INFLUENCE OF DIETARY PROTEIN ON LIVER CONSTITUENTS OF TUBERCULOUS AND NON-TUBERCULOUS GUINEA PIGS

Thesis for the Degree of Ph. D.

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Roberta Ellen Bleiler

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This is to certify that the

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INFLUENCE OF DIETARY PROTEIN ON LIVER CONSTITUENTS OF TUBERCULOUS AND NON-TUBERCULOUS GUINEA PIGS

By

ROBERTA ELLEN BLEILER

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
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The amounts of fat, nitrogen, sulfur, solids, flavin adenine dinucleotide, flavin mononucleotide plus free riboflavin, and the activity of glutathione reductase were determined in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes. During 40- and 47-day experimental periods, purified diets containing 35 and 25 per cent protein were fed to 39 control guinea pigs and to 28 animals that had been inoculated with M. tuberculosis H37Rv. The tuberculous animals consumed greater quantities of ration than did their respective controls, but failed to utilize the feed for weight gain as well as did the non-infected guinea pigs.

The enlargement of the livers of the tuberculous guinea pigs indicated that possible structural and functional alterations had occurred in the organ during the infection. The percentages of solids in the livers of the tuberculous animals were lower and the percentages of water higher than those for the respective controls. Tuberculosis appeared to have little effect on the percentages of fat, nitrogen, and sulfur in the livers of

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the animals. Limitation of dietary protein did not significantly alter the concentrations of these constituents in the livers of the guinea pigs.

Estimation of the activity of liver glutathione reductase suggested that mechanisms responsible for maintaining reduced glutathione in the livers of the guinea pigs were not disturbed either by the presence of tuberculosis or by the restriction of dietary protein. A higher value for the percentage of flavin mononucleotide plus free riboflavin fraction in the liver was found for guinea pigs which received the lower protein ration than for those consuming the 35 per cent protein diet. The concentration of flavin adenine dinucleotide per gram of liver tissue decreased during tuberculosis in the guinea pigs. It is possible that alterations occurred in the flavin adenine dinucleotide-linked enzyme systems of the livers of the tuberculous animals. The relationship between riboflavin retention and nitrogen storage in liver was not maintained during tuberculosis.

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Roberta Ellen Bleiler

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INTRODUCTION

Among bacterial infections, tuberculosis is unique. The ability of tubercle bacilli to remain viable in a host for long periods of time without forcing an immediate all-or-nothing struggle reflects the intractability of the disease. The efficiency of the host's defense mechanisms decreases due to the chronic nature of the infection. Known chemotherapeutic agents, at least in concentrations sufficiently low to prevent cell toxicity in certain body organs are tuberculostatic, rather than tuberculocidal. The host is not capable of destroying the attenuated parasites, rapidly.

A healthy body can avoid mechanistic incompetency more easily than can one that is poorly nourished; moreover, tissue which was destroyed during the progress of the tuberculous infection must be regenerated. Herein lies the importance of nutrition with regard to both preventative and curative measures in tuberculosis.

REVIEW OF LITERATURE

Relationship between Protein Metabolism and the Liver

The relationship between protein metabolism and the liver can be considered from two approaches; that is, the effect of the liver on protein metabolism or, conversely, that of protein metabolism on the composition and structure of the liver. For example, the presence of hepatic disease, in spite of the ingestion of adequate amounts of nutritious foods, may lessen protein synthesis (Cannon, '50); on the other hand, protein malnutrition tends not only to produce a fatty liver, but also to promote pathological alterations of the liver, the central organ in protein metabolism (György, '54).

Injury to the hepatic parenchyma may occur when dietary patterns are altered adversely. Fatty infiltration of the liver was the first noticeable change in a choline deficiency which could be reversed with the feeding of methionine or other sulfur-containing amino acids (György, '54). Hawk and Elvehjem ('53) have indicated that protein possesses a lipotropic action beyond that attributable to its content of sulfur-containing amino

acids, particularly that of methionine. According to Himsworth ('47), every dietary component except carbohydrate has been indicated as the cause of liver injury at one time or another.

Diet-induced massive necrosis appears to result from multiple deficiencies, while singular dietary limitations may cause cirrhosis of the liver (György, '55). The recognition of dietary liver cirrhosis and dietary liver necrosis as separate and distinct entities does not preclude the possibility that fibrosis may succeed necrosis or that the death of cells may occur during the cirrhotic process (Daft, '54; György, '55). Furthermore, György ('55) suggested that preventative measures against cirrhosis and necrosis include all substances which fall under the terminology, lipotropic factors. Treatment of diseases of the liver by diets high in protein was based on the potentialities of protein in abetting regenerative processes, in reducing fatty infiltration, and in regressing fibrosis.

Siess and Stegmann ('50) suggested that, in a period of growth, the weight of the liver remains a constant fraction of the total body weight, but that a high level of dietary protein will increase the weight of the liver, slightly. According to Kosterlitz ('54), the entire

cytoplasm of the liver cells may change with alterations in the dietary protein; the concentrations of protein within individual liver cells of rats increased with greater protein intake, but varied with the quality of the nutrient. Widdowson and McCance ('57) demonstrated that the amounts of nitrogen present per 100 grams of liver in young rats depended upon the percentages of protein in the rations; when dietary protein was increased from 1.5 to 15.8 to 45 per cent, the nitrogen content per 100 grams of liver rose from 2.4 to 3.0 to 3.5 grams, respectively.

Protein deficiency in the diet has produced marked changes in the enzyme pattern and structure of the liver; these alterations were especially noticeable in young growing rats in comparison with adults (Srinivasan and Patwardhan, '55). Lévy ('53) reviewed the influence of the nutritional state upon the activity of enzymes, and concluded that, on low protein diets, the majority of liver enzymes diminish in proportion to the amount of the nutrient lost from the liver. Knox et al. ('56) observed that when dietary protein was limited, the liver enzyme concentrations which did change were decreased uniformly; the concentrations of the catalysts increased when the diet contained extra protein or amino acids. Some changes in liver enzyme

activities in relation to alterations in protein intake are given in Table 1; the results agree generally with the observations of Lévy (loc. cit.) and Knox et al. (loc. cit.). Cytochrome oxidase, however, demonstrated an increased concentration in the liver of rats when a protein-free diet was fed, according to Wainio et al. ('52).

In 1931, Hopkins and Elliott proposed that a system capable of reducing oxidized glutathione existed in animal tissues. Meldrum and Tarr ('35) described an enzymatic reduction of oxidized glutathione in a coupled reaction with glucose-6-phosphate dehydrogenase (Zwischenferment) of blood cells or yeast in the presence of a triphosphopyridine nucleotide preparation, and suggested that a glutathione reductase must have been present in order to explain the results. The enzyme was purified partially from wheat germ and higher plants, and studied in Zwischenferment systems (Conn and Vennesland, '51; Anderson, et al., '52); materials which contained glutathione reductase activity were extracted from animal tissues by Rall and Lehninger ('52). The catalyzing ability of glutathione reductase in mammalian erythrocytes was about the same in Zwischenferment systems as in the presence of reduced triphosphopyridine nucleotide, but the requirement for a

Table 1

Changes in liver enzyme activity with dietary protein alterations, reported in literature

Enzyme	Direction of change of liver enzyme activity from control during dietary variation			Investigator
	Protein depletion	Protein free	Balanced diet pair-fed with protein free	
cytochrome oxidase	decrease	increase	decrease	Lévy and Legrand, '53 Wainio et al., '52
xanthine oxidase	same decrease	decrease	decrease	Wainio et al., '52 Meikleham et al., '51
pyruvate oxidase	same		same	Wainio et al., '52
succinic oxidase	decrease decrease		same	Lévy and Legrand, '53 Wainio et al., '52
succinic dehydrogenase	decrease		same	Wainio et al., '52
d-amino acid oxidase	decrease		same	Wainio et al., '52
DPN-cytochrome c	decrease		decrease	Wainio et al., '52
uricase	decrease		decrease	Wainio et al., '52
alkaline phosphotase	decrease	decrease		Rosenthal et al., '52
arginase		decrease		Rosenthal et al., '50
rhodanase		decrease		Rosenthal et al., '50
adenosinepyrophosphotase		decrease		Rosenthal et al., '50
alanine transaminase		decrease		Awapara, '53
choline oxidase	decrease			Richert and Westerfeld, '52
esterase	decrease	decrease		Meikleham et al., '51

cofactor was evident when diphosphopyridine nucleotide served as the hydrogen donor (Francoer and Denstedt, '54). According to Racker ('55), fractionation of the acetone powders from beef liver enzyme with alcohol and isoelectric precipitation increased the glutathione reductase activity over that in the original extract; spectrophotometrically, the concentrate demonstrated a more rapid reaction with triphosphopyridine nucleotide than with diphosphopyridine nucleotide between pH 5.8 and 6.5, and showed a typical absorption spectrum of a protein. The relationship between dietary protein and glutathione reductase activity in the liver is unknown.

Sulfur is a component of glutathione, a peptide functioning primarily in the maintenance of sulfhydryl groups in a reduced physiological state (Barron, '49; Bricas and Fromageot, '53). The sulfur-containing amino acids, which form an integral and essential part of the dietary protein, provide the majority of the body sulfur.

Since a dietary deficiency of sulfur-containing amino acids had been suggested as a cause of necrosis of the liver, Lindan and Work ('51) determined the amounts of glutathione present in necrotic livers. After nine days, a slight diminution in glutathione values was noted

in the livers of albino rats fed a necrogenic yeast diet which supplied seven per cent protein, in comparison with the values obtained for the control animals. No further lowering of liver glutathione was observed until the experimental animals became ill suddenly and the livers showed symptoms of necrosis; the glutathione values in the necrotic livers decreased to 20 per cent of those found for the livers of the control animals. Beck and Bianconi ('56) found that the non-protein sulfhydryl content of the livers of mice decreased markedly when the sulfur-containing amino acids of the diet were restricted.

The content of sulfur within the tissues of the body is most closely associated with the tissue protein, specifically with nitrogen; the usual nitrogen to sulfur ratio of both food and body tissues lies between 14 and 16 (Shohl, '39). According to Shohl (loc. cit.), the average weight of the liver of an adult human being is 1.8 kilograms when the total body weight is 66.2 kilograms; the sulfur of this organ represents approximately 0.03 per cent (0.5 grams) of its weight. Little has been reported in the literature on the amounts of total sulfur in body tissues since 1930.

Evidence has been accumulated to indicate that relationships exist between protein metabolism and certain

B-vitamins; riboflavin concentrations in tissues appear to depend not only upon the dietary content of the vitamin, but also upon the amount of protein in the diet. Sarett and Perlzweig ('43) studied protein and riboflavin interrelationships in rats. They reported that the riboflavin content per gram of liver appeared to bear a direct relationship to the amount of nitrogen present per gram of tissue when the values for the high dietary protein groups were compared with those for the low protein animals, despite the fact that some of the high protein rats had low vitamin supplementation. Although the total riboflavin present in the liver was slightly higher in both groups receiving the larger amounts of vitamins than in the livers of the respective low-vitamin animals, total nitrogen retention appeared to be the dominant factor with respect to the storage of riboflavin in the liver. The investigations of Czaczkes and Guggenheim ('46) demonstrated that the amount of riboflavin in the livers of rats increased when dietary casein was raised from 15 to 34 per cent. Riesen et al. ('46) showed that greater amounts of nitrogen were retained in the livers of rats and that the riboflavin content increased from 14 to 25 to 29 micrograms per gram of tissue as the casein content of the diet was augmented progressively from 8 to 18 to 50 per cent, respectively.

Riboflavin exists in the liver in three forms: free riboflavin which accounts for the smallest fraction; flavin adenine dinucleotide (FAD), the largest portion; and flavin mononucleotide (FMN). FAD and FMN are coenzymes; specifically, they are considered to be prosthetic groups, since the apoenzyme and coenzyme moieties are firmly attached each to the other. The primary function of the flavoproteins is in electron transport systems.

In 1949, Bessey et al., employing acid hydrolysis in a fluorometric procedure for the determination of esterified riboflavin in animal tissues, reported that the FAD varied from nearly 90 per cent of the total riboflavin in the skeletal muscles to about 70 per cent in the kidney. During an imposed riboflavin deficiency, the concentrations of all three fractions decreased; the percentage decrease of the FMN appeared to be greater than that of either FAD or free riboflavin, but the investigators noted large variations between groups of animals. Free riboflavin in tissues is low, thus making measurement difficult by this technique; generally, this fraction and FMN are expressed together. Trufanov ('46) found that the livers of rats fed a lower protein diet than the control animals for 50 days failed to synthesize a normal quantity of FAD.

Nutrition in Tuberculosis

Good nutrition appears to be one of the primary requisites in resistance to tuberculosis, according to the myriad reports concerned with the disease. Studies in countries involved in both World War I and World War II adduced evidence which strongly supported this theory, and furthermore established an inverse relationship between economic level and the incidence of the disease (Faber, '38; Sneedin, '46; Rich, '51). Economic status influences food consumption, particularly that of protein; Faber (loc. cit.) had associated the rise in the mortality rate of tuberculosis in Denmark during World War I with dietary protein deficiency. Cannon (loc. cit.) attributed the increased susceptibility to infection displayed by starving persons to a depletion of protein reserves and to the inability to fabricate new supplies of antibody globulins because of an inadequate intake of essential amino acids; according to Rich (loc. cit.), a dietary protein deficiency probably has an adverse effect even upon acquired resistance.

In an attempt to establish the protein requirement in tuberculosis, McCann ('22) maintained ten bed patients in nitrogen equilibrium by feeding 60 to 90 grams of

protein daily. According to Brewer et al. ('50), the retentions of nitrogen by six women with moderately advanced, active tuberculosis were similar to those of healthy college women when the protein intakes were comparable; these patients were able to store some nitrogen when the daily protein consumption was 80 grams. Tui et al. ('54) reported that tuberculous patients do not require higher nitrogen intakes than normal subjects to achieve nitrogen equilibrium, and attributed the hypoproteinemia in chronic tuberculosis to prolonged low food consumption rather than to physiological catabolism. With tuberculous girls in their adolescent years as subjects, Johnston ('53) observed a correlation between adequate storage of nitrogen and a decrease in the progress of the infection.

In animal experimentation, Hornemann ('13) noted a lesser development of tuberculous lesions in infected swine that received a high protein diet than in those fed a high carbohydrate or mixed diet. The extensiveness of disease and mortality rate of tuberculous rats of the Wistar strain decreased as dietary protein increased from 15 to 40 per cent (Koerner et al., '49). In contrast, Metcoff et al. ('49) studied three groups of infected rats fed diets which were (1) adequate, (2) protein

deficient, and (3) initially adequate, then severely deficient, respectively, and obtained results which favored the concept that dietary protein deficiency does not alter, significantly, the susceptibility of the rats nor the course of the disease.

Following tuberculous injection, Swiss albino mice fed a diet of whole wheat and dried milk survived longer than those on a cornmeal, gelatin, and butter regime; the effects of a change from a good to an inadequate diet resulting in increased susceptibility to infection may reflect a non-specific physiological disturbance rather than a defined dietary deficiency, according to Dubos and Pierce ('48). Dubos et al. ('55) demonstrated that the susceptibility of mice to experimental bacterial infection can be increased consistently by various procedures designed to disturb animal metabolism. For instance, the fasting mouse manifested a decreased resistance within 36 hours after the beginning of the test period, and this alteration in susceptibility was further aggravated by feeding only glucose or an incomplete diet to the animal. Dubos ('55) hypothesized that metabolic disturbances which cause either a depletion of the glycogen reserves of the body or a reduction in the glycolytic activity in inflammatory cells possibly with the accumulation of certain

polycarboxylic acids and ketones in the tissues can effect a decrease in resistance to tuberculous infection in mice. Furthermore, this investigator also reported that the resistance of mice appeared to be independent of the dietary protein level, within wide limits; whereas mice fed a diet very low in protein and high in carbohydrates proved markedly susceptible to tuberculosis, supplanting part of the carbohydrates with fat without deviating the protein content served to return their resistance to normal. Thus, the evidence provided no indication that protein deficiency alone could accelerate the course of tuberculosis. Dubos (loc. cit.) discussed diet and resistance to tuberculosis in man and concluded:

> It may be worth noting in this respect that investigators who have tried to correlate deficiency in nutritional factors with susceptibility to tuberculosis on the basis of epidemiological studies of the human disease have emphasized the importance of proteins of animal source. As deficiency in foods of animal origin is almost always accompanied by other dietary limitations, it seems possible that these, rather than low protein intake, are responsible for the increase in susceptibility to tuberculosis which has been observed in certain epidemiological situations, for example, during war time.

According to Farber and Miller ('43) from observations on 400 tuberculous patients, very ill subjects often demonstrated multiple vitamin deficiencies which may

correlate in degree with the severity of the disease. Ascorbic acid metabolism appears to be disturbed during tuberculosis; it is generally conceded that the need for this vitamin by the tuberculous person is increased greatly.[1] The urinary levels of thiamine, riboflavin, and niacin were found to be lower in tuberculous subjects when compared with the norm reported by Sartory et al. ('51), and the extent of diminution was proportional to the gravity of the infection, in agreement with Farber and Miller (loc. cit.). On the other hand, Brewer et al. (loc. cit.) reported that the riboflavin metabolism of six women with moderately active, advanced tuberculosis was similar to that of college-age women, and that the urinary excretion of riboflavin was related directly to the dietary intake of the vitamin, but inversely to the retention of nitrogen. The values for pyridine nucleotides in erythrocytes of tuberculous patients were less than those for non-tuberculous patients; the values for pyridine nucleotides in the red blood cells and livers of experimentally-infected guinea pigs fell below those for the control animals (Pätiälä, '54). When nicotinic acid amide and

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[1] Reviews include Perla and Marmorston ('37); Sweany et al. ('41); Shaw et al. ('50).

Coenzyme I were administered, orally, to infected mice, the spread of tuberculosis was suppressed; this effect was more marked when the treatment with niacin derivatives was instituted prior to the inoculation of the mice (McKenzie et al., '48; Fitzpatrick, '55).

The pathological characteristics of the general hosts, man, cattle, birds, and voles to the types of tubercle bacilli, human, bovine, avian, and murine, respectively, differ; the various strains can produce disease in the other hosts (Soltys et al., '52). The locations and nature of primary lesions in the hosts may be dissimilar. In contrast to the pulmonary type of tuberculosis common in man and the rabbit, the lungs of guinea pigs were affected only in more advanced and nonspecific tuberculosis, while the spleen, liver, and lymphatic system demonstrated extensive necrosis; these symptoms were found also in rats (Willis, '25; Krause, '26; Wessels, '41; Lack, '56). Tuberculosis is a chronic disease with manifestations arising from areas involved in the infectious process, i.e., those which have tubercles, as well as from those without lesions. Chaudhuri and Martin ('54) measured the activity of succinic dehydrogenase in the tissues of guinea pigs which had been infected with virulent and nonvirulent strains of M. tuberculosis. Whether or not morphological

lesions were present in the tissues of the organs studied, the catalytic ability of the enzyme was altered in the spleen, kidney, and the liver in relation to that for the control animals. In man, hepatic functions were altered in pulmonary tuberculosis (Steidl and Heise, '33; Hurst et al., '47). Gillman and Gilbert ('54) reported that, among Africans, tuberculosis contributed to widespread destruction of hepatic tissue even when the extent of infection of the liver was minimal. Ban ('55) found liver disease to be a prominent factor in 60 patients with active pulmonary tuberculosis. Liver biopsies revealed a generalized necrotic state of the organs; in some cases, the tissue was cirrhotic and particularly tended towards fatty degeneration. Proliferation of cells was noted also.

Sarin et al. ('57) presented data on 100 tuberculous patients; pulmonary tuberculosis was diagnosed in 91 of these cases. Of the patients who had pulmonary tuberculosis, 55 showed abdominal symptoms as anorexia, constipation, and diarrhea; 33 had enlarged livers. Derangement of liver function was found in 31 of the 100 cases of tuberculosis, and there appeared to be some correlation between hepatomegaly and malfunction of the liver. Fatty infiltration accompanied the cirrhotic changes which occurred in the livers of 27 patients. The appearance

of fatty livers was attributed to the toxicity of the tuberculous infection, associated with malnutrition and metabolic disturbances.

A preliminary experiment was designed in this laboratory to investigate the influence which protein consumption may have upon enzyme activity in the livers of rats. This was followed by a study of tuberculous and non-tuberculous guinea pigs which consumed purified diets differing in casein content. The amounts of fat, nitrogen, sulfur, and the activity of glutathione reductase in the livers of the guinea pigs were determined. The concentration of riboflavin, as flavin adenine dinucleotide and flavin mononucleotide plus free riboflavin was measured also.

PRELIMINARY EXPERIMENT

Influence of Dietary Protein upon the Concentrations of Riboflavin-containing Coenzymes in the Livers of Rats

The riboflavin content of the liver appears to depend upon both dietary protein and dietary riboflavin. Sarett and Perlzweig (loc. cit.) reported that rats fed a diet higher in protein retained more riboflavin in the livers than did those which received lesser amounts of protein. Bessey et al. (loc. cit.) demonstrated that the tissue concentrations of flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and free riboflavin decreased when the amounts of riboflavin in the diet were low. In this laboratory, the concentrations of FAD and FMN plus free riboflavin were determined in the livers of rats fed diets containing different percentages of protein.

Thirty male, albino rats[1] were distributed among three groups: Group I was given an eight per cent casein diet; Group II, the controls, received a diet containing

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[1] Sprague-Dawley, Madison, Wisconsin. Shipping weight, 100 grams.

20 per cent casein; the pair-fed animals, Group III, received the same diet as animals in Group II, but the amounts were restricted to the intakes of the respective pair-mate animals in Group I. The basic diet was a characteristic, semi-synthetic ration for rats; cerelose was employed as a caloric equivalent to casein in the low protein diet. The diets supplied similar percentages of riboflavin. At the end of the 35-day experimental period, the animals were sacrificed. A modification of the fluorometric procedure of Bessey et al. (loc. cit.) was used for the determination of riboflavin-containing coenzymes in the livers.

The data for one animal are unavailable because of a laboratory accident. The mean values for the nucleotides of riboflavin in the livers of rats are given in Table 2 and the individual data are presented in Appendix Table 1. The average amount of riboflavin, both as FAD and FMN plus free riboflavin, which was stored in the livers of the animals that received eight per cent casein, was significantly less than the quantity found in the livers of either group of rats fed the higher protein ration (t-test; $P \leq 0.01$). There were no significant differences between the mean values for the riboflavin

Table 2

Mean values for the nucleotides of riboflavin in the livers of rats fed eight per cent and 20 per cent protein rations

Group	Dietary casein	Animals	FAD	FMN plus free riboflavin	Total riboflavin
	%	no.	ug./g.	ug./g.	ug./g.
I Low protein	8	9	17.6 (13.0-23.4)	3.5 (2.5-4.7)	21.1
II Control	20	10	27.0 (21.3-31.8)	7.3 (5.3-10.8)	34.3
III Control: pair-fed to low protein	20	10	31.9 (26.1-37.2)	6.5 (4.5-9.7)	37.4

fractions in the livers of the two groups of animals that received the 20 per cent casein diet.

When the low protein ration was fed, the mean values in micrograms per gram of liver were 17.6 and 3.5 for FAD and FMN plus free riboflavin, respectively. The mean values for FAD and FMN plus free riboflavin in micrograms per gram of liver were 27.0 and 7.3, respectively, for the controls (Group II), and 31.9 and 6.5, respectively, for the animals in Group III which were pair-fed to the low protein rats. The animals in Group III, consuming ration in amounts equal to those of the pair-mate rats in Group I, received 245 grams of the 20 per cent casein diet per animal over the 35-day period; the corresponding controls consumed 400 grams of the 20 per cent casein ration per animal during the same length of time.

The data agree with those presented by Sarett and Perlzweig (loc. cit.); the livers of their rats fed high protein-high vitamin diets retained more riboflavin than did those of animals which consumed low protein-high vitamin rations. In the present study, when the protein content of the diet was decreased from 20 to eight per cent, the concentration of riboflavin in the livers of the rats diminished. The livers of the animals which received the

20 per cent casein ration in amounts similar to those given to the low protein rats contained more riboflavin than did the livers of the animals consuming eight per cent casein, even though dietary riboflavin was the same. The FAD and FMN plus free riboflavin appeared to decrease in proportion to the total riboflavin.

It appeared of interest to investigate possible changes in riboflavin fractions in the livers of tuberculous guinea pigs when the protein in the purified ration was decreased from 35 to 25 per cent.

PROCEDURE

General Experimental Plan

The original experimental plan included 72 young adult guinea pigs,[1] males, to be distributed equally among 12 study groups. Diets supplying two different quantities of protein were to be fed during two experimental periods to controls, to tuberculous animals, and to non-tuberculous guinea pigs, pair-fed with the infected animals. Because of the limitations in handling large numbers of animals, the guinea pigs were managed in three sets of 24 each, over a seven-month period. After the completion of the first phase of the study, it became apparent that the tuberculous animals consumed more feed, consistently, than did the non-tuberculous guinea pigs which were allowed the same amounts of rations as their pair-mates; thus, the non-tuberculous animals, pair-fed with the tuberculous guinea pigs, did not differ appreciably in food consumption from the control animals. No pair-fed animals were included in the second and the third sets; there were 40

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[1] Rockland Farms, New York. Shipping weight, 250 grams.

control animals and 32 tuberculous guinea pigs in the final experimental design, as shown in Table 3.

A 12-day adaptation period preceded the experimental periods. The guinea pigs were fed ground rabbit pellets[1] and cabbage for two days; distilled water was given ad libitum throughout the study. In order to accustom the animals to the consumption of a purified diet, a mixture of ground rabbit pellets and the 35 per cent protein ration which was used during the experimental periods was fed for ten days. By gradually increasing the percentage of the purified diet in the mixture and correspondingly decreasing that of the pellet derivative and the amount of cabbage, the guinea pigs appeared to adjust to the consumption of the purified diet, alone, by the end of the adaptation period.

The animals were then distributed so that the average weight of each group approximated the others as nearly as possible. On the morning of the first experimental day, the animals which were to be in the tuberculous group were transferred to a special laboratory and injected between the dermal and the peritoneal layers of the right inguinal

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[1] Millett Elevator & Coal Company, Millett, Michigan.

Table 3

Experimental Design

Group	Animals
	no.
Control	
35 per cent protein	
40 days	10
47 days	10
25 per cent protein[1]	
28 days	10
35 days	10
Tuberculous	
35 per cent protein	
40 days	8
47 days	8
25 per cent protein[1]	
28 days	8
35 days	8

[1] The experimental periods were either 40 or 47 days in length; the animals consumed the ration containing 35 per cent protein for 12 days and received the 25 per cent protein diet for the last 28 and 35 days, respectively.

region with 0.1 milligrams of tubercle bacillus H37Rv, mammalian strain, prepared in 0.85 per cent saline. The Michigan Department of Health donated the culture from which a transfer was made to modified Proskauer-Beck medium for propagation of the organism (Soltys et al., loc. cit.). The remaining animals served as controls.

After 12 days of the experiment had elapsed, one-half of the tuberculous animals and one-half of the control guinea pigs were fed a 25 per cent protein diet, while the remaining animals continued to consume the 35 per cent protein ration. When 28 additional days had passed, one-half of the number of animals within each of these four groups was sacrificed, thus completing a 40-day experimental period. The other guinea pigs were continued for seven days and were sacrificed at the end of a 47-day experimental period.

Care of Animals

The tuberculous animals were housed in an air-conditioned, windowless room; the control animals were kept in a separate laboratory which had windows on two sides. The temperatures in these rooms at several hours were recorded for a one-week period; these are given in Appendix Table ii.

The animals were maintained individually in wire cages. An eight-ounce water bottle with a glass tip extending inward through the mesh was attached to the outside of each cage and a wide-mouthed feeding dish to the inside. Clean bottles were provided every other day and were rinsed well with distilled water on the alternate days; the glass tips were washed daily when a fresh supply of distilled water was given. The cages and collection trays were replaced at least once a week, the feed dishes as often as necessary. All equipment was washed in hot, disinfectant[1] water and rinsed well with distilled water.

Diets

The purified diets for the guinea pigs in this study were modifications of those used by Reid and Briggs ('53) and Heinicke et al. ('55). The 25 per cent and the 35 per cent protein diets, fed ad libitum, differed only in the amounts of casein and cerelose in the mixture. Table 4 and Table 5 present the composition of the diets.

The amounts of ration consumed daily by each animal were recorded throughout the experiment. The guinea pigs were weighed twice weekly.

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[1] Phenol Coefficient 25.

Table 4

Composition of the guinea pig diets

Nutrient	35 per cent casein diet		25 per cent casein diet	
	g./kg.	%	g./kg.	%
Vitamin-free casein[1]	350.0	35.00	250.0	25.00
Roughage (Alphacel)[1]	150.0	15.00	150.0	15.00
Corn oil	40.0	4.00	40.0	4.00
Cerelose	381.5	38.15	481.5	48.15
Potassium acetate	25.0	2.50	25.0	2.50
Magnesium oxide	5.0	0.50	5.0	0.50
Salts (P-H)[1]	40.0	4.00	40.0	4.00
Vitamin preparation	8.5	0.85	8.5	0.85

[1] Nutritional Biochemical Corporation, Cleveland.

Table 5

Vitamin[1] preparation for the guinea pig diets

Nutrient	Amount in ration
	mg./kg.
Choline chloride	3000.00
Ascorbic acid	3000.00
Inositol	2000.00
p-aminobenzoic acid	100.00
Thiamine hydrochloride	25.00
Riboflavin	30.00
Pyridoxine hydrochloride	25.00
Calcium pantothenate	80.00
Nicotinic acid	200.00
Biotin	1.00
Pteroylglutamic acid	10.00
Vitamin B_{12}	0.04
Alpha-tocopherol acetate	20.00
Menadione	2.00

[1] Vitamin A and Vitamin D were supplied weekly from Upjohn Super D Concentrate in 2000 and 200 unit amounts, respectively.

Sacrifice of the Animals and Tissue Preparation

The animals were sacrificed by a heart injection of chloroform on the morning following the last experiment day. As rapidly as possible, the livers were removed from the guinea pigs, blotted gently with filter paper to rid the tissue of superfluous blood, and weighed. During the autopsies of the tuberculous guinea pigs, smears were secured from organ and/or lymph node material and stained[1] to permit examination for infiltration by tubercle bacilli.

Each liver was homogenized with distilled water of a known volume and temperature; the weight of the water used was between one and two times that of the liver. For this procedure, a micro-Waring blendor was employed (Comar et al., '44; Benne, '55); it was regulated by means of a voltage controller[2] set at 60. After the homogenate was sampled for immediate determination of glutathione reductase activity and per cent solids, the remaining material was transferred into a small, brown- or green-glass container, the opening of which was subsequently

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[1] Acid-fast staining by the modified Ziehl-Neelsen method (Conn, '27).

[2] This voltage controller operates on a 115-volt input. Fisher Scientific Company, Pittsburgh.

sealed with parafilm and tightly capped. This homogenate was frozen for use later in the following analyses: total sulfur, nitrogen, and riboflavin-containing coenzyme groups.

Analytical Methods

Glutathione reductase activity

A modification of the methods employed by Racker ('49, '50, and '55) to concentrate glutathione reductase from biological materials was used for the preparation of the enzyme from guinea pig liver. The procedure was standardized to permit the calculation of relative enzymatic activity in the livers.

Glutathione reductase was determined as the rate of decrease of the optical density of a test system containing the extract, oxidized glutathione, reduced triphosphopyridine nucleotide,[1] and buffers. The test system of 1.7 milliliters was prepared in 0.5-centimeter silica cells from reagents which were previously warmed to 37.5° C. Optical density was followed at 340 millimicrons in a Beckman DU spectrophotometer[2] using a tungsten light source.

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[1] Acknowledgment is made to Sigma Chemical Company, St. Louis, for supplying reduced triphosphopyridine nucleotide at a discount.

[2] National Technical Laboratories, South Pasadena.

Satisfactory proportionality was demonstrated between enzyme concentration and rate of oxidation of reduced triphosphopyridine nucleotide in this type of test system by Racker ('55).

The values used for expressing glutathione reductase activity were calculated by means of the following equation:

$$R = \frac{\Delta O.D.}{s} \times \frac{1}{\frac{3/25\ E}{\%\ solids\ (w)}}$$

R is the rate for the enzyme in one gram of liver solids.

ΔO.D. is the change in optical density.

s is the number of 15-second intervals which elapsed during the change in optical density.

E is the amount of liver extract expressed in milliliters in the test system.

w is the grams of wet liver in the aliquot of original homogenate.

The mathematical factor, 3/25, arises from the dilution procedure employed during the extraction.

Per cent solids

Aliquots of the fresh liver homogenate were dried for 24 hours in an oven regulated to maintain 100° C. Air was circulated through the oven by a motor-driven fan.

Per cent fat

The aliquot of homogenate used for the determination of the per cent solids also served as the sample for the per cent fat. Purified ether was employed for the extraction process in the Goldfisch fat apparatus.[1]

Total sulfur

The principal steps involved in the determination of total sulfur are two: (1) the conversion of all sulfur derivatives to sulfate; (2) the precipitation of barium sulfate. In this laboratory, the liver sulfur derivatives were oxidized in the Parr oxycalorimeter;[2] a turbidimetric method, patterned after the suggestions of Treon and Crutchfield ('42) for the quantitative measurement of sulfates, was used to complete the analysis.

An aliquot of the thawed homogenate was weighed into a calorimeter capsule and dried. The material was ignited and the washings transferred to a beaker to be treated with bromine water. The excess bromine was evaporated and the neutralized solution was made to a volume of 250 milliliters. Barium chloride was added to the samples and the

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[1] Laboratory Construction Company, Kansas City, Missouri.

[2] Parr Instrument Company, Inc., Moline, Illinois.

turbidity was expressed as optical density units in the Beckman DU spectrophotometer[1] at 540 millimicrons. The amount of sulfur present in the sample solution was obtained as sulfate directly from the standard sodium sulfate curve; sulfur represents one-third of the sulfate value.

Nitrogen

The boric acid modification (Scales and Harrison, '20) of the Kjeldahl method was employed to measure the nitrogen content of the livers of the guinea pigs.

Riboflavin-containing coenzymes

The amounts of flavin adenine dinucleotide and those of flavin mononucleotide plus free riboflavin in the livers of the guinea pigs were determined by a modification of the fluorometric procedure proposed by Bessey *et al.* (*loc. cit.*). The protein material in the livers was precipitated by trichloroacetic acid and removed by cold filtration. A Coleman 12A photofluorometer[2] was used to measure the fluorescence of the deproteinized liver sample against an internal standard.

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[1] National Technical Laboratories, South Pasadena.

[2] Coleman Electric Company, Inc., Maywood, Illinois.

RESULTS AND DISCUSSION

Statistical Treatment of Results

The statistical procedure used most frequently to aid in the interpretation of the results was analysis of variance. The experimental variables were: disease treatment; dietary protein; and length of experimental period.

In the analysis, the disease treatment segregated the tuberculous guinea pigs from the non-tuberculous animals which were designated as the controls. The rations described by the term dietary protein were composed of purified materials containing either 35 or 25 per cent casein. The lengths of experimental periods were 40 and 47 days, since approximately six weeks are allowed to elapse for the development of tuberculous infection in guinea pigs used in diagnostic work. As was mentioned previously, the animals placed in the low protein group received the 35 per cent protein diet for the first 12 experimental days, and the 25 per cent protein ration for the last 28 and 35 days of the 40- and 47-day periods,

respectively. This plan was employed because it seemed doubtful that tuberculous guinea pigs could survive the experimental periods if fed only the low protein diet throughout the experiment.

Five of the original 72 animals which began the experimental periods failed to survive for the required lengths of time; Table 6 presents the numbers of guinea pigs within each group for which data are available. The control animal, for which data are missing, died 10 days after the beginning of the experimental period; the cause of death appeared to be starvation. Autopsies of the four tuberculous animals which survived approximately three weeks of the experiment showed that severe gastro-intestinal disturbances had occurred; it is not known whether failure to utilize the purified rations or the presence of disease was responsible for the abnormality. In order to avoid non-orthogonality in the statistical analyses, the averages of values obtained from determinations made for the surviving animals within each group were used for the unknown values.

Since separate housing units were employed for the control and for the tuberculous animals, the possibility that confounding may have been introduced into the study

Table 6

Tuberculous and non-tuberculous animals which survived the experimental periods on different protein intakes

Group	Animals planned for group	Animals surviving in group
	no.	no.
Control		
35 per cent protein		
40 days	10	10
47 days	10	10
25 per cent protein[1]		
28 days	10	9
35 days	10	10
Tuberculous		
35 per cent protein		
40 days	8	8
47 days	8	5
25 per cent protein[1]		
28 days	8	7
35 days	8	8

[1] All animals received the 35 per cent protein diet during the first 12 experimental days; the 25 per cent protein ration was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

was explored. Two preliminary statistical analyses were completed, one from the data for the guinea pigs which received the 35 per cent protein diet and another from the results for those consuming the lower protein ration; the form for the analysis of variance is shown in Table 7. The variability among the animals was estimated by the error mean square. The results indicated that the guinea pigs belonged to the same statistical population; that is, no significant effects due to housing facilities had been superimposed upon experimental values.

Another analysis of variance demonstrated that the subdivision of the animals into three sets of 24 each for study over a seven-month period caused no significant differences among the data for the guinea pigs.

It is doubtful, therefore, that the separation of the diseased animals from the controls and the distribution of the groups for study over seven months have affected the results beyond that which is usually attributed to experimental error. That the experiment may be considered as a statistical unit has been justified; the final form for the analysis of variance employed in the interpretation of the results is given in Table 8.

Table 7

Form for the analysis of variance employed in the evaluation of the effects of separated housing units upon the experimental results

25 per cent protein diet		35 per cent protein diet	
	df		df
Total	35	Total	35
Disease treatment	1	Disease treatment	1
Length of period	1	Length of period	1
Interaction	1	Interaction	1
Error	32	Error	32

Table 8

Final form for the analysis of variance employed in evaluating the experimental results

	df
Total	71
Disease treatment (T)	1
Dietary protein (P)	1
Length of period (D)	1
Interaction TP	1
Interaction TD	1
Interaction PD	1
Interaction TPD	1
Error	64

Observations at Autopsy

The guinea pigs which had been inoculated with M. tuberculosis H37Rv showed definite involvement of miliary tuberculosis: the enlarged lymph nodes contained caseous material; the spleens had amassed tubercles and almost had doubled in size; the livers gave evidence of infiltration by the bacilli and of enlargement. Slides made from the lymph node and/or splenic tissue of the tuberculous animals demonstrated the presence of red rods, identified as tubercle bacilli.

The disease was more widespread among the tuberculous animals surviving through the 47-day period than among the 40-day guinea pigs. Also, the extent of tuberculous lesions was greater among the animals that received the 25 per cent protein diet than among those which consumed the 35 per cent protein ration. For the guinea pigs receiving low dietary protein, symptoms of the infection frequently included fluid accumulation in the intestinal region; in some cases, far advanced tuberculosis was indicated by lung involvement. On the other hand, no distinct differences were observed for the control animals between the 25 and 35 per cent protein intakes or between the two

experimental periods; all of the control animals appeared healthy at autopsy.

Weight Gain and Food Intake

The mean gains in body weight and average food intakes of both the control and tuberculous animals are presented in Table 9; values for individual animals are presented in Appendix Table iii. Data for the guinea pigs which consumed the 25 per cent protein ration during 28 and 35 days of the 40- and 47-day periods, respectively, include the values from the first 12 experimental days on the 35 per cent protein diet.

The average gain in weight of the animals which were given the 35 per cent protein ration was greater than that of the guinea pigs which received the 25 per cent protein diet. This was true of the tuberculous animals as well as the controls. The differences were found to be statistically significant by analysis of variance ($P \leq 0.01$).

The tuberculous animals which received the 35 per cent protein diet for 40 and 47 days had average weight gains of 166 ± 45[1] and 172 ± 138 grams, respectively. These weight

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[1] Numbers following mean values represent standard deviations.

Table 9

Average weight gain and food consumption for tuberculous and non-tuberculous guinea pigs on different protein intakes

Group	Animals[1]	Weight gain	Food intake	Weight gain per food intake
	no.	g.[2]/animal	g.[2]/animal	g.[2]/100 g.
Control				
35 per cent protein				
40 days	10	160±61	835±63	19±7
47 days	10	181±46	1000±79	18±4
25 per cent protein[3]				
28 days	10	126±51	706±105	18±7
35 days	10	137±56	903±166	15±4
Tuberculous				
35 per cent protein				
40 days	8	166±45	1214±169	14±4
47 days	8	172±38	1342± 93	13±3
25 per cent protein[3]				
28 days	8	104+47	881±231	12±3
35 days	8	112±35	1158±243	10±4

[1] Calculated for the surviving 67 animals.

[2] Numbers following mean values represent standard deviations.

[3] All animals received the 35 per cent protein ration during the first 12 experimental days; the 25 per cent protein ration was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

gains appeared to be similar to those of control animals which received the same diet. The consumption of the lower protein ration by the diseased animals resulted in smaller mean gains in weight than were observed for the respective controls; however, no real differences existed.

The tuberculous animals which received the 35 per cent protein ration during the experimental periods consumed the largest amount of food. The control guinea pigs fed the lower protein diet after the first 12 experimental days consumed the smallest quantity of food of any of the groups of animals. Analysis of variance indicated that both the presence of infection and the consumption of the higher protein ration resulted in higher values for total food intake ($P \leq 0.01$).

The gain in weight per 100 grams of food consumed by each of the guinea pigs was calculated as an index of food utilization; the mean values are given in Table 9. Tuberculous animals which received the 35 per cent protein diet for 47 days gained 172 ± 38 grams while consuming 1342 ± 93 grams of feed; this represented a mean weight gain of 13 grams per 100 grams of food. When the control animals received the same dietary treatment during the corresponding period of time, the average weight gain was 181 ± 46 grams,

and the mean food intake, 1000 ± 79 grams; the calculated index of food utilization was 18 grams of weight gained per 100 grams of food consumed. The mean values thus determined as indices of food utilization indicated that the tuberculous animals required more food than did the controls to gain weight at a similar rate. The consumption of the 25 per cent protein diet appeared to result in slightly smaller gains in weight per 100 grams of food during the respective experimental period than did the 35 per cent protein ration.

The studentized range was used to test whether these differences were significant; the results of the analysis are shown in Figure 1. Each group of animals is represented by a vertical line. The tip of the arrow head represents the average utilization value calculated from the grams of weight gained per 100 grams of food consumed by the animal. The cross-bar indicates the adjusted values obtained by subtraction of the correction factor derived from the standard error of the mean and the studentized range table at the five per cent level. When the bar of one group shows a value equal to or greater than that corresponding to the arrow tip of another group, the differences between the means of the two groups are significant.

Figure 1

Figure 1

Comparison of the weight gains of guinea pigs per 100 grams of food consumed, by means of the studentized range

35-C40; 35 per cent protein, control, 40-day

35-C47; 35 per cent protein, control, 47-day

35-TB40; 35 per cent protein, tuberculous, 40-day

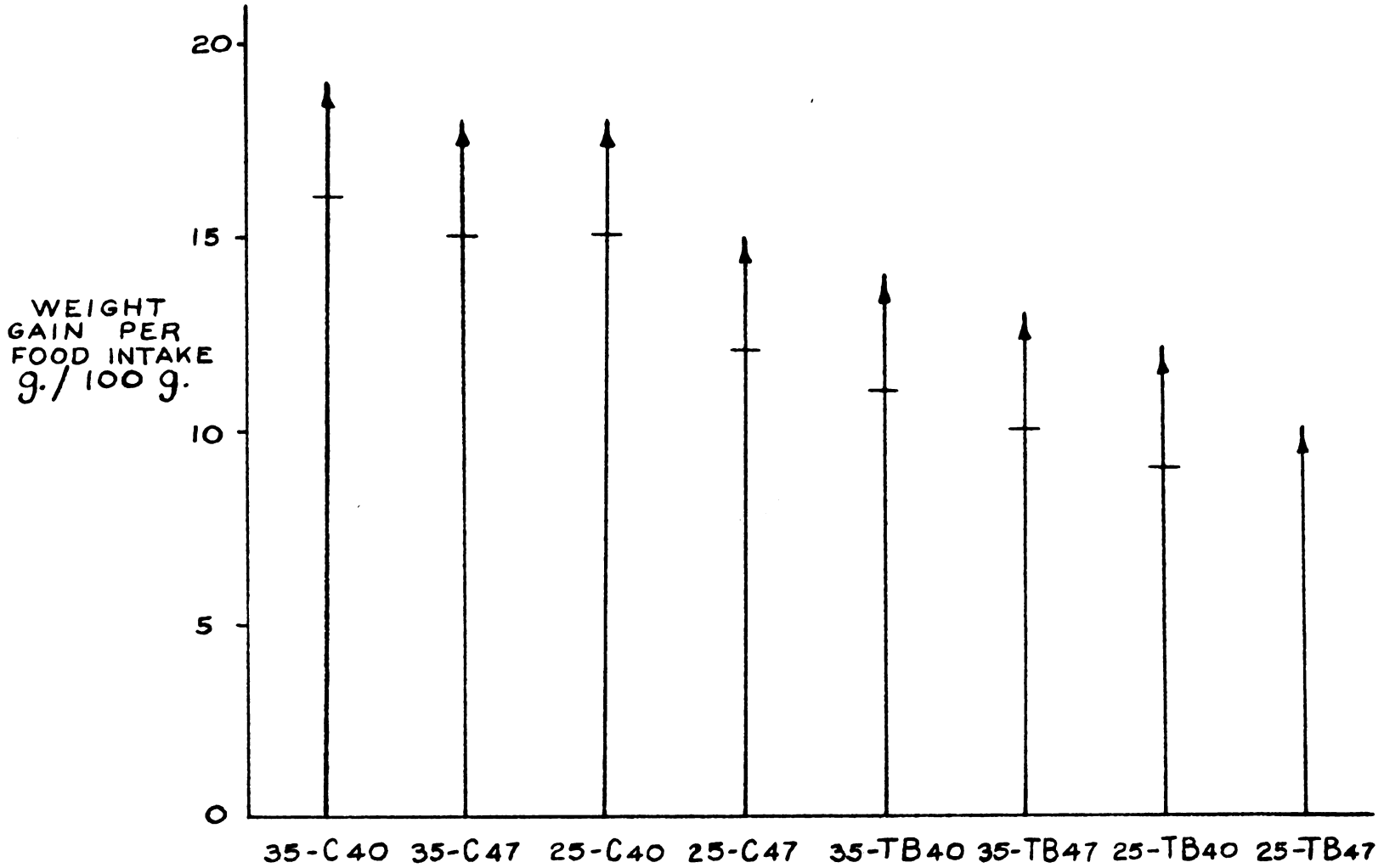
35-TB47; 35 per cent protein, tuberculous, 47-day

25-C40; 25 per cent protein, control, 40-day

25-C47; 25 per cent protein, control, 47-day

25-TB40; 25 per cent protein, tuberculous, 40-day

25-TB47; 25 per cent protein, tuberculous, 47-day


WEIGHT GAIN PER FOOD INTAKE g./100 g.
20
15
10
5
0
35-C40
35-C47
25-C40
25-C47
35-TB40
35-TB47
25-TB40
25-TB47

The performance of the control animals was found to be superior to that of the comparable groups of tuberculous guinea pigs which received the same dietary treatment throughout a similar length of time. There were significant differences between the weight gains per unit of food intake for the animals given the 35 per cent protein ration and those which received the diet supplying 25 per cent protein when the experimental period was continued for 47 days following the inoculation of the test animals. However, this was not true for the corresponding groups on experiment for 40 days.

The group of animals with the poorest weight gain per 100 grams of food consumed was the tuberculous animals which were fed the 25 per cent protein ration for the last 35 days of the 47-day experimental period. Although the mean gain in weight per 100 grams of food intake for these animals was not significantly different from that for diseased guinea pigs receiving the same ration for 28 of 40 days, the value was significantly less than for all other groups, either experimental or control.

Symptoms of anorexia, which may accompany tuberculosis in human beings, were not observed in the tuberculous guinea pigs. Both the presence of tuberculosis and the

restriction of dietary protein apparently impaired the utilization of food for weight gain. The effect of these treatments appeared to be more pronounced over a longer period of time.

Liver Fat and Solids

Fat

An examination of the mean values for fat in the livers of the guinea pigs indicated that the protein content of the diet had a greater influence upon the amount of fat which was deposited than did either the disease treatment or the length of the experimental period. These data are given in Table 10 and the individual values in Appendix Table iv. The control animals which were given the 35 per cent protein diet for 40 days were found to have a mean value of 0.46 ± 0.09 grams of fat in their livers; the livers of the respective tuberculous animals contained 0.52 ± 0.10 grams of fat. When the 25 per cent protein ration was fed to tuberculous animals during the last 28 days of the 40-day period, the amount of fat accumulated in the livers averaged 1.05 ± 0.36 grams. An analysis of variance indicated that no significant differences existed between the mean values.

Table 10

Mean weights and percentages of fat in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

Group	Animals[1]	Fat	
		Weight	Per cent of liver weight
	no.	g.[2]	
Control			
35 per cent protein			
40 days	10	0.46±0.09	2.4
47 days	10	0.65±0.24	3.3
25 per cent protein[3]			
28 days	10	0.71±0.40	4.1
35 days	10	0.81±0.25	4.4
Tuberculous			
35 per cent protein			
40 days	8	0.52±0.10	2.0
47 days	8	0.77±0.19	3.2
25 per cent protein[3]			
28 days	8	1.05±0.36	4.8
35 days	8	0.69±0.19	3.5

[1] Calculated for the remaining 67 animals.

[2] Numbers following mean values represent standard deviations.

[3] All animals received the 35 per cent protein ration during the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

The mean values for the percentages of fat in the livers were larger for the four groups of animals that received the lower protein diet than for the guinea pigs which consumed the 35 per cent protein ration. Among the groups of animals to which the 35 per cent protein diet was fed, the livers of the controls on experiment for 47 days contained the greatest percentage of fat, i.e., 3.3; for the groups which received the diet supplying 25 per cent protein, the mean percentages for fat accumulation in the livers ranged from 3.5 to 4.8. These differences, however, were not significant.

Harper et al. ('55) considered a fat deposit between 2.9 and 4.5 per cent of the total weight of the liver of albino rats to be adequate and not excessive. Although the consumption of the 25 per cent protein ration under the conditions of the current study may have caused a slightly greater retention of fat per unit of liver tissue than did the 35 per cent protein ration, it does not appear that the diminution of lipotropic factors was sufficient to result in fatty livers.

Solids

Table 11 and Appendix Table v include the mean values and individual data, respectively, for dry matter in the livers of the guinea pigs. The total solid material determined in the livers of tuberculous animals which consumed the 35 per cent protein diet for 40 days averaged 6.6±0.6 grams, representing 25.2 per cent of the weight of the organ. The livers of the corresponding control guinea pigs contained 5.2±0.8 grams of solids, which was 26.5 per cent of the organ weight. The mean weight of dry matter in the livers of the control guinea pigs that received the 25 per cent protein diet for 28 of 40 days was 4.5±1.1 grams; this value was 24.9 per cent of the weight of the liver.

It was found by analysis of variance that there was a significantly greater amount of solid material deposited in the livers of the tuberculous animals than in those of the controls ($P \leq 0.01$); however, the percentage deposition was greater in the livers of the control animals than in those of the tuberculous guinea pigs ($P \leq 0.01$). The total amount of dry material found in the livers of the animals was higher also when the ration supplied 35 per cent protein than when the 25 per cent

Table 11

Mean weights of solids and percentages of solids, fat-free solids, and water in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

Group	Animals[1]	Solids			Water,[3] per cent of liver weight
		Weight	Per cent of liver weight	Fat-free[2]	
	no.	g.[4]		%	
Control					
35 per cent protein					
40 days	10	5.2±0.8	26.5	24.1	73.5
47 days	10	5.1±0.8	25.4	22.1	74.6
25 per cent protein[5]					
28 days	10	4.5±1.1	24.9	20.8	75.1
35 days	10	5.1±0.7	27.3	22.9	72.7
Tuberculous					
35 per cent protein					
40 days	8	6.6±0.6	25.2	23.2	74.8
47 days	8	5.7±0.8	24.6	21.4	75.4
25 per cent protein[5]					
28 days	8	5.4±0.9	24.1	19.3	75.9
35 days	8	5.0±0.9	25.0	21.5	75.0

[1] Calculated for the surviving animals.
[2] Calculated: per cent solids - per cent fat.
[3] Calculated: 100 - per cent solids.
[4] Numbers following mean values represent standard deviations.
[5] All animals received the 35 per cent protein diet during the first 12 experimental days; the 25 per cent protein ration was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

protein ration was fed; the differences between the means were found to be significant by analysis of variance ($P \leq 0.01$). The differences between the mean percentages of solids accumulated in the livers of the animals consuming either ration were not significant.

Since the weight of the solids in the liver probably depends to some degree upon the total weight of the liver, the comparisons of the mean percentages of solid material seem to be more indicative of the effects of the experimental factors upon the livers of the animals than do average weights of dry matter. The percentage of dry matter was less in each tuberculous group of animals than in the corresponding control animals. This was true also of the percentages of fat-free solids. By calculation, then, the percentages of water in the livers of the control guinea pigs were less than those in the livers of the respective tuberculous animals.

These results suggest that a dilution effect may have occurred in the liver during tuberculosis. It is possible that tuberculosis interferes with the structure within liver cells and thus may cause a decrease in the effective function of the organ.

Liver Weight and Body Weight

The mean weights of the animals and of the livers are given in Table 12; Appendix Table vi contains the individual data. The mean weights of the groups of guinea pigs that received the ration supplying 35 per cent protein were approximately the same; however, the mean weights of the livers of the tuberculous animals exceeded those for the respective controls. When the 25 per cent protein diet was given to control animals for the last 28 days of the 40-day period, the mean weight of the livers was found to be 17.9 grams while that of the livers of the tuberculous guinea pigs was 22.3 grams; the mean body weight for the controls was approximately 25 grams greater than that for the tuberculous animals. The livers of the tuberculous animals receiving the lower protein ration during the last 35 days of the 47-day period had a mean weight of 20.1 grams; the body weights averaged 405 grams.

These data and the gross examinations of the animals upon sacrifice suggested that the presence of the tuberculous infection caused enlargement of the liver. Since the individual body and liver weight values overlapped considerably, an attempt was made to evaluate the data statistically.

Table 12

Average body weights and liver weights for the guinea pigs

Group	Animals	Weight of liver	Weight of animal
	no.	g.	g.
Control			
35 per cent protein			
40 days	10	19.6 (16.0-26.5)	437 (356-512)
47 days	10	20.2 (14.2-25.2)	458 (376-550)
25 per cent protein[1]			
28 days	9	17.9 (9.3-22.8)	406 (267-510)
35 days	10	18.6 (15.4-21.8)	438 (334-516)
Tuberculous			
35 per cent protein			
40 days	8	26.2 (21.5-37.0)	447 (350-560)
47 days	5	23.5 (16.2-28.9)	436 (331-470)
25 per cent protein[1]			
28 days	7	22.3 (18.0-28.7)	380 (262-476)
35 days	8	20.1 (15.9-24.3)	405 (344-468)

[1] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

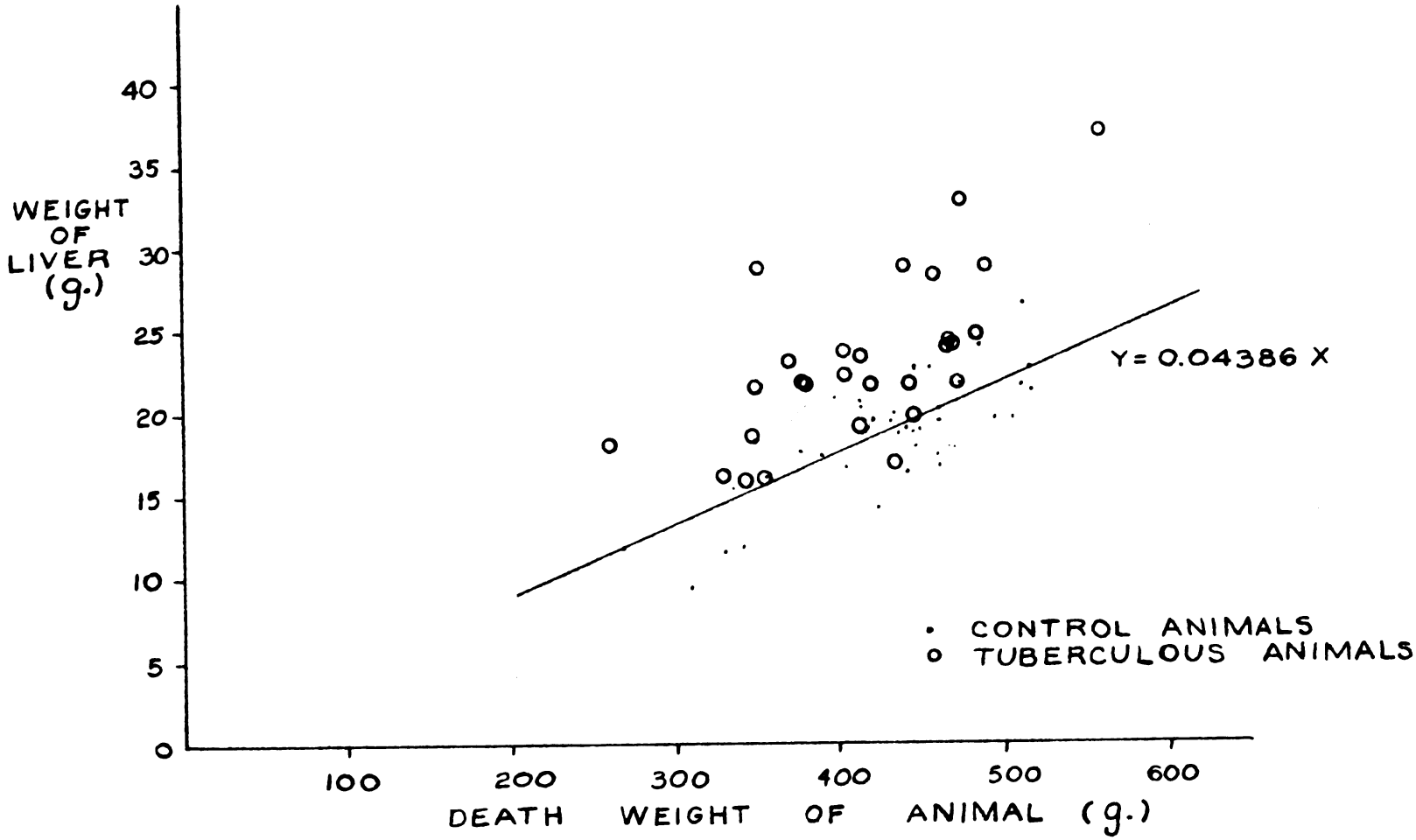
A predicting equation, $y = 0.04454\ x$, was calculated from the body and liver weight data of the control guinea pigs which were fed the 35 per cent protein ration; y represented the weight of the liver in grams and x the death weight of the animal. The theoretical weight of the liver of each animal was then calculated, according to the equation. The actual weights of the livers of the tuberculous animals on both protein rations differed significantly from the predicted weights (Chi Square; $P \leq 0.05$). The actual weights of the livers of the control guinea pigs which received the 25 per cent protein ration were approximately the same as those predicted on the basis of body weight.

Therefore, the liver and body weight data determined for both groups of control animals were used to calculate a second regression equation, $y = 0.04386\ x$, as shown in Figure 2. For the tuberculous guinea pigs, the actual weights of the livers deviated positively from those predicted by means of this regression equation, with one exception. It was thus established by a Chi Square test and a t-test ($P \leq 0.01$), that the livers of the tuberculous animals were enlarged with respect to body size, in comparison with the control animals.

Figure 2

Figure 2

Relation between liver weight and body weight of tuberculous and non-tuberculous animals


WEIGHT OF LIVER (g.)
40
35
30
25
20
15
10
5
0
100
200
300
400
500
600
DEATH WEIGHT OF ANIMAL (g.)
Y = 0.04386 X
• CONTROL ANIMALS
o TUBERCULOUS ANIMALS

The data in Table 12 show that the tuberculous animals receiving the 25 per cent protein ration weighed less than the corresponding controls, but the livers weighed more than those of the non-tuberculous guinea pigs. A failure to deposit body tissue or destruction of body tissue during tuberculosis may result in the appearance of heavier liver weights in relation to body weights. There appears to be no reason to doubt that hepatomegaly occurred during tuberculosis; however, the wasting process of the disease may also be a factor in determining how enlarged the liver seems to be with respect to body size.

Himsworth and Glynn ('45) studied dietary massive necrosis of the liver in rats. During acute necrosis, the livers were enlarged, and it was demonstrated that the injured organs were heavier in proportion to body weight than were those of the control animals. The investigators noted that the increase in size and weight of the affected livers was due to edema, which appeared to be confined to the liver. Furthermore, approximately the same amounts of fat and a lesser concentration of protein were demonstrated to be present in the necrotic livers with respect to those in the livers of the control animals.

It was shown in the present study that the percentages of water in the livers of the tuberculous animals were greater than those in the livers of the respective controls. A tendency for more of the solid material to be deposited as fat during the consumption of a lower protein ration was also indicated; however, the differences between the mean percentages of fat in the livers of the animals consuming the 35 per cent protein diet and those receiving the 25 per cent protein ration were not significant. Ban (loc. cit.) reported that certain tuberculous patients were found to exhibit necrosis with possible cirrhosis and fatty degeneration of the liver upon biopsy. György ('55) described many causative agents in necrosis, but explained that a decrease in the consumption of lipotropic factors may result in fatty livers and, in some cases, cirrhosis of the liver. Changes attributable to degenerative processes in the liver may occur suddenly, according to Himsworth ('47). Liver cells proliferate readily; it is possible for the parenchymal cells to regenerate following necrosis, but the capacity for this process is dependent upon the type and severity of the injury. Himsworth (loc. cit.) also explained that atrophy of the necrotic liver may succeed the stage characterized

by edema and may lead to postnecrotic cirrhosis. The data from the present study, shown in Table 12, indicate that the mean weights of the livers of the tuberculous animals on experiment for 47 days were less than those of the livers of the corresponding guinea pigs which were sacrificed upon the fortieth experimental day.

The lack of histological evidence precludes the distinction of the type of liver injury that apparently occurred during the progress of the tuberculous infection in the guinea pigs. The results indicate that, during tuberculosis, the infectious process may be primarily responsible for injury to the hepatic parenchyma; the livers of the tuberculous animals were possibly necrotic and edemic. An extension of the experimental period may have resulted in atrophy of these livers. There were no indications that fatty infiltration into the livers of the tuberculous animals was greater than that into the livers of the control animals receiving the same quantity of protein. Moreover, the data did not suggest that the amount of fat in the livers of the tuberculous animals increased when the length of the experimental period was increased from 40 to 47 days. However, the infiltration of fat into portal spaces may be more marked in tuberculosis when dietary protein is restricted.

Liver Nitrogen

The average values for the retention of nitrogen in the livers of the guinea pigs are given in Table 13; the individual data are presented in Appendix Table vii.

Analysis of variance showed that the tuberculous animals had significantly more nitrogen in their livers than did the respective controls ($P \leq 0.01$), and that the consumption of the 35 per cent protein ration resulted in a greater deposition of total nitrogen in the livers than did the lower protein diet ($P \leq 0.01$). The control animals which were fed 25 per cent protein for the last 28 days of the 40-day period had an average of 557 ± 126 milligrams of nitrogen in the livers; the highest mean was 752 ± 88 milligrams, for the tuberculous guinea pigs receiving 35 per cent protein for 40 days.

When the nitrogen values were expressed as milligrams per gram of wet tissue, the livers of the control animals contained significantly greater amounts of nitrogen than did those of the tuberculous guinea pigs ($P \leq 0.01$); per gram of wet tissue, dietary protein did not cause significant differences between mean nitrogen values for the livers. No significant differences were

Table 13

Mean weights of nitrogen in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

Group	Animals[1]	Nitrogen		
		Total	Per gram of wet tissue	Per gram of solids
	no.	mg.[2]	mg.[2]	mg.[2]
Control				
35 per cent protein				
40 days	10	617± 93	31.5±1.7	119±11
47 days	10	643±109	31.9±1.2	126±10
25 per cent protein[3]				
28 days	10	557±126	31.4±2.0	126±11
35 days	10	577± 87	31.0±3.8	115±17
Tuberculous				
35 per cent protein				
40 days	8	752± 88	28.9±1.8	115±10
47 days	8	742±136	31.5±1.1	128± 8
25 per cent protein[3]				
28 days	8	658± 89	29.6±2.8	124±11
35 days	8	618± 85	31.0±2.6	124±12

[1] Calculated for the surviving animals.

[2] Numbers following mean values represent standard deviations.

[3] All animals received the 35 per cent protein ration during the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

observed between mean values when liver nitrogen was expressed as milligrams per gram of solids.

That the analysis of variance showed that the tuberculous guinea pigs stored less nitrogen per gram of tissue than did the control animals may be attributable to the values obtained for the tuberculous animals on experiment for 40 days. These means were lower than those for the corresponding tuberculous guinea pigs on experiment for 47 days. When 35 per cent protein was given to tuberculous animals on experiment 40 and 47 days, the livers retained per gram of wet tissue 28.9±1.8 and 31.5±1.1 milligrams of nitrogen, respectively. The corresponding values for the tuberculous guinea pigs fed the 25 per cent protein ration were: 29.6±2.8 milligrams when the animals received the low protein diet for the last 28 days of the 40-day period; 31.0±2.6 milligrams, when the animals received the low protein diet for the last 35 days of the 47-day period. Himsworth and Glynn (loc. cit.) demonstrated that the concentrations of protein in the livers of rats fed necrogenic diets were less than those in the livers of the control animals; the animals that survived this acute dietary necrosis had livers which were chemically indistinguishable from those

of the unaffected animals. In the current experiment, the feeding of a 25 per cent protein diet for 28 and for 35 days apparently did not alter the liver nitrogen values per gram of solids or per gram of wet tissue with respect to the values for the corresponding animals which received the higher protein ration. Nitrogen appeared to comprise a similar fraction of the dry matter in the livers of the experimental animals, regardless of the imposed treatment.

The percentages of nitrogen deposited in the livers of the guinea pigs were not altered by the decrease in dietary protein from 35 to 25 per cent. The presence of tuberculosis appeared to have influenced the percentage composition of the wet liver tissue, which possibly was a reflection of the increased moisture content of the organ.

Liver Sulfur

As shown in Table 14, the lowest mean value for sulfur per gram of dry material in the livers was 6.5 ± 0.6 milligrams; this was determined for the tuberculous animals which received the 35 per cent protein ration for 40 days. The highest value, 7.5 milligrams for sulfur per gram of liver solids, was found for both the control and tuberculous animals consuming the 25 per cent protein diet during

Table 14

Average values for sulfur in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes; nitrogen-sulfur ratios

Group	Animals[1]	Sulfur per gram of solids	Total sulfur	Total nitrogen[2]	N/S ratio
	no.	mg.[3]	mg.[3]	mg.[3]	
Control					
35 per cent protein					
40 days	10	7.1 ± 0.3	37 ± 9	617 ± 93	16.7:1
47 days	10	7.3 ± 0.6	36 ± 5	643 ± 109	17.9:1
25 per cent protein[4]					
28 days	10	7.5 ± 1.0	34 ± 11	557 ± 126	16.4:1
35 days	10	6.7 ± 0.4	33 ± 6	577 ± 87	17.5:1
Tuberculous					
35 per cent protein					
40 days	8	6.5 ± 0.6	43 ± 10	752 ± 88	17.5:1
47 days	8	7.3 ± 0.9	43 ± 8	742 ± 136	17.2:1
25 per cent protein[4]					
28 days	8	7.5 ± 0.9	40 ± 7	658 ± 89	16.4:1
35 days	8	7.2 ± 0.8	36 ± 6	618 ± 85	17.2:1

[1] Calculated for the surviving animals.
[2] Data from Table 13.
[3] Numbers following mean values represent standard deviations.
[4] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

28 of 40 days; the standard deviations for this mean were 1.0 and 0.9, respectively. The range for the means was small and no group average varied from the over-all mean of 7.1 milligrams by more than nine per cent; no significant differences between means for sulfur per gram of liver solids were found. The individual data are given in Appendix Table viii.

The deposition of total sulfur in the livers of the guinea pigs was significantly higher when the percentage of protein in the ration was 35 than when 25 per cent protein was fed ($P \leq 0.05$); significantly larger amounts of total sulfur were determined in the livers of the guinea pigs which had been inoculated with tubercle bacilli than in the livers of the control animals ($P \leq 0.01$). The data in Table 14 show that when the values for the total nitrogen in the livers were high, values for total sulfur in the livers also were augmented. When the 35 per cent protein diet was fed to tuberculous animals for 40 days, the livers contained an average of 43 ± 10 milligrams of sulfur and 752 ± 88 milligrams of nitrogen; the values for the corresponding controls were 37 ± 9 and 617 ± 93 milligrams, respectively. The livers of the tuberculous guinea pigs which consumed the 25 per

cent protein ration for 28 days of the 40-day experimental period accumulated 40±7 milligrams of sulfur and 658±89 milligrams of nitrogen. The mean ratios of nitrogen to sulfur in the livers of the animals within the eight study groups ranged only from 16.4:1 to 17.9:1.

The ratio of nitrogen to sulfur in organic matter approximates 16:1. Folin ('05) found that the ratios of nitrogen to sulfur in the urine of thirty human subjects decreased slightly when the protein in the diet was decreased. In severe wasting illnesses, the nitrogen to sulfur ratio in urine may increase markedly, since the excretion of nitrogen increases with degeneration of tissues. It was found in this laboratory[1] that the urinary sulfur excreted by eight patients with terminal tuberculosis was not excessive when an excretion of one gram per day was considered as the norm.

The relationship between nitrogen and sulfur in the livers of the guinea pigs apparently was not disturbed during tuberculosis or during restricted protein intake.

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[1] Unpublished data, Department of Foods and Nutrition, Michigan State University.

Glutathione Reductase Activity in Liver

The values calculated for the glutathione reductase activity in the livers of the guinea pigs were expressed as rates[1] per gram of solids, per gram of fat-free solids, and per gram of nitrogen; the mean values for the eight study groups are given in Table 15. No significant differences were found among these means by statistical analysis; the individual results varied greatly as can be seen in Appendix Table ix.

The glutathione reductase activity in the livers of the tuberculous animals which received the 25 per cent protein diet for 35 days of the 47-day period, when expressed as the mean rate per gram of solids, was 0.82 ± 0.52; that of the respective control animals was 0.46 ± 0.17. For these two groups of guinea pigs, the average rates calculated per gram of fat-free solids and per gram of nitrogen were: tuberculous, 0.94 ± 0.58 and 6.5 ± 3.7, respectively; control, 0.55 ± 0.19 and 4.2 ± 1.8, respectively.

There appears to be no evidence that glutathione reductase activity decreased during restricted protein

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[1] The basic equation for calculating glutathione reductase activity is given on p. 33.

Table 15

Average rates for glutathione reductase activity[1] in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

Group	Animals[2]	Rate[3] per gram of		
		solids	fat-free solids	nitrogen
	no.			
Control				
35 per cent protein				
40 days	10	0.53±0.55	0.60±0.65	4.5±4.5
47 days	10	0.37±0.49	0.44±0.22	2.9±1.1
25 per cent protein[4]				
28 days	10	0.33±0.29	0.38±0.32	2.7±2.4
35 days	10	0.46±0.17	0.55±0.19	4.2±1.8
Tuberculous				
35 per cent protein				
40 days	8	0.46±0.27	0.51±0.30	4.1±2.3
47 days	8	0.52±0.62	0.60±0.27	4.1±1.8
25 per cent protein[4]				
28 days	8	0.48±0.29	0.61±0.38	3.9±2.3
35 days	8	0.82±0.52	0.94±0.58	6.5±3.7

[1] The basic equation for calculating glutathione reductase activity is given on p. 33.

[2] Calculated for the surviving animals.

[3] Numbers following averages represent standard deviations.

[4] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

intake. The results presented both by Wainio et al. (loc. cit.) and by Knox et al. (loc. cit.) indicated that, even during severe dietary protein limitation, not all enzymes demonstrated decreased activities.

The mean values for glutathione reductase activity per gram of nitrogen were slightly higher for the tuberculous animals than for the respective control guinea pigs, except when 35 per cent protein was given for 40 days. Reduced glutathione is a hydrogen donor and may help protect ascorbic acid in the liver from oxidation. It is known that ascorbic acid metabolism is disturbed during tuberculosis.

Under the experimental conditions imposed upon the guinea pigs, it appears that tuberculosis did not interfere with the mechanisms which function in maintaining liver glutathione in a reduced form.

Riboflavin-containing Coenzymes in Liver

Mean values for flavin adenine dinucleotide (FAD), for flavin mononucleotide (FMN) plus free riboflavin, and for the sums of the two fractions designated total riboflavin in the livers of the guinea pigs are presented in Table 16. Individual data are given in Appendix Table x.

Table 16

Table

Mean values for riboflavin in the livers of tuberculous and

Group	Animals[1]	Liver riboflavin		
		Total B_2	FAD	FMN plus free B_2
		ug.[2]	ug.[2]	ug.[2]
Control				
35 per cent protein				
40 days	10	585±112	520±112	65±21
47 days	10	647±117	574±104	73±21
25 per cent protein[3]				
28 days	10	531±125	460±128	71±15
35 days	10	548± 96	471± 86	77±21
Tuberculous				
35 per cent protein				
40 days	8	609± 81	536± 83	73±21
47 days	8	622± 65	542± 66	80±13
25 per cent protein[3]				
28 days	8	558± 82	476± 78	82±17
35 days	8	532± 89	452± 76	80±27

[1] Calculated from the surviving animals.

[2] Numbers following mean values represent standard

[3] All animals received the 35 per cent protein diet diet was fed for the last 28 and 35 days of the 40-

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non-tuberculous guinea pigs on different protein intakes

Per gram of tissue			Per gram of solids		
Total B_2	FAD	FMN plus free B_2	Total B_2	FAD	FMN plus free B_2
$\gamma g.^2$	$\gamma g.^2$	$\gamma g.^2$	$\gamma g.^2$	$\gamma g.^2$	$\gamma g.^2$
29.8±3.5	26.5±4.2	3.3±1.0	113±16	100±18	13±4
32.2±4.1	28.6±3.6	3.6±1.0	127±16	113±13	14±4
29.9±3.3	25.6±3.7	4.3±2.0	120±17	103±17	17±8
29.4±3.3	25.2±3.2	4.2±1.0	108±15	93±13	15±5
23.7±3.3	20.9±3.3	2.8±0.7	94±13	83±13	11±3
27.1±2.7	23.5±2.4	3.6±0.9	109± 7	95± 7	14±3
25.2±3.4	21.4±2.8	3.8±1.1	105± 9	89± 6	16±5
26.7±3.7	22.7±3.5	4.0±1.0	107±13	91±14	16±3

deviations.

for the first 12 experimental days; the 25 per cent protein and 47-day periods, respectively.

Effect of dietary protein

The consumption of the 25 per cent protein diet caused a decrease in the mean amounts of FAD and total riboflavin present in the livers of the guinea pigs in comparison with values determined for the animals fed the higher protein ration. An analysis of variance indicated that these differences were significant ($P \leq 0.01$). There appears to have been no significant effect of dietary protein restriction upon the mean amounts of FAD and total riboflavin stored either per gram of liver tissue or per gram of solids. Limitation of dietary protein resulted in slightly higher mean values for FMN plus free riboflavin in the livers of the guinea pigs; the means for this fraction expressed per gram of wet tissue and per gram of solids in the livers of the guinea pigs consuming the 25 per cent protein diet were significantly higher than those found for the livers of the animals which received the 35 per cent protein ration ($P \leq 0.01$).

The control animals which received the 35 per cent protein diet for 40 days had an average of 585 ± 112 micrograms of riboflavin in the livers; the values for FAD and FMN plus free riboflavin were 520 ± 112 and 65 ± 21 micrograms, respectively. When the protein content of the diet

was limited to 25 per cent for 28 of 40 experimental days, the livers of the guinea pigs accumulated 531±125 micrograms of riboflavin; there were 460±128 micrograms of FAD and 71±15 micrograms of FMN plus free riboflavin. For these two groups which differed only in the percentage of protein in the ration, the mean values for FMN plus free riboflavin per gram of tissue were: 35 per cent protein, 3.3±1.0 micrograms; 25 per cent protein, 4.3±2.0 micrograms.

From the data given in Table 17, it can be seen that the livers of the animals which received the 35 per cent protein diet contained more nitrogen as well as more riboflavin than did those of guinea pigs consuming the 25 per cent protein ration. Dietary protein appeared to have little effect upon the storage of nitrogen and total riboflavin either per gram of wet tissue or per gram of solids. When the milligrams of riboflavin were expressed per milligram of nitrogen, it became evident that the retention of riboflavin in the livers of the guinea pigs was directly related to the storage of nitrogen. It would then appear that the tissue retention of riboflavin is related to protein consumption insofar as the storage of nitrogen is influenced by dietary protein. Sarett and Perlzweig

Table 17

Ratio of riboflavin to nitrogen in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

Group	Animals[1]	Total riboflavin[2]	Total nitrogen[3]	Riboflavin-nitrogen ratio
	no.	mg.	mg.	mg./mg.
Control				
35 per cent protein				
40 days	10	585	617	0.95
47 days	10	647	643	1.01
25 per cent protein[4]				
28 days	10	531	557	0.95
35 days	10	548	577	0.95
Tuberculous				
35 per cent protein				
40 days	8	609	752	0.81
47 days	8	622	742	0.84
25 per cent protein[4]				
28 days	8	558	658	0.85
35 days	8	532	618	0.86

[1] Calculated for the surviving animals.
[2] Data from Table 16.
[3] Data from Table 13.
[4] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

(loc. cit.) proposed the existence of a direct relationship between riboflavin retention in tissue and both nitrogen storage and protein consumption. The FAD content in the livers of the animals seemed to parallel that of total riboflavin.

That the amount of FMN plus free riboflavin per gram of wet tissue and per gram of solids in the livers of the guinea pigs increased when dietary protein was restricted is contrary to the data reported in the Preliminary Experiment for rats. When the dietary protein was decreased from 20 to 8 per cent without alteration in the quantity of ration fed to the rats, the FMN plus free riboflavin fraction expressed as micrograms per gram of liver tissue decreased proportionately with total riboflavin and FAD. Bessey et al. (loc. cit.) found that a decrease in riboflavin consumption was reflected in a diminution of FMN plus free riboflavin values per gram of tissue in the livers of rats. The guinea pigs in the current study, which received the 25 per cent protein diet, had slightly smaller food intakes than the respective guinea pigs on the higher protein ration; thus, they consumed less protein and smaller amounts of riboflavin than did the animals fed the 35 per cent protein ration.

Constituents in the livers of guinea pigs and of rats will not necessarily respond in a like manner to similar external stimuli. On the other hand, in the livers of the guinea pigs, the FAD fraction and that of FMN plus free riboflavin did not appear to react similarly to imposed stress. Since the protein deficiency apparently was not severe, the FMN plus free riboflavin in the livers of the experimental animals receiving the 25 per cent protein ration may have reflected only a temporary alteration.

Effect of disease treatment

The riboflavin retention, either as FAD or as FMN plus free riboflavin, in the livers of the tuberculous guinea pigs was not significantly different from that in the livers of the control animals. However, per gram of wet tissue, the livers of the control animals which received the 35 per cent protein ration for 40 days contained 29.8 ± 3.5 micrograms of riboflavin of which 26.5 ± 4.2 micrograms were FAD, while the values for the corresponding tuberculous guinea pigs were 23.7 ± 3.3 and 20.9 ± 3.3 micrograms, respectively. That the retentions of total riboflavin and FAD per gram of tissue were less during tuberculosis can be seen from the mean values given in

Table 16. These differences were significant ($P \leq 0.01$). This also was true for the total riboflavin and FAD values expressed per gram of solids ($P \leq 0.01$).

It can be seen in Table 17 that the livers of all of the control guinea pigs retained more riboflavin per milligram of nitrogen than was found in the livers of any tuberculous group; the differences between means were significant ($P \leq 0.01$). The control animals that received 35 per cent protein for 40 days stored 0.95 milligrams of riboflavin per milligram of nitrogen; for the corresponding tuberculous guinea pigs, the mean value was 0.81 milligrams of riboflavin per milligram of nitrogen. When the dietary protein was decreased to 25 per cent for the last 28 of 40 days, the livers of the control and tuberculous animals contained 0.95 and 0.85 milligrams of riboflavin per milligram of nitrogen, respectively.

The relationship between the retention of riboflavin and that of nitrogen in the liver appears to have been disturbed during tuberculosis. The tuberculous guinea pigs consumed more ration than did the corresponding controls receiving similar percentages of protein and riboflavin in the ration. The storage of riboflavin per milligram of nitrogen did not appear to be increased

during tuberculosis when dietary protein was increased from 25 to 35 per cent. That the differences may be effects of simple dietary deficiency is not indicated by the data.

Since it was the FAD fraction of the riboflavin that appeared to decrease per gram of liver tissue and per gram of solids during tuberculosis, alterations may have occurred in physiological mechanisms of the liver which require FAD as a coenzyme. Consideration of the nitrogen data determined under these experimental conditions indicates that the apoenzyme may not be as limiting a factor in FAD-linked enzyme systems as the coenzyme during tuberculosis in guinea pigs.

Effect of the length of the experimental period

The control and tuberculous guinea pigs which received the 35 per cent protein diet were found to have slightly higher values for liver riboflavin after the 47-day experimental period than following the 40-day period. This was also true for the control animals fed 25 per cent dietary protein. When the diet supplied 25 per cent protein to tuberculous guinea pigs, the riboflavin in the livers decreased slightly from the fortieth to the forty-seventh experimental day. An analysis of variance showed that the

small changes in the average riboflavin values in the livers of the guinea pigs due to the length of time on experiment were not significantly different.

Interrelationships

The experimental data indicate that the restriction of protein in the diets of tuberculous guinea pigs accelerated the course of the disease, but did not affect, to a marked degree, the particular liver constituents under investigation in this study.

The gross examination of the tuberculous animals suggested that the limitation of dietary protein propagated the infection at a more rapid rate than was found when dietary protein was adequate. In general, the tuberculous guinea pigs on 25 per cent protein appeared to be more debilitated and gave evidence of more widespread infection than did the corresponding tuberculous animals fed 35 per cent protein.

The stress which restriction of dietary protein imposed upon the tuberculous guinea pigs was most apparent in the influence on the utilization of food for weight gain. The weight gained by the tuberculous guinea pigs which received the high protein diet was approximately the

same as the increase recorded for the controls fed the same ration. The consumption of the low protein ration by diseased animals resulted in a lesser mean gain in weight than was observed for the respective controls. In the tuberculous animals there was a decreased ability to utilize food for weight gain; the restriction of protein in the ration seemed to exaggerate this stress. Limitation of food consumption which has been reported to be characteristic of human beings with far advanced pulmonary tuberculosis was not apparent with the infected guinea pigs.

The mean liver weights of the tuberculous animals were greater than those of the control guinea pigs; however, the percentages of solids in the livers were decreased during tuberculosis. Nitrogen and sulfur appeared to remain a constant fraction of liver solids regardless of disease treatment or dietary protein. The glutathione reductase activity of the liver probably was not disturbed during tuberculosis. Abnormal fat deposits were not produced in the livers of the tuberculous guinea pigs. During tuberculosis, the concentrations of riboflavin, specifically as the FAD fraction, per gram of wet tissue and per gram of solids were markedly decreased; limitation of protein in the ration was reflected in an augmentation of FMN plus

free riboflavin per gram of wet tissue and per gram of solids. From these observations, it could be postulated that two opposing stresses were placed upon the livers of the tuberculous animals which received the low protein diet. The data indicate, however, that limitation of dietary protein did not cause alterations in the riboflavin fractions in the livers of the tuberculous guinea pigs beyond those which resulted when similar restrictions in diet were imposed upon control animals.

Thus the restriction of protein in the rations fed to tuberculous guinea pigs did not appear to exaggerate the stress placed upon the constituents of the livers by the presence of the infection. This does not preclude the fact that the consumption of a low protein ration by tuberculous guinea pigs may have caused stress in the livers of the animals, beyond that caused by the presence of tuberculosis alone. The homeostatic mechanisms may have been capable of counteracting the imposed dietary protein limitation so that the various effects of the disease treatment were not magnified by inadequate diet.

Dubos ('55) reported that experiments with mice provided no indication that protein deficiency alone could accelerate the course of tuberculosis, and that the

resistance of mice appeared to be independent of the protein content of the diet, within wide limits. The resistance to tuberculosis was judged by the cumulative numbers of mice surviving at the close of specified time intervals. The guinea pigs in the present experiment were sacrificed rather than permitted to survive until the imposed stresses effected the loss of life. That the limitation of dietary protein increased the progress of the infectious process in the guinea pigs can be implied only from observations of the physical conditions of the animals.

SUMMARY AND CONCLUSIONS

The concentrations of certain liver constituents were determined for 28 tuberculous and 39 non-tuberculous guinea pigs which were fed 35 and 25 per cent protein during 40- and 47-day experimental periods. Autopsies of the tuberculous guinea pigs revealed that the consumption of the lower protein ration resulted in more advanced and diffuse tuberculous infection, as judged by gross symptoms.

The amounts of ration, containing either 25 or 35 per cent protein, that were consumed by guinea pigs inoculated with tubercle bacilli, exceeded those consumed by the respective non-tuberculous controls. However, the utilization of feed, which was represented as grams of weight gained per 100 grams of food intake, was significantly less by the injected animals than by the controls.

The percentages of solids in the livers of the tuberculous animals were less and the percentages of water higher than those values determined for the respective controls; the percentages of fat were similar. Comparison

of the relationships existing between body weights and liver weights of tuberculous animals and of their controls demonstrated that enlargement of the liver occurred during tuberculosis. This incongruity in liver size with respect to body weight appeared to reflect the accumulation of excessive amounts of fluid in the livers with possible structural and functional alterations. These data indicated that the liver injury manifested by the tuberculous guinea pigs may have been necrosis, without fatty infiltration.

The ratio of nitrogen to sulfur in the livers of the guinea pigs was not altered during tuberculosis or by restriction of dietary protein. Nitrogen comprised a similar fraction of liver solids for all guinea pigs; this was true also of sulfur.

Limitation of dietary protein and the presence of tuberculosis infection probably did not cause disturbances in mechanisms responsible for maintaining reduced glutathione in the livers of the animals.

The decrease in total riboflavin and the FAD content of the livers of guinea pigs fed a diet supplying only 25 per cent protein was not observed when the values were expressed per gram of tissue and per gram of solids; the

FMN plus free riboflavin fraction increased with dietary restriction.

The amount of riboflavin present in the livers of the guinea pigs per milligram of tissue was less for the tuberculous than for the control animals. The FAD fraction of riboflavin decreased per gram of tissue and per gram of solids during tuberculosis. It is possible that alterations occurred in FAD-linked enzyme systems of the livers of the tuberculous animals.

When the experimental period of 40 days was lengthened to 47 days, no significant changes in the concentrations of liver constituents were noted beyond those found for the shorter time interval.

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APPENDIX

LIST OF TABLES

Table 1

Individual determinations of the nucleotides of riboflavin in the livers of rats

Group	Dietary casein	FAD	FMN plus free riboflavin	Total riboflavin
	%	µg./g.	µg./g.	µg./g.
I Low protein	8	16.6	2.8	19.4
		18.5	3.4	21.9
		19.9	3.7	23.6
		19.1	3.0	22.1
		lost	lost	lost
		13.8	4.6	18.4
		14.4	3.8	18.2
		14.0	4.0	18.0
		20.4	3.4	23.8
		22.1	3.0	25.1
II Control	20	26.1	8.0	34.1
		27.2	10.7	37.9
		26.7	5.7	32.4
		21.8	8.8	30.6
		27.6	7.1	34.7
		28.3	5.5	33.8
		26.5	6.2	32.7
		29.8	6.7	36.5
		25.0	7.7	32.7
		30.6	6.6	37.2
III Control: pair-fed to low protein	20	30.1	7.8	37.9
		32.1	9.0	41.1
		31.1	5.5	36.6
		30.7	5.8	36.5
		31.2	5.2	36.4
		30.6	6.0	36.6
		37.2	8.8	46.0
		31.5	6.0	37.5
		34.8	6.2	41.0
		29.6	5.1	34.7

Table 11

Temperatures in the animal rooms recorded over a weekly period

Date	Hour	Housing for tuberculous animals	Hour	Housing for control animals
		°F.		°F.
2/25/57	8 AM	68		
	1 PM	72	12 N	80
	5 PM	74	6 PM	82
			10 PM	77
2/26/57	8 AM	72	6 AM	73
	12 N	72	12 N	78
	5 PM	74	6 PM	74
2/27/57	8 AM	70	6 AM	68
	1 PM	74	12 N	74
	5 PM	74	6 PM	77
2/28/57	8 AM	66	6 AM	63
	11 AM	66	12 N	73
	6 PM	70	6 PM	78
3/1/57	8 AM	70	6 AM	68
	1 PM	66	12 N	76
	5 PM	70	6 PM	78
3/2/57	9 AM	70	6 AM	67
	1 PM	69	12 N	68
	5 PM	76	6 PM	68
3/3/57	8 AM	68	6 AM	62
			12 N	68

Table iii

Table 111

Individual weight gain and food consumption of tuberculous and non-tuberculous guinea pigs on different protein intakes

	Control				Tuberculous			
	Animal[1]	Weight gain	Food intake	Food utilization	Animal[1]	Weight gain	Food intake	Food utilization
		g.	g.	g./100g.		g.	g.	g./100g.
35 per cent protein								
40 days	101	178	856	21	121	199	1092	18
	102	232	884	26	122	181	1146	16
	103	216	901	24	123	219	1211	18
	104	188	927	20	124	123	1062	12
	111	118	795	15	321	222	1438	15
	112	152	770	20	322	90	1336	7
	113	176	851	21	323	146	1429	10
	114	189	751	25	324	146	997	14
	301	17	758	3				
	302	138	860	16				
47 days	105	258	1137	23	125	162	1263	13
	106	196	973	20	126	198	1368	14
	107	241	1063	23	127	238	1465	16
	108	174	1091	16	128	101	1442	7
	115	197	1002	20	a	172	1342	13
	116	168	916	18	b	172	1342	13
	117	192	949	20	327	160	1174	14
	118	144	951	15	c	172	1342	13
	305	103	892	12				
	306	140	1031	14				

25 per cent protein[2]								
28 days	d	126	706	18	21	48	718	7
	2	115	615	19	22	186	800	23
	3	173	660	26	23	82	704	12
	4	88	672	13	24	43	584	7
	11	132	707	19	e	104	881	12
	12	201	742	27	222	128	1025	12
	13	162	676	24	223	122	1299	9
	14	39	539	7	224	121	1038	12
	201	64	911	7				
	202	162	831	19				
35 days	5	220	1072	20	25	150	1505	10
	6	191	901	21	26	60	865	7
	7	96	685	14	27	174	1024	17
	8	85	656	13	28	123	868	14
	15	201	1089	18	225	107	1362	8
	16	43	716	6	226	81	1033	8
	17	153	914	17	227	89	1260	7
	18[3]	137	903	15	228	115	1350	8
	205	105	1064	10				
	206	138	1027	13				

[1] Small letters represent the animals missing from the group; the values are the means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

[3] Data missing; mean values for group substituted.

Table iv

Table iv

Individual weights and percentages of fat in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

	Control			Tuberculous		
		Fat			Fat	
	Animal[1]	Weight	Per cent of liver weight	Animal[1]	Weight	Per cent of liver weight
		g.			g.	
35 per cent protein						
40 days	101	0.43	2.2	121	0.50	2.0
	102	0.58	2.2	122	0.42	1.5
	103	0.49	2.5	123	0.62	1.9
	104	0.39	2.1	124	0.43	2.0
	111	0.40	2.0	321	0.70	1.9
	112	0.35	2.1	322	0.41	1.9
	113	0.40	2.0	323	0.56	2.6
	114	0.40	2.0	324	0.52	2.4
	301	0.62	3.9			
	302	0.55	2.9			
47 days	105	0.73	2.9	125	0.92	3.2
	106	0.53	2.2	126	0.84	3.5
	107	0.50	2.2	127	1.15	4.0
	108	0.52	2.7	128	0.44	2.7
	115	1.16	6.9	a	0.77	3.2
	116	0.93	4.1	b	0.77	3.2
	117	0.75	4.0	327	0.49	2.5
	118	0.56	3.2	c	0.77	3.2
	305	0.37	2.6			
	306	0.46	2.3			

25 per cent protein[2]						
28 days	d	0.71	4.1	21	1.52	5.3
	2	0.37	1.8	22	1.40	6.4
	3	0.37	1.8	23	0.96	5.2
	4	0.32	3.4	24	1.39	7.7
	11	1.04	5.8	e	1.05	4.8
	12	1.61	7.4	222	0.77	3.3
	13	0.71	3.1	223	0.80	3.4
	14	0.90	7.6	224	0.49	2.2
	201	0.56	2.9			
	202	0.48	2.9			
35 days	5	0.85	3.9	25	0.46	1.9
	6	0.90	4.6	26	0.87	5.4
	7	1.35	7.8	27	1.01	4.3
	8	0.73	4.0	28	0.76	4.8
	15	0.51	2.4	225	0.48	2.8
	16	0.81	5.3	226	0.64	2.8
	17	1.04	5.8	227	0.68	3.1
	18	0.76	3.9	228	0.60	3.1
	205	0.61	3.5			
	206	0.57	3.2			

[1] Small letters represent the animals missing from the group; the values are the means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table v

Table v

Weights of solids and percentages of solids and fat-free solids in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

	Control			Tuberculous		
		Solids			Solids	
	Animal[1]	Weight	Per cent of liver weight	Animal[1]	Weight	Per cent of liver weight
		g.			g.	
35 per cent protein						
40 days	101	4.9	25.3	121	6.5	26.1
	102	7.1	26.7	122	7.4	26.2
	103	5.0	25.3	123	7.9	24.2
	104	5.3	28.2	124	5.6	26.1
	111	5.5	27.0	321	9.2	25.0
	112	4.2	25.4	322	5.6	25.8
	113	5.3	26.6	323	5.6	25.6
	114	4.9	24.7	324	5.0	23.0
	301	4.0	25.0			
	302	5.8	30.6			
47 days	105	6.2	24.8	125	6.5	22.4
	106	5.7	23.5	126	6.0	24.9
	107	6.1	26.6	127	6.8	23.6
	108	5.1	26.2	128	4.3	26.7
	115	4.0	24.0	a	5.7	24.6
	116	5.6	24.4	b	5.7	24.6
	117	4.9	26.1	327	5.0	25.4
	118	4.9	27.7	c	5.7	24.6
	305	3.6	25.5			
	306	5.1	25.1			

25 per cent protein[2]						
28 days	d	4.5	24.9	21	6.3	22.0
	2	5.1	24.4	22	6.4	29.3
	3	5.0	24.4	23	4.4	23.6
	4	2.2	23.7	24	3.8	21.2
	11	4.2	23.6	e	5.4	24.1
	12	5.2	23.9	222	5.8	24.7
	13	6.3	27.4	223	6.1	25.6
	14	3.1	26.5	224	4.9	22.1
	201	5.0	25.8			
	202	4.1	24.8			
35 days	5	6.4	29.1	25	6.4	26.4
	6	5.8	29.2	26	4.4	27.2
	7	4.7	27.0	27	6.1	25.9
	8	5.0	27.6	28	4.0	24.8
	15	5.8	27.0	225	4.4	25.8
	16	4.6	30.2	226	5.2	22.4
	17	5.5	30.8	227	5.3	24.1
	18	4.5	23.3	228	4.5	23.4
	205	4.2	24.1			
	206	4.4	24.5			

[1] Small letters represent the animals missing from the group; the values are the means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table vi

Table vi

Body weights and liver weights for the guinea pigs

	Control			Tuberculous		
	Animal	Liver weight	Animal weight	Animal	Liver weight	Animal weight
		g.	g.		g.	g.
35 per cent protein						
40 days	101	19.5	460	121	24.8	486
	102	26.5	512	122	28.3	459
	103	19.6	494	123	32.8	476
	104	18.7	435	124	21.5	350
	111	20.3	411	321	37.0	560
	112	16.7	403	322	21.8	378
	113	19.8	433	323	21.7	445
	114	20.0	424	324	21.6	421
	301	16.0	356			
	302	19.0	440			
47 days	105	25.2	550	125	28.9	442
	106	24.1	485	126	24.0	470
	107	22.8	516	127	28.8	492
	108	19.4	418	128	16.2	331
	115	16.8	459	a	missing	missing
	116	22.8	445	b	missing	missing
	117	18.8	445	327	19.8	447
	118	17.6	376	c	missing	missing
	305	14.2	423			
	306	20.2	460			

25 per cent protein[1]						
28 days	d	missing	missing	21	28.7	352
	2	20.8	397	22	21.8	476
	3	20.6	410	23	18.4	348
	4	9.3	308	24	18.0	262
	11	18.0	446	e	missing	missing
	12	21.7	510	222	23.3	414
	13	22.8	453	223	23.7	404
	14	11.8	267	224	22.2	405
	201	19.5	420			
	202	16.4	440			
35 days	5	21.8	474	25	24.3	468
	6	19.7	504	26	16.0	355
	7	17.4	388	27	23.5	468
	8	18.2	349	28	15.9	344
	15	21.4	516	225	17.0	437
	16	15.4	334	226	23.0	371
	17	17.9	465	227	21.9	380
	18	19.5	429	228	19.2	414
	205	17.4	458			
	206	17.8	468			

[1] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table vii

Table vii

Individual weights for nitrogen in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

	Control				Tuberculous			
		Nitrogen				Nitrogen		
	Animal[1]	Total	Per gram of wet tissue	Per gram of solids	Animal[1]	Total	Per gram of wet tissue	Per gram of solids
		mg.	mg.	mg.		mg.	mg.	mg.
35 per cent protein								
40 days	101	639	32.7	130	121	758	30.6	117
	102	819	30.9	116	122	854	30.2	115
	103	657	33.5	132	123	917	28.0	116
	104	599	32.1	114	124	596	27.7	106
	111	604	29.8	110	321	978	26.5	106
	112	516	30.8	121	322	590	27.0	105
	113	683	34.5	130	323	654	30.1	118
	114	607	30.3	123	324	672	31.1	135
	301	493	30.9	123				
	302	552	29.2	95				
47 days	105	790	31.4	128	125	909	31.4	140
	106	768	31.9	136	126	780	32.4	130
	107	761	33.4	126	127	888	30.9	131
	108	575	29.7	113	128	475	29.4	110
	115	575	34.2	142	a	742	31.5	128
	116	701	30.8	126	b	742	31.5	128
	117	637	33.9	120	327	658	33.3	131
	118	524	29.8	108	c	742	31.5	128
	305	476	33.5	131				
	306	623	30.8	123				

25 per cent protein[2]								
28 days	d	557	31.4	126	21	784	27.3	124
	2	645	31.0	127	22	768	35.2	120
	3	698	33.9	139	23	572	31.0	132
	4	307	32.9	139	24	525	29.1	137
	11	552	30.6	130	e	658	29.6	124
	12	689	31.7	133	222	675	28.9	117
	13	640	28.0	102	223	611	25.8	101
	14	407	34.6	130	224	668	30.0	136
	201	586	30.1	117				
	202	492	30.0	121				
35 days	5	680	31.2	107	25	619	25.4	96
	6	602	30.6	105	26	536	33.4	123
	7	510	29.4	109	27	770	32.8	127
	8	483	26.5	96	28	515	32.3	130
	15	611	28.6	106	225	559	32.9	128
	16	424	27.6	92	226	683	29.7	133
	17	713	39.9	130	227	660	30.2	125
	18	581	29.8	128	228	598	31.1	133
	205	576	33.2	138				
	206	590	33.2	136				

[1] Small letters represent the animals missing from the group; the values are the means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table viii

Table viii

Individual weights for sulfur in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

	Control			Tuberculous		
		Sulfur			Sulfur	
	Animal[1]	Per gram of solids	Total	Animal[1]	Per gram of solids	Total
		mg.	mg.		mg.	mg.
35 per cent protein						
40 days	101	7.4	37	121	6.8	44
	102	7.8	55	122	7.1	52
	103	8.2	41	123	7.1	56
	104	6.9	36	124	6.2	35
	111	7.4	41	321	6.7	62
	112	5.1	22	322	5.6	32
	113	8.4	44	323	6.6	37
	114	6.5	32	324	5.8	29
	301	7.3	29			
	302	5.8	34			
47 days	105	7.5	47	125	8.7	56
	106	6.8	38	126	6.6	40
	107	5.6	34	127	7.7	53
	108	8.4	42	128	5.6	24
	115	8.4	34	a	7.3	43
	116	6.0	33	b	7.3	43
	117	8.0	39	327	7.9	40
	118	6.2	30	c	7.3	43
	305	9.2	33			
	306	6.7	34			

25 per cent protein[2]						
28 days	d	7.5	34	21	7.6	48
	2	6.4	32	22	7.8	50
	3	8.5	42	23	7.8	34
	4	6.9	15	24	8.1	31
	11	8.9	38	e	7.5	30
	12	8.3	43	222	7.8	45
	13	6.7	42	223	5.3	32
	14	5.9	18	224	8.0	39
	201	7.5	38			
	202	8.4	34			
35 days	5	6.7	42	25	5.6	36
	6	5.6	32	26	6.7	29
	7	7.0	33	27	6.9	42
	8	5.9	30	28	7.2	29
	15	3.6	21	225	7.0	31
	16	6.2	29	226	7.8	40
	17	7.1	39	227	8.8	46
	18	7.6	34	228	7.8	35
	205	9.4	39			
	206	7.7	34			

[1] Small letters represent the animals missing from the group; the values are means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table ix

Table ix

Rates for glutathione reductase activity[1] in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes

	Control				Tuberculous			
	Animal[2]	Reductase activity, rate per gram of			Animal[2]	Reductase activity, rate per gram of		
		solids	fat-free solids	nitrogen		solids	fat-free solids	nitrogen
35 per cent protein								
40 days	101	0.39	0.43	3.0	121	0.19	0.20	1.6
	102	0.13	0.14	1.1	122	0.16	0.17	1.4
	103	0.07	0.08	0.5	123	0.95	1.04	8.2
	104	0.20	0.22	1.8	124	0.23	0.25	2.2
	111	0.63	0.68	5.7	321	0.46	0.50	4.3
	112	0.87	0.94	7.2	322	0.62	0.68	5.9
	113	0.05	0.05	0.4	323	0.58	0.65	4.9
	114	0.58	0.64	4.7	324	0.55	0.62	4.1
	301	1.91	2.26	15.5				
	302	0.46	0.51	4.8				
47 days	105	0.42	0.47	3.3	125	0.22	0.26	1.6
	106	0.32	0.35	2.4	126	0.98	1.14	7.5
	107	0.10	0.11	0.8	127	0.40	0.48	3.0
	108	0.40	0.44	3.5	128	0.33	0.37	3.0
	115	0.67	0.94	4.7	a	0.52	0.60	4.1
	116	0.34	0.40	2.7	b	0.52	0.60	4.1
	117	0.42	0.50	3.2	327	0.69	0.77	5.3
	118	0.20	0.23	1.8	c	0.52	0.60	4.1
	305	0.34	0.38	2.6				
	306	0.49	0.54	4.0				

25 per cent protein[3]								
28 days	d	0.33	0.38	2.7	21	0.20	0.27	1.6
	2	0.90	0.98	7.1	22	0.22	0.28	1.8
	3	0.38	0.40	2.7	23	0.12	0.16	0.9
	4	0.34	0.39	2.4	24	0.78	1.22	5.7
	11	0.18	0.24	1.4	c	0.48	0.61	3.9
	12	0.02	0.03	0.2	222	0.48	0.54	4.1
	13	0.18	0.21	1.8	223	0.63	0.72	6.2
	14	0.08	0.11	0.6	224	0.94	1.05	6.9
	201	0.77	0.87	6.6				
	202	0.14	0.16	1.2				
35 days	5	0.56	0.66	5.2	25	0.53	0.57	5.5
	6	0.62	0.72	5.9	26	0.48	0.60	3.9
	7	0.35	0.49	3.2	27	0.52	0.62	4.1
	8	0.67	0.79	7.0	28	0.45	0.56	3.5
	15	0.55	0.60	5.2	225	0.57	0.64	4.4
	16	0.39	0.48	4.2	226	1.94	2.22	14.6
	17	0.20	0.26	1.5	227	0.87	1.00	7.0
	18	0.62	0.75	4.8	228	1.17	1.34	8.8
	201	0.25	0.29	1.8				
	202	0.38	0.42	2.8				

[1] The basic equation for calculating glutathione reductase activity is given on page 33.

[2] Small letters represent the animals missing from the group; the values are means for the surviving animals in the group.

[3] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table x

Table x

Riboflavin in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes
(micrograms per liver)

	Control				Tuberculous			
	Animal[1]	Total B_2	FAD	FMN plus free B_2	Animal[1]	Total B_2	FAD	FMN plus free B_2
		γ.	γ.	γ.		γ.	γ.	γ.
35 per cent protein								
40 days	101	534	474	60	121	568	474	94
	102	763	654	109	122	778	710	68
	103	750	707	43	123	620	528	92
	104	554	502	52	124	528	478	50
	111	590	521	69	321	706	610	96
	112	520	438	82	322	523	486	37
	113	625	581	44	323	575	503	72
	114	588	542	46	324	573	499	74
	301	378	308	70				
	302	550	476	74				
47 days	105	770	664	106	125	707	603	104
	106	704	627	77	126	680	613	67
	107	853	764	89	127	640	565	75
	108	591	517	74	128	488	398	90
	115	597	503	94	a	622	542	80
	116	636	568	68	b	622	542	80
	117	742	667	75	327	598	533	65
	118	521	468	53	c	622	542	80
	305	490	434	56				
	306	564	527	37				

25 per cent protein[2]								
28 days	d	531	460	71	21	627	544	83
	2	519	450	69	22	706	601	105
	3	665	562	108	23	508	401	107
	4	277	186	91	24	438	365	73
	11	656	606	50	e	558	476	82
	12	648	583	65	222	586	525	61
	13	612	537	75	223	524	462	62
	14	388	324	64	224	518	434	84
	201	521	460	61				
	202	494	432	62				
35 days	5	697	599	98	25	634	492	142
	6	593	506	87	26	466	388	78
	7	457	415	42	27	598	508	90
	8	514	450	64	28	383	319	64
	15	637	571	66	225	536	471	65
	16	364	304	60	226	457	395	62
	17	622	538	84	227	614	548	66
	18	522	405	117	228	568	491	77
	205	566	483	83				
	206	512	441	71				

[1] Small letters represent the animals missing from the group; the values are means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table x (continued)

Table x (continued)

Riboflavin in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes
(micrograms per gram of wet liver)

	Control				Tuberculous			
	Animal[1]	Total B_2	FAD	FMN plus free B_2	Animal[1]	Total B_2	FAD	FMN plus free B_2
		ug.	ug.	ug.		ug.	ug.	ug.
35 per cent protein								
40 days	101	27.4	24.3	3.1	121	23.0	19.2	3.8
	102	28.8	24.7	4.1	122	27.5	25.1	2.4
	103	38.2	36.0	2.2	123	18.8	16.0	2.8
	104	29.6	26.8	2.8	124	24.5	22.2	2.3
	111	29.1	25.7	3.4	321	19.1	16.5	2.6
	112	31.1	26.2	4.9	322	24.0	22.3	1.7
	113	31.6	29.4	2.2	323	26.4	23.1	3.3
	114	29.4	27.1	2.3	324	26.5	23.1	3.4
	301	23.7	19.3	4.4				
	302	29.0	25.1	3.9				
47 days	105	30.5	26.3	4.2	125	24.4	20.8	3.6
	106	29.2	26.0	3.2	126	28.3	25.5	2.8
	107	37.4	33.5	3.9	127	22.2	19.6	2.6
	108	30.5	26.7	3.8	128	30.2	24.6	5.6
	115	35.5	29.9	5.6	a	27.1	23.5	3.6
	116	28.0	25.0	3.0	b	27.1	23.5	3.6
	117	39.5	35.5	4.0	327	30.3	27.0	3.3
	118	29.7	26.7	3.0	c	27.1	23.5	3.6
	305	34.3	30.4	3.9				
	306	27.8	26.0	1.8				

25 per cent protein[2]								
28 days	d	29.9	25.6	4.3	21	21.8	18.9	2.9
	2	25.0	21.7	3.3	22	32.4	27.6	4.8
	3	32.2	27.2	5.0	23	27.5	21.7	5.8
	4	29.7	20.4	9.3	24	24.1	20.1	4.0
	11	36.5	33.7	2.8	e	25.2	21.4	3.8
	12	29.9	26.9	3.0	222	25.0	22.4	2.6
	13	26.8	23.5	3.3	223	22.1	19.5	2.6
	14	33.0	27.6	5.4	224	23.3	19.5	3.8
	201	26.7	23.6	3.1				
	202	30.1	26.3	3.8				
35 days	5	32.0	27.5	4.5	25	26.0	20.2	5.8
	6	30.2	25.6	4.6	26	29.1	24.2	4.9
	7	26.3	23.9	2.4	27	25.4	21.6	3.8
	8	28.3	24.8	3.5	28	24.1	20.1	4.0
	15	29.9	26.8	3.1	225	31.5	27.7	3.8
	16	23.7	19.8	3.9	226	19.8	17.1	2.7
	17	34.8	30.1	4.7	227	28.0	25.0	3.0
	18	26.8	20.8	6.0	228	29.6	25.6	4.0
	205	32.6	27.8	4.8				
	206	28.8	24.8	4.0				

[1] Small letters represent the animals missing from the group; the values are means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

Table x (continued)

Table x (continued)

Riboflavin in the livers of tuberculous and non-tuberculous guinea pigs on different protein intakes
(micrograms per gram of liver solids)

	Control				Tuberculous			
	Animal[1]	Total B_2	FAD	FMN plus free B_2	Animal[1]	Total B_2	FAD	FMN plus free B_2
		γ.	γ.	γ.		γ.	γ.	γ.
35 per cent protein								
40 days	101	108	96	12	121	88	74	14
	102	108	93	15	122	105	96	9
	103	151	142	9	123	78	66	12
	104	105	95	10	124	94	85	9
	111	108	95	13	321	76	66	10
	112	122	103	19	322	93	86	7
	113	118	110	8	323	103	90	13
	114	119	110	9	324	116	101	15
	301	94	77	17				
	302	95	82	13				
47 days	105	123	106	17	125	109	93	16
	106	125	111	14	126	114	103	11
	107	141	126	15	127	94	83	11
	108	117	102	15	128	113	92	21
	115	148	125	23	a	109	95	14
	116	114	102	12	b	109	95	14
	117	152	136	16	327	119	106	13
	118	107	96	11	c	109	95	14
	305	134	119	15				
	306	111	104	7				

25 per cent protein[2]								
28 days	d	120	103	17	21	99	86	13
	2	103	89	14	22	110	94	16
	3	132	111	21	23	117	92	25
	4	125	86	39	24	114	95	19
	11	155	143	12	e	105	89	16
	12	125	113	12	222	102	91	11
	13	98	86	12	223	86	76	10
	14	124	104	20	224	105	88	17
	201	104	92	12				
	202	122	106	15				
35 days	5	109	94	15	25	99	77	22
	6	103	87	16	26	107	89	18
	7	98	89	9	27	98	83	15
	8	103	90	13	28	97	81	16
	15	110	99	11	225	122	107	15
	16	79	66	13	226	88	76	12
	17	113	98	15	227	117	104	13
	18	115	89	26	228	126	109	17
	205	135	115	20				
	206	117	101	16				

[1] Small letters represent the animals missing from the group; the values are means for the surviving animals in the group.

[2] All animals received the 35 per cent protein ration for the first 12 experimental days; the 25 per cent protein diet was fed for the last 28 and 35 days of the 40- and 47-day periods, respectively.

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