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DIETARY PROTEIN AND RESISTANCE OF MICE TO TRICHINELLA SPIRALIS

CLARE K. KWAN, EDWARD D. WAGNER AND ALBERT SANCHEZ
Department of Microbiology, School of Medicine, Loma Linda University, Loma Linda, California and The International Nutrition Research Foundation, Riverside, California

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The primary factors related to resistance to infection are: the virulence of the parasite and the host's genetic makeup, age and environment. Nutrition, which is an important environmental factor, and its relation to infection has been recently reviewed.[1] The interaction of nutrition and infection has been studied primarily with viruses, bacteria, and protozoa. Only during recent years have studies been carried out with helminthes.

Nutrition is associated with natural or acquired resistance of the host helminthiasis.[2] The relationship of vitamins and infection has been studied with *Ascaridia* and chickens,[3,4] human *Ascaris* and pigs,[5] *Ancylostoma caninum* and dogs,[6] *Trichinella spiralis* and rats [7-9] The importance of minerals has been shown with *Ancylostoma caninum* and dogs.[6] Protein deficiencies were associated with a decreased resistance of mice to *Trichinella*[10] or *Hymenolepsis*[11] and of rats to *Nippostrongylus muris*.[12]

The present study was undertaken to determine the effect of the level of dietary protein and the age of mice on the resistance to *Trichinella spiralis*. The synergism between malnutrition and infectious agents is especially important during weaning and the succeeding early years of life, particularly in infants in areas of the world where these two factors are prevalent. For this reason, each group of mice was given a different diet and infected at intervals up to six weeks post weaning in order to simulate an infant's early post weaning period. A very casual comparison of the average life span of the mouse with the human shows that every mouse day is equal to one human month. In terms of human life, this experiment covers a period of approximately six years post weaning. This report illustrates the fact that more study should be given to factors contributing to helminthiasis and other infectious diseases in order that the problem may be approached with the greatest degree of success.

Methods and Materials

Diets and Experimental Groups: Three hundred weanling Swiss Webster mice were placed on Purina Chow for three days post weaning and then segregated by weight into four equal groups. On the basis of growth rate a 9% protein diet is submininal for the mouse. The minimal protein requirement for mice is approximately 14% but lactating mice require a higher level (16%).[13,14] An 18% protein level (casein) is near optimal but levels higher than 30% protein inhibit growth.[15] Commercial laboratory feeds contain between 17 and 25% protein. Consequently, low (8%), medium (24%), and high (48%) protein levels and Purina Chow (23% protein) were chosen in these experiments. The test diets were isocaloric and contained protein from casein at the given levels, corn oil 10%, vitamin mixture 2%, U.S.P. salt mixture XIV 4%, and the balance was dextrin. The vitamin mixture contained: thiamine hydrochloride 0.2 g, pyridoxine 0 3 g, calcium pantothenate 2 g, nicotinic acid 2 g, para-aminobenzoic acid 15 g, i-inositol 10 g, folic acid 0.025 g, biotin 0 01 g, menadione 0.1 g, riboflavin 0.4 g, vitamin A 4 g, vitamin D 0.2 g, vitamin E 6 g, and alphacel to make one kilogram. Food and water were given *ad libitum*.

Resistance of mice to infection may be altered by the length of time the animals are on the diet.[16] Therefore, the animals in each of the four dietary groups were subdivided into three experimental groups. The mice were inoculated with *Trichinella spiralis* after being on the diets the following periods of time: Experiment I at

the beginning of the dietary period, Experiment II at three weeks, and Experiment III at six weeks.

Inoculation and Recovery of Organisms: *Trichinella spiralis* larvae were obtained from the skeletal muscles, the tongue, the masseter muscles, intercostal muscles, and the diaphragm of rats. The artificial digestion method was employed to recover these organisms. The digestive juice contained the following: concentrated HCl 8 ml, pepsin (1 : 10,000) 6 g, and water 1000 ml. The rat muscles were chopped and ground for one minute in an electric blender with 100 ml of digestive juice. This mixture was placed in a one liter flask with additional digestive juice and incubated for five or six hours with occasional manual shaking. Undigested cartilage and pieces of bone were removed by filtration through a six layer thickness of moistened gauze. The filtrate was placed in 15 ml centrifuge tubes and larvae were recovered by sedimentation. The supernatant liquid was decanted and the sediment washed twice with 0.85% saline solution and centrifuged at 2000 r.p.m. The washed larvae were added to a suspension medium (nutrient broth), containing 2 g tryptose, 15 g gelatin and 100 ml distilled water. An aliquot was taken, and a total larvae count was made.

The lethal dose for mice infected with *Trichinella spiralis* is approximately 50 larvae per mouse gram weight according to the results obtained in our laboratory. Since the purpose of this experiment was the recovery of the adult and larval stages of the worm, a sublethal dose of 33 larvae per mouse gram weight was administered by the oral gavage method. The average weight of mice on Experiment I (inoculation at beginning of dietary period) was 12 grams. A dose of 400± 15 larvae was administered to mice on this and subsequent experimental periods without respect to weight. The mice were given water *ad libitum* but food was removed for a period of eight hours prior to inoculation.

Seventy-two hours after infection, (after an eight-hour fast,) four animals were sacrificed. The small intestine was removed, divided by slitting longitudinally into halves, washed with 0.1% NaOH, and centrifuged for 2 minutes at 2000 r.p.m. After decanting, the sediment and water used to rinse the centrifuge tubes were placed in a petri dish, and the worms were removed with a pipette as they were counted by use of a stereoscopic microscope. The method described for the recovery of adult *Trichinella spiralis* is similar to the one described by Larsh and Kent.[17]

At the end of four weeks after infection the remainder of the mice were sacrificed. Each mouse was examined in the following manner. The feet and tail were cut off and discarded; it was skinned, eviscerated, cut into small pieces, and incubated with occasional manual agitation for 6 to 12 hours with 150 ml of artificial digestive juice. The material was then suspended in 5 ml of distilled water. The total count was established by taking 0. 1 ml aliquot of the suspension, placing it on a counting chamber and counting the larvae under a stereoscopic microscope.

Growth Response: Experimental mice were matched by weight with control mice which were not infected during the course of the experiment. Growth response on the various diets was determined by twice weekly weighings of both the control and experimental mice on the various diets.

Results and Discussion

The effect of varying protein intake on mice infection with *Trichinella spiralis* was determined by (1) the number of adult worms recovered from the intestine 72-hours after infection, (2) the number of encysted larvae recovered by digestion of muscles four weeks after infection, and (3) by the weight gain observed in the infected and non-infected animals during the course of the dietary period. When diet alters the resistance of the host to the parasite, this may be shown by the number of ingested larvae that have become established and developed to adult worms. In like manner host resistance may be involved in permitting a greater or smaller number of larvae to migrate and encyst. For the purpose of the study, resistance is interpreted as the ability of the host to resist the establishment and propagation of the parasite. More weight is given to the data on the recovery of larvae than on the recovery of adults since it is of greater importance to find

larvae encysted in the muscle than to detect the presence of adult worms in the intestines. The latter may be termed the infected state, whereas the former is actually the disease. In Experiment I fewer infective larvae were recovered than in Experiments II and III since the incubation period used to digest the muscles of rats on the first experiment was longer and thus produced less than 50% infective larvae but over 90% in the other two when the digestion period was reduced to 5 or 6 hours. However, the relative order of infection is similar in all three experiments.

The mice in each group weighed an average of 12 g at the beginning of the dietary period. They were weighed twice weekly but their weights at selected times only were reported.

Several deaths occurred among the animals on the low protein diet before infection. Deaths occurred in several animals of the various groups after inoculation. Since the parameters of evaluation did not include the number of deaths and mortality was not high, these data were not included in the results. The critical mortality period was between the eighth and eleventh day after infection. A loss in weight followed inoculation and a gradual but slow gain in weight occurred some time after the eighth day.

Experiment I: Table I shows the effect of diet on resistance to infection and on weight gain of mice infected at the beginning of the dietary period. The average weight gain of mice was similar ($P = >0.05$) for all groups except the one on the 8% protein diet ($P = <0.001$). There was no difference in weight gain between the non-infected and infected mice. There was no statistical significant difference between any of the groups ($P = <0.05$ to >0.10) in the number of adult worms recovered. The number of encysted larvae was only slightly significantly greater ($P = 0.05$) in the 8% than in the 24% protein group. The statistical significance is minimized by the great individual variations in number of larvae in mice infected with *Trichinella spiralis*. However, the relative order of encysted larvae was the same in this experiment as in Experiments II and III. The 8% protein diet afforded the least resistance to infection (greatest number of encysted larvae), 24% protein afforded the greatest resistance, 48% protein and laboratory chow (23 % protein) were intermediate between the 8 and 24% protein diets

Experiment II: Table II shows the effect of diet on resistance to infection and on weight gain of mice infected three weeks after the beginning of the dietary period. The weight gain of mice on the low protein diet was again the lowest

Table I. *The average weight, number of adult Trichinella Spiralis recovered from the intestine, and the number of larvae recovered from the muscles of mice on different levels of protein and infected at the beginning of the experimental dietary period*

Protein level and source	Average weight (in grams)						Number of mice	Average number of adult worms	Number of mice	Average number of larvae.[1]
	Non-infected			Infected						
	No. of mice	Initial	28 days	No. of mice	Initial	28 days				
8%, casein	15	12	22	20	12	22	4	103±17	14	9,000±1750
24%, casein	15	12	35	20	12	32	4	76±18	13	4,500±1310
48%, casein	15	12	35	20	12	32	4	58±5	15	6,200±870
Purina chow (23%)	15	12	36	20	12	35	4	67±9	10	6,400±1290

[1] Mean ± standard error of the mean

($P = < 0.001$) while weight gain was similar for the other three groups ($P = > 0.05$) A sharp decrease in weight was noticed in mice infected with *Trichinella spiralis*, and thereafter these mice gained weight at a slower rate than the controls.

The only significant difference in the number of worms recovered was between 8% and the 48% protein groups ($P = < 0.01$). However, this may be due in part to the ralatively few number of animals tested in this phase of the study. There was a significant difference ($P = < 0.01$) in the number of larvae recovered from animals in the 24% protein groups when compared with the 8% and 48 % protein groups but not very significant ($P = < 0.05$) when compared with the laboratory chow group.

Experiment III: Table III shows the effect of diet on resistance to infection and weight gain of mice infected six weeks after the beginning of the dietary period when compared with the 24% protein group. The weight of mice was low ($P = < 0.01$) for the group on the 8% protein diet but slightly higher for the other two diets ($P = 0.05$) A decrease in weight after infection was noticed with these mice as withyounger ones in the previous experiments (I & II) There was no significant difference between the groups in thenumber of worms recoverd from the intestines.

Due to the great individual variations the number of larvae recovered was slightly significantly different ($P = < 0.05$) only between mice on the 8 and 24% protein diets. However, the relative resistance afforded by the diets was typical of the previous two experiments.

Comparisons of Experiments I, II and III: A typical growth response is seen in figure 1 as well as the sharp decrease in weight of mice infected with *Trichinella*. When the growth data for the three experiments were combined, the weight gain of mice on the 8 % protein diet was significantly lower ($P = < 0.001$), laboratory chow higher ($P = < 0.01$), and 48% protein similar ($P = > 0.05$) when compared with the 24% protein diet. Regardless of the diet, the weight loss at the time of infection was not regained in four weeks post infection. It is suggested that the reason for mice ingesting less food and losing weight after infection is that helminthiasis causes anorexia and a decreased ability to digest protein which is attributed to anti-enzymes and mechanical damage to the intestinal mucosa.[18-20] This reduction in protein digestion has its peak between 4 and 12 days after infection.

There was no significant difference between groups in the number of adult *Trichinella* recoverd. However, the mice

Table II. *The average weight, number of adult Trichinella Spiralis recovered from the intestine and the number of larvae recovered from the muscles of mice on different levels of protein and infected three weeks after the beginning of experimental dietary period*

Protein level and source	Average weight (in grams)						Number of mice	Average number of adult worms.[1]	Number of mice	Average number of larvae[1]
	Non-infected			Infected						
	No. of mice	21 days	49 days	No. of mice	21 days	49 days				
8% casein	15	18	27	21	17	19	2	15±10	3	55,700±13591
24% casein	15	30	38	24	31	30	5	74±27	16	21,00±6380
48% casein	15	32	39	19	31	30	5	90±8	11	40,000±2046
Purina Chow (23%)	15	34	37	20	31	29	5	35±18	14	35,700 1695

1. Mean ± standard error of the mean

Table III. *The average weight, number of adult Trichinella Spiralis recovered from the intestine and the number of larvae recovered from the muscles of mice on different levels of protein and infected six weeks after the beginning of experimental dietary period*

Protein level and source	Average weight (in grams)						Number of mice	Average no. adult worms[1]	number of mice	Average No. of larvae.[1]
	Non-infected			Infected						
	No. of mice	42 days	70 days	No. of mice	42 days	70 days				
8% casein	10	26	29	20	27	23	3	13±1	11	41500±5090
24% casein	10	36	37	20	35	31	4	19±6	13	28300±4377
48% casein	10	35	40	20	36	31	4	17±7	14	35000±6981
Purina chow (23%)	10	37	42	17	33	33	4	27±9	12	34100±4595

1. Mean±standard error of the mean.

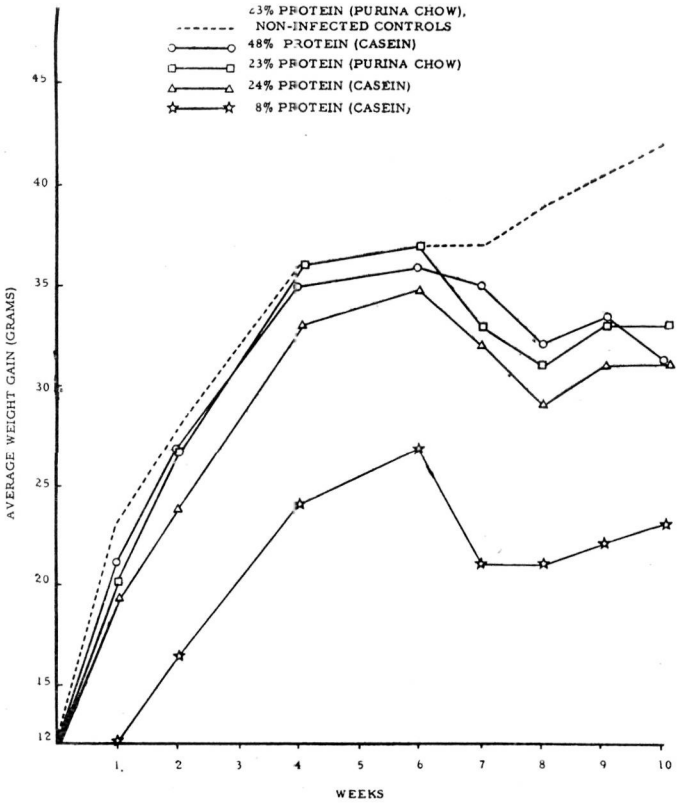

Fig. 1. The effect of diet on the weight gain of mice inoculated after six weeks on the experimental diet (Experiment III).

infected at the beginning of the dietary period had a greater number of adult worms than mice infected after six weeks (compare data in tables I and III). These data support the observation that young mice (26 to 32 days old) harbored more adult *Trichinella spiralis* than adult mice (115 to 171 days old).[21] Data from histological studies of intestines suggest that goblet cells which produce mucus may be a factor in the elimination of worms from the host since the number of these cells increases with age of rats and chickens, and in turn decrease the number of adult worms.[22] The number of adult worms recovered may also be dependent upon the phenomenon of adaptation to a diet. This phenomenon of adaptation has been noted with mice and quality and quantity of protein in resistance to infection,[16] in growth studies with chickens,[23] in nitrogen balance studies with rats,[24] and in utilization of high fat or high carbohydrate reducing diets with mice and human subjects.[25]

The data for these experiments were grouped together and compared as a whole in Figure 2. (Only the data for Experiment II and III were taken because of a difference in the number of *Trichinella* inoculated per mouse in Experiment I due to reasons which have been already explained; however, the relative order in number of larvae was the same between groups for all three experiments). There was a significant difference ($P = <0.01$) between the 24% protein and 8% as well as between the 8% and 48% protein groups. A slight significant difference existed ($P = 0.05$) between the

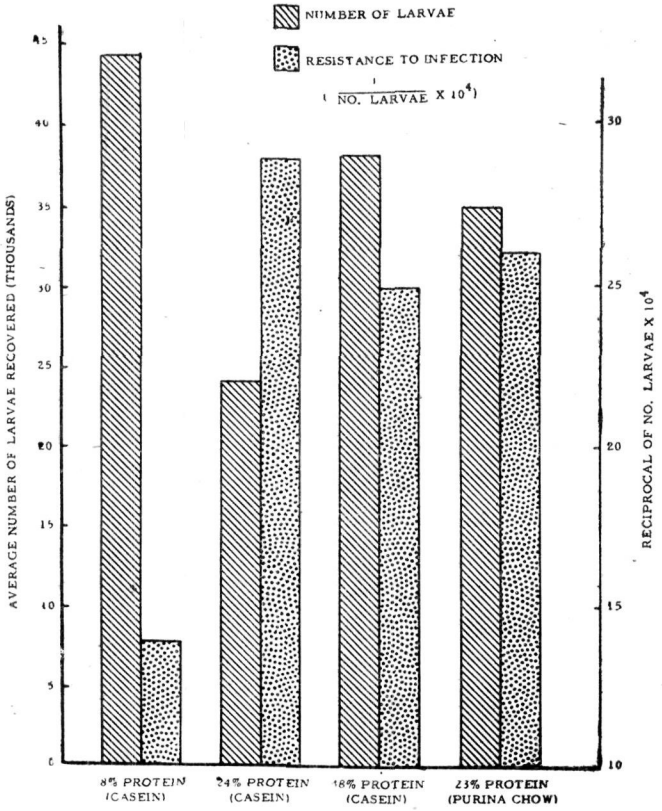

Fig. 2. The composite results (Experiments II and III) of the effect of diet on the number of larvae recovered or the resistance to infection (reciprocal of the number of larvae recovered).

24%protein and laboratory chow groups.

The data for the number of larvae recovered from the mice are directly proportional to susceptibility and inversely proportional to resistance. Therefore, resistance to infection can be represented as the reciprocal of the number of encysted larvae as shown in Figure 2. For growth, as well as for resistance to infection, an 8% protein diet of casein is suboptimal in the levels of dietary protein. A decrease in weight of mice on protein levels above 30%, as reported by others,[15] was noticed in this study. A 24% protein level produced good growth and afforded the best resistance to infection. The 48% protein diet of casein and the laboratory chow (23% protein) produced good growth but afforded significantly less resistance than the 24% protein diet. A near optimal level for normal mouse nutrition has been determined to be 18%.[15]

A low protein diet has been known to decrease the resistance of the host to infection. On the other hand, the machanism for a decrease in resistance with a high protein level as compared to a medium protein level has not been reported. More study is needed to determine the effects and mechanism of high protein diets. It is known, however, that high protein diets decrease the longevity of rats,[26-29] alter rat enzyme patterns, [30-31] decrease physical endurance as measured by the swim test with rats[32] and mice[33], and increase the vitamin A requirement in relation to resistance to infection[34] as compared with lower protein diets.

The resistance to infection afforded by Purina Chow (23% protein) was similar to the diet containing 48% rather than 24% protein. The data show that there is a difference in protein quality as determined by resistance to infection. Protein quality has been known to be important in resistance to infection.[16] However, on the basis of growth, the laboratory diet was as good as any level of casein tested (figure 2). Growth rate was not related to resistance of infection in much the same way that logevity is not directly proportional to growth rate [26-29] These differences illustrate the importance of experiments such as the present which are designed to evaluate proteins by parameters other than growth.

The relationship between diet and infectious diseases has been adequately described.[1] In many areas of the world undernutrition is common, while in the United States overnutrition has become a significant aspect of malnutrition. For this reason, a wide range of protein levels was selected to determine the relative importance of a low, medium, and high protein diet with respect to resistance to infection. It can be seen from this study that protein malnutrition, undernutrition or overnutrition is a contributing factor to helminthiasis.

Programs for decreasing malnutrition, especially protein malnutrition have been instituted in various parts of the world, such as India and Central America The programs generally consist of producing concentrated protein foods of good quality from blends of local plant protein sources which can be used in supplementing the diets especially of infants, children, and lactating mothers. It would appear that such programs, together with education in the rudiments of hygiene and an adequate diet, would reduce the incidence of infectious diseases by decreasing the prevalence of the two synergistic factors, parasites and malnutrition.

Summary

1. Three hundred Swiss Webster albino mice were divided equally by weight and placed on the following diets : 8, 24, and 48% protein from casein and a commercial laboratory diet (control, 23% protein), The dietary groups were then divided into three experimental periods according to the time of infection with *Trichinella spiralis* after the beginning of the dietary period : (1) zero time, (2) three weeks, (3) six weeks, The parameters of evaluation were: weight gain, number of adult worms recovered from the lumen of the intestines 72 hours after infection, and the number of encysted larvae recovered from the muscles four weeks after infection.

2. The weight of mice was lowest on the 8% protein diet in the three experimental periods. The weight gain was similar for the medium (24%) and high protein (48%),

as well as the control, but these were significantly higher than the group on the low protein (8%) level.

3. The number of adult worms recovered was similar between the groups. However the total number of worms was less in the older animals than those infected at the beginning. The possible reasons for these results are discussed.

4. The number of encysted larvae varied widely between dietary groups. The number of larvae recovered from mice of the 8% protein group was greatest (least resistance to infection), least for the 24% group, and intermediate for the 48% protein and control (23% protein) groups.

5. The importance of the quantity and quality of dietary protein and their relation to infectious disease is discussed.

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