|---|---|---|---|---|---|---|---|
| | Original | At infection time | (Gain) | 2 d. | 3 d. | 4 d. | 5 d. |
| | *gm.* | *gm.* | *gm.* | (out of 8 mice) | | | |
| 20 C | 27.6 | 33.1 | (5.5) | 0 | 2 | 6 | 7 |
| 8 C | 27.6 | 32.6 | (5.0) | 3 | 5 | 6 | 7 |
| Pellets | 26.7 | 31.9 | (5.2) | 0 | 1 | 6 | 7 |

Male mice; infected i.v. with 0.1 ml. *Myco. fortuitum* (Penso).

TABLE V

| Diet (14 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | |
|---|---|---|---|---|---|---|
| | Original | At infection time | (Gain) | 18 d. | 21 d. | 27 d. |
| | *gm.* | *gm.* | *gm.* | (out of 8 mice) | | |
| 20 C | 23.5 | 29.0 | (5.5) | 1 | 2 | 6 |
| 8 C | 26.4 | 30.3 | (3.9) | 6 | 7 | 7 |
| Pellets | 23.6 | 28.9 | (5.3) | 1 | 1 | 6 |

Male mice; fed pellets for 2 weeks (after weaning) before being placed on experimental diets.

Infected i.v. with 0.05 ml. *Myco. fortuitum* (Penso).

Increase in casein content of the diet from 8 to 20 per cent clearly brought about a marked increase in resistance, thus demonstrating the importance of the quantitative aspect of protein intake. On the other hand, pellets were much less effective in inducing resistance to infection than was diet 20 C, even though their protein content is high (21 per cent or more). As reported

under Methods, the proteins of pellets are derived from many sources, especially of vegetable origin, and are probably inferior to casein in nutritional value. It is of interest to mention in passing recent publications by Hedgecock from which it appears that mice fed commercial pellets developed immunity more slowly than mice fed casein diets supplemented with mixtures of certain fatty acids (17).

In addition to the casein, diets 20 C and 8 C contained 3.75 per cent gelatin and small amounts of nitrogenous constituents present in potato starch. The

TABLE VI

| Diet (14 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | | |
|---|---|---|---|---|---|---|---|
| | Original | At infection time | (Gain) | 21 d. | 28 d. | 35 d. | 42 d. |
| | *gm.* | *gm.* | *gm.* | (out of 16 mice) | | | |
| 20 C | 26.1 | 32 | (5.9) | 1 | 4 | 7 | 9 |
| 8 C | 26.4 | 31.1 | (4.7) | 5 | 14 | 15 | 16 |
| Pellets | 26.1 | 29.8 | (3.7) | 4 | 15 | 15 | 16 |

Male mice; infected i.v. with 0.1 ml. *Myco. tuberculosis* (Vallée).

TABLE VII

| Diet (30 days before infection | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | | |
|---|---|---|---|---|---|---|---|
| | Original | At infection time | (Gain) | 30 d. | 45 d. | 60 d. | 75 d. |
| | *gm.* | *gm.* | *gm.* | (out of 16 mice) | | | |
| 20 C | 19.5 | 26.6 | (7.1) | 0 | 0 | 3 | 13 |
| 8 C | 19.7 | 28.3 | (8.6) | 2 | 4 | 9 | 14 |
| Pellets | 20.1 | 26.0 | (5.9) | 1 | 2 | 11 | 13 |

Female mice; infected i.v. with 0.02 ml. *Myco. tuberculosis* (Vallée).

fact that diet 8 C, on which animals were highly susceptible, contained as much gelatin and potato starch as did diet 20 C on which they were resistant, makes it unlikely that these materials played any role in resistance to infection.[1]

*3. Effect of Amino Acid Supplementation on Weight Gain of Uninfected Animals.*—Experiments were instituted to test the effect on susceptibility to infection of diets containing amino acids instead of protein. The amino

[1] In more recent experiments, gelatin and potato starch have been omitted from the diets. As will appear from results to be published in the near future, this technical change has not affected the pattern of differences in susceptibility to infection associated with the casein content of the diets.

TABLE VIII

| Diet (13 days before infection) | Weight per mouse at time of infection | Cumulative deaths at indicated days (d.) postinfection | | |
|---|---|---|---|---|
| | | 2 d. | 6 d. | 10 d. |
| | *gm.* | | | |
| | | | (out of 8 mice) | |
| 20 C | 26.3 | 2 | 5 | 5 |
| 8 C | 26.5 | 3 | 6 | 7 |
| 8 C + AA | 25.1 | 1 | 5 | 5 |
| Pellets | 26.3 | 3 | 6 | 6 |
| (16 days before infection) | | 4 d. | 6 d. | 10 d. |
| | | | (out of 8 mice) | |
| 20 C | 27.8 | 1 | 5 | 6 |
| 8 C | 27.6 | 3 | 7 | 8 |
| 8 C + AA | 25.9 | 2 | 4 | 5 |
| Pellets | 26.5 | 4 | 6 | 7 |

Female mice; infected i.v. with 0.05 ml. *Staph. aureus* (Giorgio).

TABLE IX

| Diet (15 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | | |
|---|---|---|---|---|---|---|---|
| | Original | At infection time | (Gain) | 5 d. | 8 d. | 15 d. | 22 d. |
| | *gm.* | *gm.* | *gm.* | | | | |
| | | | | | (out of 8 mice) | | |
| 20 C | 15.9 | 25.1 | (9.2) | 1 | 3 | 5 | 6 |
| 8 C | 15.9 | 28.2 | (12.3) | 3 | 5 | 6 | 7 |
| 8 C + AA | 16.4 | 26.2 | (9.8) | 1 | 2 | 5 | 6 |
| (37 days before infection) | | | | 5 d. | | 18 d. | |
| | | | | | (out of 8 mice) | | |
| 20 C | 15.3 | 34.0 | (18.7) | 1 | | 5 | |
| 8 C | 15.3 | 35.3 | (20.0) | 1 | | 4 | |
| 8 C + AA | 14.9 | 31.2 | (16.3) | 0 | | 2 | |

Male mice; fed bread and milk for 3 days (after weaning) before being placed on experimental diets.

Infected i.v. with 0.05 ml. *Myco. fortuitum* (Penso).

acid mixture (AA) selected for this purpose was one that had been shown by other investigators to be highly effective in supporting the regeneration of blood constituents when fed to rats subjected to repeated bleedings. (16). Its composition is presented in Table II.

It was found, however, that mice refused to eat diets containing this amino acid mixture without protein. On the other hand, the AA mixture could be

rendered more acceptable by adding to it 8 per cent casein. In view of this fact, the amino acid mixture AA was added to diet 8 C in a proportion of 12 per cent of the total weight in order to bring the nitrogen content to a level approximately similar to that of diet 20 C.

Even under these conditions, the AA mixture imparted to the diet 8 C certain characteristics which decreased its acceptability by mice. This was particularly noticeable during hot humid days when the great hygroscopicity of diet 8 C + AA changed its physical characteristics. As a rule, mice fed the 8 C + AA diet increased in weight somewhat less rapidly at first, and less

TABLE X

| Diet (8 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | | | |
|---|---|---|---|---|---|---|---|---|
| | Original | At infection time (Gain) | | 3 d. | 5 d. | 14 d. | 21 d. | 28 d. |
| | *gm.* | *gm.* | *gm.* | | | | | |
| | | | | (out of 8 mice) | | | | |
| 20 C | 14.3 | 18.1 | (3.8) | 0 | 3 | 3 | 3 | 4 |
| 8 C | 13.5 | 17.7 | (4.2) | 3 | 4 | 5 | 6 | 8 |
| 8 C + AA | 13.7 | 13.7 | (0) | 0 | 0 | 0 | 0 | 0 |
| (16 days before infection) | | | | 4 d. | | 7 d. | | 14 d. |
| | | | | (out of 16 mice) | | | | |
| 20 C | 14.1 | ? | ? | 2 | | 4 | | 6 |
| 8 C | 14.1 | 22.2 | (8.1) | 3 | | 10 | | 12 |
| 8 C + AA | 13.9 | 17.9 | (4.0) | 0 | | 1 | | 2 |

Female mice; fed 2 days (after weaning) on bread and milk before being placed on experimental diets.

Infected i.v. with 0.05 ml. *Myco. fortuitum* (Penso).

regularly, than did those fed 8 C (see Tables VIII, IX, and X). Indeed, marked loss of weight was often observed during the first few days following the change to the casein amino acid diet.

*4. Effect of Dietary Amino Acid on Resistance to Infection.*—The effect of supplementation with AA on susceptibility to infection was tested by techniques identical with those reported in the preceding section. It was found that mice receiving diet 8 C + AA for 8 days or longer before infection exhibited much greater resistance to *Staph. aureus* and to *Myco. fortuitum* than did mice fed diet 8 C for the same length of time (Tables VIII, IX, and X). Indeed, supplementation with the amino acid mixture AA rendered diet 8 C at least as effective as diet 20 C (which contains 20 per cent casein) in inducing resistance to infection. Experiments are underway to determine the kind of amino acids, and the amount required, to elicit the increase in resistance to infection brought about by supplementation with the AA mixture.

It is worth emphasizing again that the increase in resistance to infection caused by addition of 12 per cent AA to diet 8 C occurred despite the fact that mice fed the supplemented diet had gained weight less rapidly than had those receiving diet 8 C without amino acid supplementation.

*5. Effect of Time of Administration of Experimental Diets on Susceptibility to Infection.*—In all experiments reported in the present paper, the mice used were young, most of them having been put on the experimental diets less than 2 weeks after weaning. Under these conditions, the change in susceptibility to infection could be detected as shortly as 1 week after the beginning of the experiment (Table X). It has been repeatedly observed, however, that more prolonged feeding of any particular diet did not necessarily magnify its effect on susceptibility, as illustrated in Tables IX and XI. In these experiments, profound differences in response to *Staph. aureus* and to *Myco. fortuitum* were observed when mice on diets 8 C and 20 C were tested 14 days after being put on the diets. However, these differences became less pronounced or disappeared altogether when the infection tests were carried out later—namely when the animals received the challenge infection after having been fed the experimental diets for 37 days (Tables IX and XI).

TABLE XI

| Diet (14 days before infection) | Weight per animal | | | Cumulative deaths at indicated days (d.) postinfection | | | |
|---|---|---|---|---|---|---|---|
| | Original | At infection time (Gain) | | 4 d. | 5 d. | 8 d. | 14 d. |
| | *gm.* | *gm.* | *gm.* | | | | |
| | | | | (out of 16 mice) | | | |
| 20 C | 24.4 | 28.2 | (3.8) | 1 | 2 | 3 | 4 |
| 8 C | 23.7 | 26.5 | (2.8) | 3 | 4 | 7 | 10 |
| Pellets | 23.6 | 25.8 | (2.2) | 1 | 3 | 6 | 6 |
| (37 days before infection) | | | | 3 d. | 5 d. | 7 d. | 15 d. |
| | | | | (out of 8 mice) | | | |
| 20 C | 23 | 28.7 | (5.7) | 2 | 3 | 4 | 6 |
| 8 C | 23 | 29.5 | (6.5) | 2 | 3 | 4 | 6 |
| Pellets | 21.2 | 24.4 | (3.2) | 2 | 4 | 6 | 6 |

Female mice; infected i.v. with 0.05 ml. *Staph. aureus* (Giorgio).

It does not seem profitable to discuss here the nature of the adaptive mechanisms which progressively obliterated the effects of composition of the particular diets used on susceptibility to infection with *Staph. aureus* and *Myco. fortuitum*. Suffice it to point out that an analogous situation has been observed in earlier experiments carried out in this laboratory. In brief, it was then found that mice rendered susceptible to infection by quantitative dietary

restriction eventually recovered their resistance while being maintained on the same restricted regimen (18).

SUMMARY

Groups of young albino mice were fed continuously four different types of diets and were compared with regard to (1) rate of weight gain; (2) resistance to experimental bacterial infections.

The protein content of the four diets was as follows: (*a*) pellets: a minimum of 21 per cent "crude" protein (according to the manufacturer); (*b*) diet 20 C: 20 per cent casein; (*c*) diet 8 C: 8 per cent casein; (*d*) diet 8 C + AA: 8 per cent casein supplemented with 12 per cent of a mixture of essential amino acids. All diets provided an adequate supply of minerals and vitamins. They were administered *ad lib.*

Three strains of pathogens virulent for mice were used for the infection tests, namely: *Staphylococcus aureus*, *Mycobacterium fortuitum*, and *Mycobacterium tuberculosis bovis*. The bacteria were injected by the intravenous route.

The experimental regimens were begun at different times before infection, and were continued until death of the animal, or until termination of the experiment.

It was found that mice on the 8 C diet exhibited much greater susceptibility to infection than did mice on the 20 C diet; mice receiving pellets were intermediate between these two groups. The infection-enhancing effect of the 8 C diet could be entirely corrected by amino acid supplementation (diet 8 C + AA). Indeed, mice fed diet 8 C + AA proved the most resistant to infection.

The fact that animals fed pellets (which contain a minimum of 21 per cent protein) consistently died faster following infection than did animals fed diets 20 C or 8 C + AA suggests that qualitative characteristics of the protein in the regimen are as important as the quantity of protein fed in determining susceptibility to infection.

The differences in susceptibility exhibited by the mice on the four experimental diets were the same whatever the species of bacterial pathogen used for the infection test, the size of the infective dose, and the duration of the disease.

There was no apparent relation between the effects of the diets on the weight curves of the animals, and on resistance to infection. Mice on diet 8 C (which were most susceptible) gained weight as rapidly as those on 20 C and more rapidly than those fed 8 C + AA (which were most resistant).

All the tests reported in the present paper were carried out with young mice, which were placed on experimental diets within 1 to 2 weeks after weaning. Preliminary experiments suggest that the relation between dietary factors and susceptibility to infection was more difficult to bring out

in older animals. There was evidence also that this relation was most apparent during the first weeks that the animals were fed the experimental diets, and became less striking after several weeks.

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