17115. Experimental Studies on Nutrition in Tuberculosis. The Role of Protein in Resistance to Tuberculosis.*†

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The view is widely prevalent, and well supported by demographic evidence, that a high level of dietary protein is protective against invasion by tuberculosis.[1-3] Recent experimental and other evidence has shown that protein metabolism is of basic importance in natural and acquired resistance to infection in general,[4] and shown that it may be of importance in tuberculosis.[5] The investigation here recorded was carried out to determine if a difference in susceptibility to tuberculosis can be detected in experimental animals nourished on diets of variable protein content.

In the stock of albino rats at the Wistar Institute of Anatomy and Biology, at the time these experiments were initiated, there were 3 colonies of animals which had lived for a number of generations on diets containing respectively 15, 25 and 40% of protein. Metabolic and growth studies on these animals have been reported by McCoy,[6] who found that rats raised on a diet containing 40% protein grew more rapidly and were more prolific than rats on diets with smaller protein content. Appearance and behavior differences were striking. The animals on a diet of 15% protein were sluggish and sleepy, while those on the 40% diet were active and playful. The former had ruffled and the latter sleek and shining hair. The 25% animals were between the two extremes.

The base diet furnished these animals was composed as follows:

	g
Whole wheat flour	3675
Skimmed milk powder	800
Whole milk powder	1200
Soy bean meal	400
Alfalfa leaf meal	300
Irradiated yeast	300
Dried liver	200
Calcium carbonate	25
Bone ash	80
Sodium chloride, iodized	20
	7000

From this base, 15, 25, and 40% diets were prepared as follows:

Composition of Diet, and Protein Level in Per Cent.

Base	15 4200 g	25 4200 g	40 4200 g
Cod liver oil	240	240	240
Dextrin	1560	960	—
Casein	—	600	1560
	6000	6000	6000

Animals from the 3 groups were used for the experiments here recorded. Approximate equalization was effected within each protein group of experimental animals as respects age, weight and sex. All animals were injected intravenously with virulent tubercle bacilli of bovine type. Two separate experiments were carried out. The dosage was more accurately calibrated in the second than the first. In the first, animals of approximately equal weight were selected at the start and each received 0.1 mg tubercle bacilli in the internal jugular vein. In the second experiment, the injection carried out by the same route was so regulated that each animal received approximately the same dosage per unit lung weight. Lung weights were esti-

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1 Faber, K., Tuberculosis and Nutrition, *Acta Tuberc. Scandinav.*, 1938, **12**, 287.

2 Kirschner, M., *Z. f. Tuberk.*, 1921, **34**, 228.

3 Schröder, G., *Beitr. z. Klin. d. Tuberk.*, 1930, **75**, 61.

4 Cannon, P. R., *J. Am. Med. Assn.*, 1945, **128**, 360; Some Pathologic Consequences of Protein and Amino Acid Deficiencies, Charles C. Thomas, Springfield, Ill., 1948.

5 Ratcliffe, H. L., *Am. Rev. Tuberc.*, 1946, **54**, 389.

6 McCoy, R. H., *J. Biol. Chem.*, 1940, **133**, lxiv.

TABLE I.
Tuberculosis in Rats on Diets with Varying Percentage of Protein (Exp. 1).

% protein	Days of life	No. animals	Extent tuber.	Predom. type cellular react.	Avg degree localization	Avg No. bacilli	% with pneumonia
15	78-150	10	2.4	Epithelioid	Poor	Large	30
	167	2	3.0	"	"	"	100
25	47-150	9	2.7	"	"	"	44
40	78-150	5	2.4	"	"	Moderate	40
	196-293	7	2.4	Regr. mono.	"	"	100

TABLE II.
Tuberculosis in Rats on Diets with Varying Percentage of Protein (Exp. 2).

% protein	Days of life	No. animals	Extent tuber.	Predom. type cellular react.	Avg degree localization	Avg No. bacilli	% with pneumonia
15	14-150	10	2.1	Prog. mono.	Poor	Small	60
	180-222	4	1.8	Epithelioid	Moderate	"	25
	253-315	4	2.5	"	Poor	Moderate	50
25	14-150	4	1.5	"	"	"	50
	180-222	1	2.0	Regr. mono.	"	"	0
40	14-150	8	1.4	Prog. mono.	Good	Small	25
	180-222	6	1.5	Epithelioid	"	"	50
	253-315	10	1.3	Regr. mono.	Fair	"	20

mated from a formula worked out at the Wistar Institute.[7]

Animals were autopsied as they died or were killed at selected intervals. Sections were stained by hematoxylin and eosin, and also with carbol-fuchsin, by the technic for acid-fastness, in order to show the number of tubercle bacilli in tuberculous lesions. The most significant facts learned were with reference to pulmonary lesions, and the following analysis is based on the development of tuberculosis in the lungs.

A simple scoring system was adopted, which was based on (a) the extent of the lesion, (b) the type of cellular reaction, (c) the degree of localization of the tuberculous process and (d) the average number of tubercle bacilli present in lesions. In addition, because of what proved a significant development in the experiment, notation was made as to the presence or absence of extensive pneumonic lesions at the time of death. The extent of tuberculosis was estimated on a simple basis as follows: 1 = slight; 2 = moderate; and 3 = extensive.

[7] Donaldson, H. H., The Rat, Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, 1924, p. 212.

The results of the 2 experiments are indicated in Tables I and II.

Table I illustrates the results of the first experiment, in which 33 rats, matched according to age and litter, were used. This group of animals developed pneumonia in a high percentage of cases. All of the animals on 15 and 25% protein, including those sacrificed, died within less than 168 days. Animals were sacrificed for comparable observation on the 78th and 150th day of the experiment. Eight rats on the diet containing 40% protein outlived all of the other animals, although all but one of these died from natural causes ultimately. The last animal of the group was killed on the 293rd day.

Tuberculosis developed rapidly in all animals and the extent exceeded "moderate" in all within the first 150 days. In the animals on the 15% protein diet very extensive tuberculosis occurred after the 150th day. After the 150th day in the animals on 40% protein little or no progression occurred, and in most animals a certain amount of regression developed, evident both by decrease in the size and extent of the lesion and by a tendency to regression in the type of reaction.

In general in the earliest days of infection

a type of cellular reaction was evident which is here designated as a progressive mononuclear response. It was characterized by the early appearance of large mononuclear cells of the macrophage series with large nuclei and a small amount of cytoplasm. With the passage of time these mononuclear cells phagocytized tubercle bacilli and developed a large amount of cytoplasm, taking on the appearance of large epithelioid cells (epithelioid stage). The average type of reaction in all the animals up to the 150th day of the experiment was epithelioid. The 15 and 25% animals did not live long enough to develop the characteristic regressive type of mononuclear reaction. This was conspicuous, however, in the 40% animals, which lived much longer. This type of reaction was seen in most animals living longer than 180 days, and was particularly frequent in the animals receiving the highest concentration of protein. In this stage large mononuclear cells with a small amount of cytoplasm again became predominant and were present in far larger numbers than the typical epithelioid cells. Two explanations are possible for this phenomenon, (1) that the epithelioid cells gradually destroyed tubercle bacilli, after which the cytoplasm shrank, and (2) that epithelioid cells containing large numbers of tubercle bacilli were themselves destroyed and autolyzed, and replaced by new infiltrating large mononuclear cells, which found relatively few bacilli to phagocytize, and therefore did not develop the large amount of cytoplasm characteristic of epithelioid cells.

In the animals on diets containing 15 and 25% protein tubercle bacilli were present in enormous numbers. In the 40% animals the number of bacilli present was definitely smaller.

In the 3 groups of animals in Experiment I, nourished on varying amounts of protein, significant differences were not evident in the incidence of pneumonia. In the first 150 days from 30 to 40% of all animals developed this disease and died. The few remaining 15% animals died of pneumonia at 167 days. The 40% animals lived many months longer, but all but the one finally killed ultimately succumbed to pneumonia.

Characteristic differences occurred in the weight curves of the 3 groups of animals. These curves are not plotted in this paper, because of the complication introduced by the fact that certain animals were killed, and the fact that the weights of others were irregularly influenced by the development of pneumonia. The general characteristics of the curves, however, were as follows: Animals on the 15% protein diet increased in weight from an average of approximately 190 g at the beginning of the experiment to approximately 280 g at about the 90th day, and decreased to approximately 220 g at the 150th day. Animals of the 25% series increased from an original average weight of approximately 200 g to an average of about 300 g on the 90th day and dropped to an average of 255 g on the 133rd day. Two surviving animals weighed approximately 310 g when killed at 150 days. Animals of the 40% series increased from an average of approximately 210 g at the start to about 300 g at the 84th day, and held this until the end of the experiment.

In summary it may be said of the animals of Experiment I that the extent of tuberculosis did not vary greatly in the 3 groups. However, the animals on the 40% protein diet outlived those on diets containing lower percentages of protein, by several months, and the number of tubercle bacilli visible in the lesions was much smaller than in the animals on 15 and 25% protein diets. At times in the 15% animals the number of bacilli was enormous, and wide dissemination of bacilli was evident in some of the animals dying of pneumonia. In a number of the animals with pneumonia ulceration of bronchi by the pneumonic process and wide scattering of exudate appeared to have a definite effect in spreading tubercle bacilli.

In Table II the results of the second experiment are recorded. Forty-seven rats were used. As noted above, the dosage was accurately measured on the basis of lung weight. The extent of tuberculosis in all groups was less than that in the preceding experiment, and differences in degree of localization were more clearly evident.

In the tabulation of Experiment II the animals are divided into 3 groups, *viz.*, those dying between 14 and 150 days, those dying between 180 and 222 days, and those dying between 250 and 315 days.

The extent of tuberculosis was measured on the same scale as in the case of the animals recorded in Table I. The extent of tuberculosis was somewhat greater in the animals receiving 15 and 25% protein diets than in those on the 40% diet. Also, as in Experiment I, after the first 150 days significant increases in extent of tuberculosis did not develop in the 40% animals.

In general the animals of this experiment showed the characteristic change in cellular reaction noted in Experiment I. This was most typical in the 40% animals, which clearly exhibited a progressive mononuclear reaction up to 150 days as the predominant type of reaction, followed by a long period during which the overwhelming majority of cells present in the exudate were of the epithelioid type, with a final period, after 250 days, when the epithelioid cells diminished in number, and were replaced by large mononuclear cells with relatively little cytoplasm. The latter stage, as noted above, is designated in this paper as regressive mononuclear stage.

In this experiment, in contrast to the first, distinct variations in the degree of localization of the tuberculous lesions were noted. In general the disease was widely disseminated in the 15 and 25% protein animals, but a "fair" to "good" degree of localization was evident in animals on the 40% protein diet. The number of bacilli visible in the tuberculous lesions was smaller in this experiment than in the previous one, but, as in that experiment, the number seen in tuberculous lesions was definitely smaller in the 40% animals than in those receiving smaller amounts of protein. Highly suggestive differences in the extent of pneumonia in the animals of the three groups were observed. The incidence of pneumonia was very high in the 15% group, and, while still high in the 40% group, it was much below that found in the 15% group. The difference in frequency and extent of pneumonia was more closely correlated with the amount of protein in the diet than was the extent of tuberculosis. The impression was created that, if the diet was responsible for variation in tuberculosis, the influence was indirect, through an effect upon resistance to the common types of pneumonia endemic in rat colonies. Bacteriological studies of the pneumonic lesions were not made. Previous studies on rats of this colony showed pneumonia is frequent and the etiology variable.[8]

As in the preceding experiment, in general the 40% animals far outlived animals of the other groups. At 315 days 5 of the 40% animals which had survived, were killed to conclude the experiment. These were large animals, free from pneumonia, with one exception, with excellent coats of hair, and every appearance of good health. As noted, all of them had moderately extensive tuberculosis, but this appeared to be in the regressive stage, as indicated by the type of cellular reaction.

Weight curves were comparable to those of the preceding experiment. In Experiment II, young and old animals were used, evenly distributed within the 3 groups. Young litters ranged from 61 to 76 days of age at the time of injection, and old litters from 736 to 931 days. All of the old animals died within the first 146 days. The animals which survived 315 days, all of them within the 40% group, were from 61 to 76 day litters. Differences in the type of cellular reaction were not observed between young and old animals.

Summary. The results of Experiment II were like those of Experiment I, the chief difference being that the infection was better regulated, and variations within the 3 protein groups were more characteristic. Tuberculosis was less extensive and life was much longer in the rats on a 40% protein diet than in the other 2 groups, and the degree of localization of the disease was better. The number of bacilli in lesions was smaller in the 40% series and the extent of pneumonia was less. Corresponding with the lesser extent of pneumonia there was less dissemination of tuberculous lesions. A characteristic

[8] Rateliffe, H. L., Chapter 22 in *The Rat in Laboratory Investigation*, Griffith, J. Q., and Farris, E. J., Lippincott Company, Philadelphia, 1942.

feature of the long-lived animals was a progressive change in the cellular type of reaction from an early infiltration of mononuclears with little cytoplasm to a stage of consolidation with epithelioid cells and finally to a "regressive" type of mononuclear reaction in which lesions decreased in size, epithelioid cells were few and mononuclears with little cytoplasm predominated.

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17116. Ante-Mortem Failure of the Aural Microphonic in the Guinea Pig.*

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In the course of an extended study of the electrical response of the cochlea of the guinea pig to pure tones and to clicks we have collected incidental observations on the conditions of survival of these responses. Since these conditions are pertinent to any theory of the origin of the aural microphonic or "cochlear response" we present them herewith. Our report concerns only the aural microphonic, which apparently is closely related to the physical movements of the basilar membrane or some closely adjacent structure, and not the action potentials of the auditory nerve (or spiral ganglion) which are usually recorded with the microphonic. In general the action potentials fail equally with or before the microphonic. We have never yet observed the action potentials under conditions where the aural microphonic could not also be elicited, although sometimes a greater intensity of stimulus is necessary to make the microphonic visible on the oscilloscope.

Our sound-generating system and our pick-up amplifiers and oscilloscopes follow conventional design and will be described in detail elsewhere. We make electrical contact with the cochlea through a tiny wick applied to the round window and through fine copper or silver wires passing through or fitted like a cork into small holes drilled through the bony shell of the cochlea. A reference electrode is connected to the edge of the wound in the neck.

Mere placement of an electrode on the round window membrane does not necessarily cause a reduction of the aural microphonic over a period of many hours or even 2 or 3 days. Neither does the anaesthetic *per se* (dial in urethane), provided the dose is light enough to allow reflex muscular movements and circulation and respiration remain adequate. Fall in body temperature sometimes seems to cause a reversible reduction of the microphonic, but no more than can reasonably be explained by the associated fall in metabolic rate and changes in circulation and respiration. Such effects of temperature have been more prominent in our experiments on cats than in guinea pigs.

It is well known that the aural microphonic falls off sharply when the animal dies, but that it persists for many hours after death at a few per cent of its original strength.[1] In the guinea pig, when respiration gradually fails from too much anesthetic, the microphonic falls to this low level *before* the heart stops. The failure is partly reversible. If the animal takes a few gasping breaths the microphonic may double its voltage (and action potentials return) as the heart beats faster and more regu-

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† Now in Santiago, Chile.

‡ Traveling Fellow of Rockefeller Foundation.

1 Wever, E. G., Bray, C. W., and Lawrence, M., *Ann. Otol., Rhinol., and Laryngol.*, 1941, **50**, 317.