THE RIBOFLAVIN AND NITROGEN METABOLISM OF SIX WOMEN WITH ACTIVE TUBERCULOSIS

By

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Good nutrition has been considered an important aid in the treatment of tuberculosis. Since there have been few studies of the quantitative requirements of the disease, emphasis has been directed toward the qualitative aspects of nutrition. Consequently there have been wide differences in the dietary practices of sanatoria (Sedgwick, 1946) and in the recommended allowances for tuberculous patients (Pottenger, 1946; Baird, 1945).

The Foods and Nutrition department at Michigan State College was asked in 1945 for recommendations for food subsidies for persons with arrested tuberculosis. Since there was inadequate information concerning nutritive requirements in tuberculosis, a survey of food habits was conducted among women with active and arrested tuberculosis at the Ingham county sanatorium. The survey techniques used were similar to those which had been used in previous investigations with apparently healthy women so that data for women free from the disease were available for comparison with data for the tuberculous series. Results of the dietary survey have been reported (Brewer, 1949).

One phase of the survey consisted of measurements of the riboflavin, thiamine and ascorbic acid content of the urine of patients for three days preceding and one day following a test dose which contained three mg. riboflavin, three mg. thiamine and 400 mg.

ascorbic acid. Tests were carried out among patients who previously had received vitamins in addition to their usual diet and also among those who had had no dietary supplements. A histogram which gives the percentage excretion of a test dose of three mg. of riboflavin by the tuberculous women and by college women under similar test conditions is shown inFigure 1. These data indicated that the riboflavin excretion of tuberculous women following a test dose of the vitamin exceeded that for healthy women on their usual, self-selected diet. The test dose excretions also were higher than those for college women receiving controlled intakes of 2.73 mg. riboflavin. These values suggested the possibility of disturbed riboflavin metabolism in tuberculosis and it was felt that there was need for further study of the riboflavin nutrition of tuberculous patients under controlled conditions.

The excretion of urinary nitrogen also was studied for the patients sampled during the survey; these values have been grouped according to calculated nitrogen intakes in Table 1. If a relatively constant value for fecal nitrogen is assumed, apparent storage of nitrogen occurred at the higher nitrogen intakes. Since dietary protein has been considered an important factor in the treatment of tuberculosis and since an interrelationship of nitrogen and riboflavin metabolism has been reported (Oldham, 1946), it was planned to investigate simultaneously the nitrogen and riboflavin metabolism of tuberculous women.

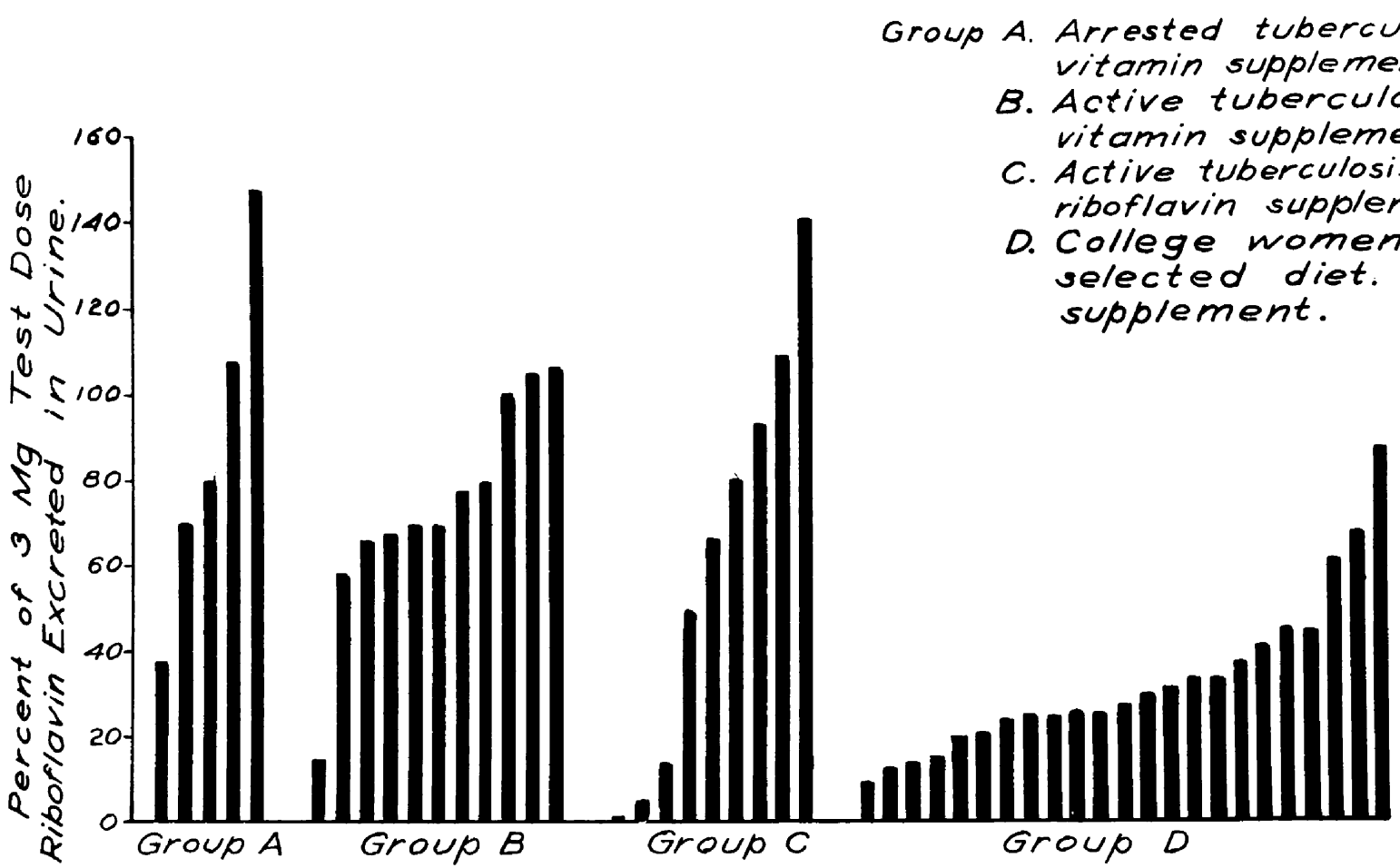

Fig. 1 The urinary excretion following a test dose of riboflavin by patients with active and arrested tuberculosis and by college woman.

TABLE 1

The average urinary nitrogen excretion of 18 women with active tuberculosis; values grouped according to calculated nitrogen intakes

Nitrogen Intake*	Number of Subjects	Urinary Nitrogen
Gms./24 hours		Gms./24 hours
5.0 - 9.99	5	6.795 (5.234 - 9.853)
10.0 - 14.99	9	8.353 (6.662 - 10.575)
15.0 and up	4	8.782 (6.728 - 11.168)

* Nitrogen Intake $= \frac{\text{Protein, gm. (by calculation)}}{6.25}$

REVIEW OF LITERATURE

Riboflavin metabolism of women.

The urinary excretion of riboflavin is directly related to the dietary intake of the vitamin. This relationship may be seen from Table 2 which gives the mean urinary excretions of riboflavin reported by various investigators for women at different riboflavin intakes. From studies of riboflavin intake and excretion, recommendations have been made for human riboflavin requirements. Earliest of these studies was the investigation of Sebrell (1941) of the riboflavin metabolism of 10 women on a basal diet containing 0.5 mg. riboflavin per 2400 calories. Symptoms of ariboflavinosis which responded to riboflavin therapy were observed for six of the 10 subjects after 89 to 232 days on the basal diet. The average urinary excretion on this diet was 77 micrograms and it was found that the excretions increased in a step-like manner when supplements of riboflavin were added to the basal diet. Whereas an intake of 0.05 mg. of riboflavin per kilogram exceeded the requirement of the adult women, an intake of 0.025 mg. per kilogram was insufficient. Sebrell evaluated the requirement for adult women at three mg. per day. Studies reported by Strong (1941) supported this recommendation.

Williams (1943) found no evidence of clinical deficiencies at intakes of 0.7 mg. per day although depletion of tissue stores of riboflavin occurred. The subjects of this study had received large amounts of riboflavin prior to the investigation. Williams estimated the riboflavin requirement of women to be 0.5 mg. per 1000 calories

TABLE 2

The urinary excretion of riboflavin by women at different riboflavin intakes

No. of Subjects	Dietary Intake of Riboflavin	Urinary Excretion of Riboflavin		Experimental Conditions	Investigator
	mg/24 hr	Mean mg/24 hr	Range mg/ 24 hr		
9	0.5 - 0.99	0.07	0.04 - 0.10	College women; controlled diet	Brewer, 1946
9		0.11	0.08 - 0.14	College women; controlled diet	Davis, 1946
12	1.0 - 1.49	0.14	0.07 - 0.25	College women; controlled diet	Brewer, 1946
9		0.23	0.14 - 0.29	College women; controlled diet	Davis, 1946
3		0.16	0.13 - 0.48	College women; controlled, synthetic diet	Hathaway, 1946
3		0.20	0.16 - 0.33	College women; controlled, natural diet	Hathaway, 1946
9	1.5 - 1.99	0.32	0.17 - 0.44	College women; controlled diet	Brewer, 1946
4	2.0 - 2.49	1.18	1.11 - 1.29	College women; controlled diet	Brewer, 1946
4	2.50 - 2.99	1.31	1.27 - 1.35	College women; controlled diet	Brewer, 1946
1		1.26		Institution patients; basal diet, supplemented	Sebrell, 1941

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The urinary excretion of riboflavin by women at different riboflavin intakes

No. of Subjects	Dietary Intake of Riboflavin	Urinary Excretion of Riboflavin		Experimental Conditions	Investigator
	mg/24 hr	Mean mg/24 hr	Range mg/24 hr		
3	3.0 - 3.49	0.99	0.79 - 1.21	Institution patients; basal diet, supplemented	Sebrell, 1941
5	3.5 - 3.99	1.17	0.57 - 1.34	Basal ration containing approximately 72 grams of protein and 3.7 to 4.2 mg riboflavin	Price, 1947
1		2.43		Institution patients; basal diet, supplemented	Sebrell, 1941
4	4.0 - 4.49	1.90	1.41 - 2.15	Institution patients; basal diet, supplemented	Sebrell, 1941
2	5.0 - 5.49	2.60	2.42 - 2.78	Institution patients; basal diet, supplemented	Sebrell, 1941
9	7.0 - 7.49	4.34	2.92 - 5.80	College women; controlled diet	Davis, 1946
3		3.4	3.1 - 3.6	College women, high milk intake, milk diet	Gardner, 1943

and he considered that the recommended allowance of 2.2 mg. made by the Food and Nutrition Board of the National Research Council (1943) for moderately active women provided a liberal margin of safety. From studies with college women, 19 to 32 years of age, Davis (1946) estimated the riboflavin requirement to be between 0.49 and 0.66 mg. per 1000 calories. Keys (1944) found that young men on a riboflavin intake of 0.31 mg. per 1000 calories showed no measurable physiological or clinical handicap over a period of five months.

Brewer and coworkers (1946) assembled riboflavin intake and urinary excretion values for apparently healthy women from the literature and plotted these on a scatter diagram together with data from this laboratory. Predicting equations were calculated from intake values from 0.5 to 2.0 mg. and from intake values of 1.3 mg. and above. The difference in slope of the two lines was statistically significant. The regression coefficient for values from 0.5 to 2.0 mg. was 0.219, and for values of 1.3 mg. and above, the regression coefficient was 0.570. The slope of the lines indicated that there was a gradual increase in urinary excretion as the dietary intake of riboflavin increased to approximately 1.5 mg. When the intake of riboflavin exceeded 1.5 mg. there resulted a rapid increase of urinary excretion of riboflavin with each unit increase in dietary riboflavin. It appeared that a daily intake of 1.3 to 1.5 mg. was an adequate amount for apparently healthy women. The present recommended allowance for riboflavin for women made by the Food and Nutrition Board of the National Research Council (1948) is 1.5 mg. This value seems to be a satisfactory estimate of the riboflavin requirement of healthy women studied without metabolic stress.

The urinary excretion of a test dose of riboflavin has been used as a concomitant measure of riboflavin nutrition with the measurement of the average daily excretion. Axelrod and coworkers (1941) were unable to relate the percent excretion of a test dose of riboflavin to variations in dietary intake. However the test dose used by these workers was of the magnitude of 200 to 400 micrograms per kilogram of body weight and later investigators considered that the size of this dose may have had a masking effect on variations in the urinary excretion. Najjar and Holt (1941) administered intravenously a test dose of 0.016 mg. riboflavin per kilogram of body weight to patients in a fasting condition. Measurements of the following four hour excretion indicated that 32 to 72 percent of the test dose was retained by healthy individuals while 81 to 93 percent was retained by persons with apparent riboflavin deficiency. A relationship of the excretion of test doses varying from two to five mg. with the average daily excretion of adults was reported by Strong and coworkers (1941). Other investigators who have used the measurement of urinary excretion after a test dose as an experimental technique for studying riboflavin nutrition of the individual have included Williams (1943), Davis (1946) and Oldham (1944). Brewer et al (1946) reported studies among college women using a test dose of three mg. of riboflavin. Statistically significant correlations were obtained when the average excretion for three days on a non-restricted diet were compared with one hour fasting riboflavin excretion, four hour excretion after the oral test dose and the 24 hour excretion after the oral test dose. Oldham (1944) also found that the one hour fasting excretion, the four hour and the 24 hour return of a test dose reflected equally well the nutritional status with respect to riboflavin. According to Berryman (1947), a

change in load test response was more closely related to the riboflavin content of animal tissues than was the fasting urinary excretion.

Johnson and coworkers (1945) studied the effects of each of three vitamins, ascorbic acid, riboflavin and thiamine on the urinary excretion of the others when the three were administered together in an oral tolerance dose. No significant effect was observed and therefore it seems that tolerance tests using mixtures of vitamins give results which are as reliable for well-fed subjects as tests that use the vitamin singly.

Keys (1945) has questioned the significance of the urinary excretion of riboflavin as a criterion for estimating riboflavin requirements since he was unable to demonstrate clinical evidence of riboflavin deficiencies even at low excretions. He has suggested that the fecal synthesis of riboflavin may provide a source of metabolic riboflavin which in effect would lower the dietary riboflavin requirement. Najjar and Holt (1944) demonstrated biosynthesis of riboflavin in adolescent boys who were kept on a synthetic diet containing only 70 to 90 micrograms of riboflavin per day for a period of three months. The average urinary excretion of riboflavin was 150 to 250 micrograms per day and the fecal excretion ranged from 200 to 600 micrograms. Intravenous injections of five to 20 mg. riboflavin caused no increase in fecal riboflavin so it was felt that the high fecal excretion on a synthetic diet did not result from destruction of tissue stores.

Davis (1946) found considerable variation in fecal excretion of riboflavin among college women; however the fecal riboflavin was relatively constant for each subject and was not influenced by the

addition of riboflavin supplement to the diet. According to Hathaway (1946) the fecal excretion of riboflavin by subjects on a diet of natural foods was 3.7 to 3.8 times greater than when the subjects were maintained on a synthetic diet and this seemed to indicate that there was a greater intestinal synthesis of riboflavin when the natural diet was used than when the synthetic diet was fed. Since the riboflavin intakes of the two diets varied from 1.09 mg. on the synthetic diet to 1.33 mg. on the natural diet, the urinary excretion values could not be used to determine whether the synthesized riboflavin was absorbed from the intestine. Hathaway suggested that there may be appreciable absorption of fecal riboflavin when the riboflavin concentration in the body is low and less absorption at other times.

Najjar and coworkers (1944) found that the administration of enemas containing 20 mg. riboflavin brought a prompt rise in the excretion of riboflavin in the urine. This was an exceedingly large amount, considerably in excess of the usual dietary intake of riboflavin or of the fecal excretion of riboflavin. In comparison, Everson (1948) reported that for eight individuals, no increase in the urinary excretion of riboflavin resulted from a retention enema of two mg.

Since the urinary excretion of riboflavin parallels the dietary intake of the vitamin and since it is doubtful that intestinally synthesized riboflavin is metabolically available, studies of the urinary excretion of riboflavin remain our best criteria for the estimation of the dietary riboflavin requirement.

Nitrogen and riboflavin interrelationships.

The increasing evidence of interrelationships among essential nutrients indicates that the quantitative requirement for an individual nutrient varies with dietary conditions. In the last few years there have accumulated data from several laboratories which demonstrate an interrelationship between the metabolism of riboflavin and of nitrogen. Sarett and coworkers (1942) reported from studies with rats that the urinary excretion of riboflavin was inversely related to the nitrogen retention and Oldham, Lounds and Porter (1946) also found in studies with college women that higher riboflavin excretions occurred when the subjects were in negative nitrogen balance than when nitrogen was being retained. Conversely, Hagedorn (1945) reported that no correlation was apparent between the nitrogen and riboflavin excretions in 24 hour urine collections of humans who were given muscle meat and cereals as the chief source of nitrogen in the diet. Urinary nitrogen values for these subjects ranged from 7.2 to 15.5 gm. of nitrogen. Mickelsen (1945) also found no parallelism between riboflavin excretion and nitrogen intake for male subjects on a controlled diet.

Studies of liver storage of riboflavin at different nitrogen intakes have provided evidence in some instances of a metabolic interrelationship of nitrogen and riboflavin. Unna and coworkers (1944) determined the concentration of riboflavin in livers of rats fed diets varying in protein content. The average weight of eight rats given a diet containing 18 percent casein for 89 to 98 days was 281 gm. and the average riboflavin content of the liver was 22.5 ± 0.8 (standard error) micrograms per gm. Ten rats fed a diet containing eight percent casein as the

only source of protein had an average body weight of 114 gm. and the riboflavin concentration in the liver was 12.5 ± 0.8 micrograms per gm. When the diet supplying eight percent casein was supplemented with 25 mg. d,l-methionine the body weight for 12 rats averaged 136 gm. and the riboflavin content of the liver was 15.6 ± 0.9 gm. L-cystine had an effect on body weight similar to that of methionine but did not exert a similar effect on the riboflavin content of the liver. Reisen, Schweigert and Elvehjem (1946) confirmed the observation that the ingestion of methionine is necessary for the retention of riboflavin in the liver of rats fed a low protein diet and that an increase in the amount of protein in the diet of rats results in increased retention of riboflavin in the liver. These workers failed to observe an inverse relationship between riboflavin excretion and protein intake. However Czackes and Guggenheim (1946) found that the protein of the diet influenced both the riboflavin content of organs (liver, kidney and muscle) and the excretion of riboflavin in the urine of rats. In these studies, dietary fat also was an influencing factor since higher riboflavin intakes were required to maintain normal concentration of riboflavin in the urine on a high fat diet than on a low fat diet.

Apparently contradictory results from different laboratories may be explained in part by differences in the quantity of nutrients fed to experimental animals. This assumption is supported by feeding trials reported by Sherman (1948; 1949). The riboflavin content of livers of rats fed various intakes of protein was determined. When the intake of protein was increased from eight percent to 32 percent,

there was an increased storage of riboflavin in the liver; when the intake of protein was increased only from 16 to 20 percent, there was no measurable difference in the riboflavin content of the livers.

Trufanov (1946) reported that synthesis of flavin-adenine-nucleotide failed to occur in the liver and tissues of rats fed for 50 days on a low protein diet but when an adequate diet was given, there was a marked synthesis of dinucleotide in the liver and muscle tissue.

There is some evidence to indicate that the riboflavin content of the diet may affect the utilization of nitrogen. Braman and co-workers (1935) observed that rats deficient in vitamin G gained less in body protein than their pair-fed controls which received a liver concentrate. Sure and Ford (1942) found that there was moderate creatinuria and large excretions of ammonia in the urine of rats deficient in riboflavin. The energy metabolism and food utilization of riboflavin deficient chicks was studied by Kleiber and Jukes (1942) who found that there apparently was no effect from riboflavin deficiency on the utilization of food energy in the chick other than the effect of a greatly decreased appetite; however the difference in the total efficiency of nitrogen utilization between the flavin-deficient and flavin-supplied chicks on equal food intake was found to be statistically significant. For every 1000 calories of gain in energy, the chicks deficient in riboflavin gained 2.3 ± 0.7 gm. nitrogen while the control chicks which had received riboflavin gained 5.1 ± 1.4 gm. on an equal food intake.

Protein requirements of women.

Estimates of the protein requirements of humans have been made from studies of the nitrogen metabolism of individuals. The retention of nitrogen has been calculated from the difference between the intake and excretion of nitrogen by the body. For the adult, the intake of nitrogen at which body equilibrium is established has been considered adequate and linear regression frequently used as a technique to ascertain from nitrogen intake and excretion data the intake which will permit nitrogen equilibrium for a group of individuals.

The early studies of the maintenance requirements for protein by humans were reviewed by Martin and Robison (1922). In 1937, Leitch and Duckworth reported a compilation of nitrogen metabolism data from the literature and estimates of the protein requirements of man. These estimates were formed by fitting regression lines of daily output on intake to positive and to negative balances. From these lines the point at which the chances of negative and positive balance were equal was calculated. The equilibrium point occurred at an intake of 48.2 to 52.4 gm. This estimate was slightly higher than the estimate formed by Sherman in 1920 from the experimental data available at that time (Sherman, 1946). From 109 studies of 47 subjects, Sherman estimated the protein requirement for equilibrium to be 44.4 gm. of protein per 70 kilograms of body weight.

The nitrogen metabolism of young college women was investigated as a part of the regional project of the North Central States (McKay, 1942). Nitrogen intakes and retentions were reported for 124 college women on their customary diet. The mean intake for this group

was 10.1 gm. Regression of nitrogen balance on intake indicated that nitrogen equilibrium might be expected at an intake of 8.69 gm.

Bricker, Mitchell and Kinsman (1945) studied the requirements of young college women for protein supplied by individual foods and various food combinations. When protein was supplied by mixed foods, it was found that 3.12 mg. nitrogen per basal calorie was required for equilibrium. The average basal calories for the nine subjects was 1300 calories and therefore the daily protein requirement for equilibrium was calculated at 25.4 gm. A similar estimate of protein requirement for adults was reported by Hegsted (1946) who studied 26 adults ranging in age from 19 to 50 years. When one-third of the protein in the diet was supplied by meat, the protein requirement was estimated to be 2.4 gm. nitrogen per square meter. Using this value, the nitrogen requirement for equilibrium for women with an average surface area of 1.66 square meters (the average for the subjects in Bricker's study) would be four gm. of nitrogen or 25 gm. of protein (protein $=$ N x 6.25).

Studies of nitrogen balances of three college women which were reported by Oldham (1947) indicated that an intake of 30 gm. of protein was inadequate for these subjects. Daily nitrogen balances were observed for 10 day periods at different nitrogen intakes. At intakes of approximately five gm., one subject was in negative nitrogen balance for nine out of ten days. The balances for the last two days were -0.66 and -0.30 gm., respectively. Another subject was in negative balance for the entire 10 days; the balances on the last two days were -0.62 and -0.42 gm. A third subject had a previous dietary history

of irregular eating habits. At an intake of approximately 5.5 gm., the balances were slightly positive for seven of the 10 days. Values for the last three days were +0.39, +0.16 and -1.71 gm., respectively. When this subject was given 20 gm. of nitrogen daily, high retentions were observed. Values for the last two days were +7.33 and +7.44 gm. The slightly positive balances at the lower intake indicated that this subject had previously subsisted on a low protein diet of approximately this introgen intake; the unusually high retention of nitrogen at intakes of 20 gm. suggested depleted stores of nitrogen.

An essential difference in the studies of Hegsted and Bricker compared with the studies of McKay is that the subjects of the former studies were maintained on nitrogen intakes sufficiently low to produce consistently negative balances whereas the studies of McKay were conducted on subjects consuming self selected diets presumably representative of their recent dietary patterns. The difference in amounts required for equilibrium by the subjects of these studies may be partly explained by this difference in experimental procedure. In McKay's study, the nitrogen intakes of 124 individuals on customary self-chosen diets ranged from 5.55 to 17.58 gm. In contrast the prediction equations in Hegsted's study were calculated from nitrogen balances for subjects all of whom received the same nitrogen intake; the only variation in nitrogen intake between subjects was that due to the small amount of nitrogen on caloric supplements which were permitted without restriction. It would be expected therefore that a line fitted to these data would

cross the zero line at some point within the upper and lower values of intake if the nitrogen balances were at all near equilibrium. For example, when the subjects on Hegsted's study were fed an all-vegetable diet containing from 2.25 to 3.12 gm. per day per square meter (as read from the published graph), the estimated intake for equilibrium was 2.88± 0.17 gm. per square meter. When the subjects were given a diet in which the percent of the protein was supplied by meat at an intake of 2.05 to 2.56 gm. nitrogen per square meter, the zero intercept of the calculated regression line was 2.41 gm. per square meter.

The subjects of the investigation reported by Bricker (1945) were studied over a wider range of intake than those of Hegsted's study although the range also was limited. The quantitative intakes were not reported but inspection of the published graphs showed that the intakes varied from approximately 0.2 to 3.8 mg. per basal calorie. When the subjects were given mixed foods as the source of protein, five subjects were in equilibrium or in slight positive balance and 10 subjects were in negative balance.

The procedure for estimating the protein value of foods for mature animals by the use of animals on negative nitrogen balance was adapted to human studies by Bricker (1945) and Hegsted (1946) from the method developed by Melnick and Cowgill (1937) for the determination of protein minima for nitrogen equilibrium in dogs. This method was based on the observation of a linear relationship between balance and percent of protein calories in the diet in the

region of nitrogen equilibrium. Allison and Anderson (1945) used a procedure similar to that of Melnick and also demonstrated that the relationship between nitrogen balance and absorbed nitrogen was linear in the region of negative balance for normal adult dogs. The linearity extended to positive balances; however the relationship became curvilinear at higher retentions.

Ohlson (1948) compared the nitrogen excretions by women 50 to 75 years of age to those reported by McKay for college age women at similar nitrogen intakes. For the older women, the mean retention of nitrogen on high protein intakes tended to be near zero and Ohlson observed that for this group, the relationship between intake and retention was found only at intakes which resulted in negative retentions. However for the younger women, there was a steadily increasing retention of nitrogen with increased dietary intakes within the limits of voluntary intake (maximum about 15 gm.). Therefore it would seem that age influences the linearity of nitrogen intake and balance and that for young adult women this linearity exists for positive as well as negative balances. For this population group, a better estimate of the requirement for equilibrium should result from studies as reported by McKay which include both positive and negative balances than studies which are limited to intakes resulting in negative balances.

Nutrition in tuberculosis

The concept that good diet is important in the treatment of tuberculosis has a historical background almost as ancient as medicine itself. Hippocrates recommended the milk of goats, asses and mares for the phthisical person and Aretaeus wrote concerning milk:

> "It is pleasant to take and is more familiar than any other food to one from a child. In color it is pleasant to see, as a medicine it seems to lubricate the windpipe, to clean, as with a feather, the bronchi and to bring off the phlegm, improve the breathing and facilitate the discharges downward. To ulcers it is sweet medicine. If one then will only drink plenty of this (milk) he will not stand in need of anything else, for it is a good thing that in a disease milk should prove both food and medicine". (Williams, 1908).

Pulmonary tuberculosis is an infectious disease which is characterized by a prolonged and chronic course. The immunologic principles are not well established. Pinner (1947) stated that the evidence concerning resistance against the invasive power of bacilli:

> "strongly suggest that the fate of a tuberculous animal is largely decided at the portal of entry and that the result of this local defense depends mainly on the rapidity with which macrophages are mobilized and on how effectively they can localize and destroy bacilli".

Much experimental work may be required to define the relation of nutrition to the resistance of infection but there is some evidence that good nutrition increases resistance to bacillary infection. Cottingham and Mills (1943) demonstrated that in rats, a deficiency of any one vitamin sufficient to retard growth also caused a reduction in the phagocytic activity of the white blood cells. This reduction was most marked in riboflavin and in pyridoxine deficiencies. It was also shown (Mills and Cottingham, 1943) that the phagocytic activity of

blood leucocytes was at an optimum with 18 percent dietary protein and declined as the protein intake was increased or decreased from this level.

The most striking evidence which supports the hypothesis that good nutrition increases resistance to tuberculosis comes from observations of increased morbidity and mortality from tuberculosis which occurred in certain European countries during the first and second World Wars and was associated with the general state of undernutrition in these countries. Faber (1938) related the high incidence of tuberculosis in Denmark following the first World War to the low consumption of protein in the diet. Similarly, in Germany there was an increase in tuberculosis which was associated with a lowering of calories from a prewar intake of 3000 to 1400 and of protein intake from a prewar average of 12 percent to an intake of eight percent at the end of the war (Bruns, 1921). Reliable statistics concerning the incidence of tuberculosis and other diseases in certain European countries during the years of the second World War are not available partly because of interruption of normal health services and also because of destruction of medical records. It has been estimated that the tuberculosis death rate in Warsaw rose 220 percent in the war years, from 155 per 100,000 population to 452 in 1941 and 500 in 1944 (Daniels, 1947). The concentration of large numbers of persons in camps resulted in marked increase of exposure; undoubtedly the poor quality of diet among these individuals was a factor in decreasing resistance to the disease. In

southern areas of France where the food situation was bad, there was a continuous rise in tuberculosis death rate during the war which amounted to an increase of 74 percent in Bouches du Rhon; however in Brittany where the food situation was relatively good, the death rate from tuberculosis fell steadily throughout the war (Daniels, 1947).

In animal experimentation, McKay (1940) found that pigs kept on a diet deficient in calcium, vitamin A and Vitamin D were more susceptible to tuberculosis than pigs on a well-balanced diet, and DuBos and Pierce (1948) reported that the susceptibility of mice to experimental infection with mammalian tubercle bacilli was influenced by the composition of the experimental diet. The resistance of white rats to experimental infection with avian tubercle bacilli was not influenced by a diet significantly low in thiamine and riboflavin (Higgins and Feldman, 1943).

According to Johnston (1947) any condition that adversely affects the nutritional state in adolescence is a factor in the development of adult type tuberculosis and the diminished ability of the adolescent to retain nitrogen and calcium may be important in conditioning unfavorable resistance to the disease when the intake is marginal.

Studies have been conducted among hospitalized patients to investigate the effect of improvement of diet on recovery from the disease. In general, these studies have been inconclusive. Ascorbic acid deficiencies, as judged by urinary excretion and blood concentration, occur among tuberculous patients (Getz and Koerner, 1941; Heise

and Martin, 1936; Chang and Lan, 1940; Webb, 1946). In some instances a parallelism has been observed between the degree of ascorbic acid deficiency and the extent of tuberculosis (Heise and Martin, 1936; Sweany et al, 1941; Getz and Koerner, 1941). Other investigators have reported that no correlation existed (Hurford, 1938; Roy and Rudra, 1941). The addition of ascorbic acid as supplement to the diet results in an increased urinary excretion of the vitamin and higher blood values (Webb, 1946; Sweany, 1941). Patients who received a dietary supplement of 100 to 300 mg. ascorbic acid daily had urinary ascorbic acid values comparable to those for active college women (Brewer, 1949).

The vitamin A nutrition of the tuberculous patient may be affected by the extent of the disease. Getz and Koerner (1941) reported that the plasma concentration of vitamin A of recently diagnosed tuberculous patients was lowered in proportion to the extent of tuberculosis and Breese, Watkins, and McCoord (1942) found that the lowered absorption of vitamin A in tuberculosis was directly related to the degree of weight loss of the patient and the severity of symptoms of intestinal tuberculosis. A lowered prothrombin concentration of the plasma (Farber and Miller, 1943) in tuberculosis may be indicative of lowered absorption of vitamin K.

Farber and Miller (1943a) reported that of 400 patients, 25 percent showed evidence of riboflavin and niacin deficiencies. The lesions responded to specific vitamin therapy. These authors considered that the state of nutrition of a tuberculous person could usually be correlated with the severity of the disease process and that the very sick

patient often demonstrated multiple vitamin deficiencies, i.e., vitamin A and K, ascorbic acid, niacin and riboflavin. Severe thiamine deficiencies were uncommon among these patients.

In a series of investigations reported from the Henry Phipps Institute, Getz and coworkers (1944) found that patients with far advanced tuberculosis showed deficiencies of ascorbic acid, serum albumin, hemoglobin, vitamin A, carotene and serum calcium.

Siebert and coworkers (1947) found a change in the protein fractions of the blood of tuberculous patients although a change in total protein content was not observed. A slight rise in the gamma globulin fraction occurred in early active tuberculosis and may have represented antibody formation. In the advanced stages, all the globulin fractions were increased and presumably this indicated tissue destruction. There was a return to a normal serum picture when healing occurred. The change in albumin-globulin ratio of the blood and an increase in fibrinogen content have been observed by other workers; these studies have been summarized by Muller (1943) who also has discussed the role of the protein constituents of the blood in the increased sedimentation rate observed in tuberculosis.

Consideration of dietary recommendations for tuberculous patients over the past century shows a trend which parallels the development of nutrition as a science. Prior to 1900, forced feeding was considered desirable, however Goodbody, Bardswell and Chapman (1908) in England demonstrated that deleterious effects resulted from overfeeding even of healthy individuals and there was a gradual trend away from the practice. Recommendations for the protein content of the diet were

influenced by the investigations of Chittenden (1907) and there was tendency toward lowering the "proteid" in the treatment of tuberculosis (Fisher, 1908; Kellogg, 1908). However there also was the concept that high protein intakes are necessary for tissue repair from "wasting diseases", particularly as a result of observations that high losses of nitrogen accompanied elevated fevers as in typhoid fever. These studies have been summarized by McCann (1922) who studied the protein metabolism of tuberculous patients.

In McCann's work, complete collections of excreta were obtained for nitrogen analyses and the nitrogen value of the weighed diets was calculated. The results indicated that the urinary nitrogen excretion of the tuberculous patients was similar to that of normal persons but that the amounts excreted per kilogram of body weight was higher for the tuberculous series. McCann considered that the "wear and tear" quota probably varied with total metabolism rather than with total body mass. Positive nitrogen balances were obtained for the ten subjects with intakes between 60 and 90 gm. of protein. McCann recommended for tuberculous patients an intake of 90 gm. of protein and 2500 calories per day with the diet supplying approximately 150 gm. of fat so that there would be minimal respiratory function.

Although dietary recommendations for tuberculosis at the present time are based on adequate protein, calories, mineral and vitamin intakes for the healthy individual, there are wide variations in quantitative recommendations. Recommended daily allow-

ances made by the California State Department of Public Health include 2500 calories, 85 to 125 gm. protein, 0.8 gm. calcium, 12.0 mg. iron, 5000 I. U. vitamin A, 1.5 mg. thiamine, 2.2 mg. riboflavin, 15.0 mg. niacin and 100 to 125 mg. ascorbic acid (Sedgwick, 1946). Pottenger and Pottenger (1946) have advocated a diet high in protective foods: protein, 231 gm., calories, 3840, calcium, 1,697 mg., phosphorus, 3,007 mg., iron, 29 mg., vitamin A, 19,337 I. U., thiamine, 4,168 micrograms, riboflavin,5,086 micrograms, nicotinic acid, 48 mg., ascorbic acid, 120 mg., vitamin D, 107 I. U. and iodine, 29 mg.

Goesl (1947) has suggested that the cost of food for the tuberculous patient should be estimated on the basis of the cost per patient per period of arrestment rather than the cost per day, and that economy of time and money result if a highly nutritious diet at a somewhat greater cost will restore a patient to health in a shorter time than a less expensive diet of poor nutritive quality.

EXPERIMENTAL PROCEDURE

Subjects

Women with moderately advanced, active pulmonary tuberculosis who were bed-patients of the Ingham county sanatorium, Michigan were subjects of the study. The ages of the patients varied from 25 to 39 years. All of the subjects were married and five of the group had one or more children. The activity of the patients varied according to restrictions placed by the house physician. Subject DW was confined entirely to bed rest; the others were permitted to walk to the bathroom once daily. Occupational therapy such as sewing, knitting or reading in bed was encouraged for all for limited hours during the day. Chest x-rays were taken at regular intervals during the study and medical records were available for examination. There was no evidence of intestinal tuberculosis among the subjects; one subject MF, however had laryngeal tuberculosis.

Experimental Plan

The usual hospital diet was served throughout the study. The subjects were observed for a preliminary period of two weeks on their usual dietary routine before the first experimental period. The preliminary period consisted of one week of weighed food records followed by one week of collections of food and excreta. For the four experimental periods, adjustments in the riboflavin and nitrogen content of the diet were made by variations in milk intake.

The milk intake for periods one and two was 300 gm. daily and for periods three and four, the intake was increased to 1200 gm. daily. During periods two and four, a supplement of five mg. riboflavin was given each day. In addition, vitamin supplements equivalent to 300 I. U. of vitamin D, 5000 I. U. of vitamin A and 300 mg. of ascorbic acid were given daily for the entire study. Each experimental period consisted of two weeks for dietary adjustment followed by a seven day balance period.

Collection and treatment of samples

Each of the subjects was supplied with a Hansen dietetics scale and was taught to weigh and record servings of all food consumed during the fourteen weeks period. Any medication or drug which was taken by the subject was recorded. During the seven day balance period, additional portions of food were served to the patient. From these, aliquots equivalent to one-fifth of the weight of food eaten were collected and separate composites were made of solid foods, liquid foods and fat foods. Aliquots of solid and liquid foods were collected into 1N sulfuric acid; aliquots of fat food were preserved without acid. Composites of liquid food and solid food were prepared from the first three days, the second three days and the last day of the balance period. Each of the composites was sampled for riboflavin analyses and an aliquot was taken for preparation of an acid digest.

Fat foods as butter and heavy cream were not included in the composites for riboflavin analysis since these foods contain negligible amounts of riboflavin and also exhibit fluorescence from extraneous

substances. A seven day composite of foods high in fat content was prepared. The water soluble portion of the composite was separated from the fat by heating and subsequent cooling. This was added to the combined aliquots of liquid food for the seven day period.

Acid digests were made of seven day composites of liquid and fat food according to the procedure of Stearns (1929). The composites were made 20 percent acid by addition of sulfuric acid and the mixture was heated at low heat on an electric hot plate until it was uniform in color and consistency. The acid digest for the solid food was passed through a fine mesh sieve, made to suitable volume and samples stored in screw-top pharmaceutical bottles for nitrogen analyses. The liquid foods were transferred without sieving to a volumetric flask, made to volume and sampled. This procedure has been used previously in this laboratory and it has been found that satisfactory sampling is obtained if the flask is inverted and rotated fifty times before the first sample is taken and an additional twenty-five times before another sample is taken.

A gelatin, no. 0 capsule filled with carmine was given to the subjects before breakfast on the first day of the balance period. A second capsule was given before breakfast on the day following the balance period. All stools were collected and the seven day fecal collection included feces from the first appearance of the carmine up to but not including the first appearance of the second marker.

As a usual procedure, the fecal collection was made by the patient herself. The stool was passed into a paraffin-lined cardboard

container and tightly covered. For subject DW who was not permitted to walk to the bathroom, the stool was collected on a square of cellophane in a bedpan and transferred to the paraffin-lined container. The fecal collections were brought to the laboratory, weighed and placed under a measured quantity of 1N sulfuric acid. When several stools for one subject were collected, the stools were blended with 50 ml. absolute ethyl alcohol and 1N sulfuric acid, transferred quantitatively to a two-liter volumetric flask and made to volume with a measured quantity of acid. From this, samples were taken for riboflavin analysis, and an aliquot was taken for preparation of an acid digest. Combined aliquots for the seven day period were brought to a concentration of 20 percent acid with sulfuric acid and digested on an electric hot plate until uniform in color and consistency. When the digestion was completed, the contents were passed through a wire sieve into a two-liter volumetric flask, made to volume, thoroughly mixed and duplicate samples were stored for nitrogen analysis.

During the seven day balance period, 24 hour urine collections were made. The urine was collected in a bedpan and transferred immediately by the nurse into a three kg. brown glass bottle which contained 50 ml. of 2.5 N sulfuric acid. When the 24 hour collection was completed, the bottle was brought to the laboratory and the urine was transferred to a glass-stoppered, graduated cylinder and thoroughly mixed. The creatinine content of the urine was measured daily as a check on the accuracy of the 24 hour collection. An aliquot representing one-tenth of the total volume was taken and stored in the refrigerator

in a brown glass bottle for a composite for riboflavin analysis. A second aliquot representing one-fifth of the total volume was taken and stored under refrigeration for preparation of a composite for nitrogen analysis.

Complete collections were made of menstrual losses for each subject for each menstruation period during the study. The used napkins were digested with 20 percent sulfuric acid and the acid digest treated as described above for fecal samples. Analyses were made of the nitrogen content of the sample.

Nitrogen losses through sputum discharge

Tissues used by patients for sputum discharge were collected into erlenmeyer flasks containing sulfuric acid. The composite for the seven day period was digested and subsequently analyzed for nitrogen content. Seven day sputum collections for three subjects contained no measurable nitrogen. Therefore sputum collections were discontinued.

Estimation of the urinary excretion of a test dose

On the morning of the seventh day of the balance period, a test dose containing three mg. riboflavin[1], three mg. thiamine and 400 mg. ascorbic acid was administered orally. The urine excreted during the following 24 hours was collected in a brown glass bottle containing 100 ml. of 5 N sulfuric acid and 100 ml. of one percent metaphosphoric acid with two ml. of one percent 8-hydroxyquinone. Analyses were made of the ascorbic acid, riboflavin and thiamine content of the urine. The thiamine data will be presented elsewhere.

[1]. Acknowledgment is made to Merck and Co. for generous supplies of thiamine and riboflavin.

Chemical methods

Aliquots of food and feces were acid-hydrolyzed, adjusted to a pH of 4.5 and incubated overnight with polidase-S enzyme (Klocke, 1947). The riboflavin content of the extract was determined by a modification of the fluorometric procedure developed by Conner and Straub (1941) with adsorption on florisil and subsequent elution with a solution of pyridine and acetic acid. An aliquot of the eluate was treated with potassium permanganate and the excess permanganate was reduced with hydrogen peroxide. The amount of fluorescence was measured in a photofluorometer[1] which was standardized against a solution of riboflavin containing 0.1 microgram riboflavin, U. S. P. standard, per ml.

The riboflavin content of the urine was determined by the same procedure as modified by Keys (1944); sample blanks were obtained by photolytic destruction of riboflavin accomplished by exposure of urine aliquots to ultraviolet light for two hours.

The nitrogen content of the metabolic digests was determined by the Gunning-Arnold modification of the Kjeldahl procedure (Association of Official Agricultural Chemists, 1940). Creatinine was determined colorimetrically using a photelometer[2]. The procedure is based on the Jaffe reaction, i. e., that when a mixture of picric acid and sodium hydroxide is added to a dilute creatinine solution, a red compound is formed which, with the yellow of the excess picric acid, produces an

1. Coleman photofluorometer, Coleman Instruments, Inc.

2. Cenco-Sheard-Sanford photelometer, Central Scientific Co.

amber colored solution (Hoffman, 1941).

Urinary ascorbic acid was measured by a modification of the procedure of Bessey (1938). In this procedure, the amount of reduction of 2,6 dichlorophenolindophenol by ascorbic acid is measured photometrically[1].

[1]. Coleman spectrophotometer, Coleman Instruments, Inc.

RESULTS AND DISCUSSION

Description of subjects

Throughout the study, attempts were made to obtain as much information concerning the subjects as was possible through laboratory tests and medical records in order to possess an index of the individual variations in nutritional state and degree of tuberculosis. A physical description of the subjects is given in Table 3 together with the average weight changes and the caloric intakes for each subject for the different periods. During periods three and four, there was some attempt by the patients to limit amounts of high fat and carbohydrate foods in the diet either because of appetite restrictions or because of a desire to control weight. Nevertheless, the average caloric intake was increased approximately 400 calories when the milk intake was increased from 300 to 1200 gm. daily. Weights of the subjects were relatively constant throughout the experiment. There was a gain of six pounds for RH. DW was the only subject who was considered underweight. Her average weight was 48.7 kg. and her height was 166 cm.

Accessory biochemical tests throughout the study were the determinations of hemoglobin, red blood cell counts, urinary creatinine and urinary ascorbic acid. These values have been compared with values for healthy women. In this instance, health is defined as freedom from apparent signs of illness or nutritional deficiencies.

TABLE 3

Description of subjects

Subject	Age	Height	Weight			Average Caloric Intake*		
			Initial	End of Period 2	End of Period 4	Preliminary	Periods 1 and 2	Periods 3 and 4
	years	cm.	kg.	kg.	kg.	Cal./day	Cal./day	Cal./day
SW	26	159	52.7	51.8	53.2	1856	1416	1988
H	25	172	59.1	60.0	61.8	2612	1920	2318
G	33	158	58.2	58.2	59.1	1722	1678	1803
B	35	166	62.7	62.7	63.6	1968	1930	2479
F	39	161	64.6	64.1	64.6	1866	1624	1942
DW	34	166	48.2	48.6	49.6	1952	1813	2326

* By calculation.

The average hemoglobin and red blood cell counts are presented in Table 4. Subject DW had received an iron-containing supplement prior to the study and during the preliminary period. Since the hemoglobin and red blood cell counts for this subject were within the range for healthy individuals at the beginning of the first experimental period, the iron supplement was discontinued. Values for college women who were apparently in good health were reported by Ohlson and coworkers (1944) from a study of 4550 individuals. The mean hemoglobin concentration for the group was 13.4 ± 0.017 (standard error) gm. per 100 ml. of blood and the mean red blood cell count was 4.56 ± 0.009 million cells per cmm. The range for hemoglobin values represented by the mean plus or minus two standard deviations was 11.08 to 15.72 gm. per 100 ml. In the preliminary period, the value for B was below this range, but all values for the tuberculous individuals were within these limits during the experimental periods. The corresponding range for red blood cell counts for healthy women was 3.80 to 5.32 million cells per cmm. and the values for tuberculous subjects were within this range. The individual variations from the end of period two to the end of period four were not consistently in the direction of an increase or decrease of values.

The average creatinine excretions for the subjects are given in Table 5. The mean values for each individual remained constant during the successive periods. The average excretion for subject DW was lower than that of the other subjects but all values were within the range of 0.89 to 1.48 gm. per 24 hours which was the range observed by

TABLE 4

The erythrocyte count* and hemoglobin** of the blood

of six women with active tuberculosis

Diet Period	Subject SW		Subject H		Subject G		Subject B		Subject F		Subject DW	
	R.B.C.	Hb.	R.B.C.	Hb.	R.B.C.	Hb.	R.B.C.	Hb.	R.B.C.	Hb.	R.B.C.	Hb.
Preliminary period	5.11	15.3	4.05	13.5	4.64	15.8	4.74	10.5	4.71	12.0	4.81	12.5
At end of period 2	4.57	14.5	3.93	13.2	4.10	14.2	4.57	12.9	4.56	14.8	4.78	13.1
At end of period 4	4.61	14.5	3.71	12.8	4.61	14.8	5.02	11.8	5.19	13.2	-	-

* Erythrocyte count expressed as million cells per cmm.

** Hemoglobin expressed as gm. per 100 ml.

TABLE 5

Creatinine excretions of six women with active tuberculosis

on different intakes of nitrogen and riboflavin

Period	Average Daily Excretion of Creatinine in Urine, Gm. per 24 Hr.						
	SW	H	G	B	F	DW	Mean
Preliminary: Usual diet			1.26 ± 0.34*	1.01 ± 0.07	1.19 ± 0.05	0.93 ± 0.17	1.10
Per. 1. 300 gm. milk daily			0.96 ± 0.33	1.05 ± 0.19	1.26 ± 0.04	0.92 ± 0.18	1.05
Per. 2. 300 gm. milk and 5 mg. riboflavin daily	0.97 ± 0.11	1.32 ± 0.14	1.15 ± 0.18	1.08 ± 0.07	1.26 ± 0.06	0.90 ± 0.05	1.11
Per. 3. 1200 gm. milk daily	1.03 ± 0.13	1.17 ± 0.21	1.22 ± 0.15	1.16 ± 0.07	1.37 ± 0.10	0.99 ± 0.46	1.16
Per. 4. 1200 gm. milk and 5 mg. riboflavin daily	1.07 ± 0.11	1.23 ± 0.06	1.09 ± 0.38	1.13 ± 0.16	1.29 ± 0.07	0.94 ± 0.14	1.13

*Standard deviation

Smith (1942) for the creatinine excretion of six healthy women. Friedemann (1948) found that the average creatinine values were increased when men were given a high protein diet following a low protein diet. This increase was not apparent for the subjects of this study, probably because the protein intake of the first two periods was not restricted below an intake of 40 gm. for any of the subjects.

Low excretions of urinary ascorbic acid are frequent among tuberculous patients on an unsupplemented diet. For this reason, a dietary supplement of 300 mg. ascorbic acid was given to the subjects daily. It would be expected from previous work (Brewer, 1949) that this intake would be adequate to provide a satisfactory state of vitamin C nutrition for the subjects and that the excretion of ascorbic acid following a test dose would be within the range of 25 to 85 percent of the dose per 24 hours, comparable to that for healthy women. It is apparent from Table 6 that the subjects were excreting urinary ascorbic acid according to the pattern established for healthy women.

The descriptive data which have been presented indicate that the subjects were in a satisfactory state of ascorbic acid nutrition, that the red blood cell counts and hemoglobin of the blood were essentially that of healthy individuals and that the creatinine excretions were similar to the values reported for individuals free from disease. In addition daily supplements of vitamin A and vitamin D were provided so that the intake of these nutrients although not large was adequate for non-tuberculous individuals.

TABLE 6

Urinary ascorbic acid excretion

of six women with active tuberculosis

Period	Percent Excretion of a Test Dose						
	SW	H	G	B	F	DW	Avg.
Preliminary: Usual diet				42	61	61	55
Per.1. 300 gm. milk daily			74	36	41	78	57
Per.2. 300 gm. milk and 5 mg. riboflavin daily	25	100	54	94	74	39	64
Per.3. 1200 gm. milk daily	22	72	38	66	96	30	54
Per.4. 1200 gm. milk and 5 mg. riboflavin daily	29	78	28	66	100	60	60

Additional information concerning the subjects is given in the temperature records, record of sputum tests and the individual case records which are included in the Appendix. Medical treatment for the individuals differed prior to the study and in some cases operative procedures were used following the experimental period. During the study, however, the treatments were uniform with the exception that for subject DW there was a bronchoscopy on Monday mornings with some aspiration of fluid and subject MG received streptomycin during period four. Sputum tests were positive for all subjects during the study. There were fluctuations in the daily temperatures, but if 98.6° F. is assumed to be a normal temperature for non-ambulatory individuals at three o'clock in the afternoon, the tuberculosis was essentially afebrile. There was no measurable change in the chest x-rays. Since each of the experimental periods was limited to three weeks and since the clinical tests failed to show progress or lack of progress from the disease, it was not possible to relate the dietary routines to recovery from the disease.

Nitrogen metabolism of tuberculous women

The average retentions of nitrogen for the preliminary period and the four experimental periods are given in Table 7. The mean intake of nitrogen for the preliminary period was 10.10 gm. per day. The milk intake for this period was not controlled so these data were not used for statistical analyses. There was an average difference of 4.4 gm. of nitrogen or 28 (N x 6.25) gm. of protein for periods three and four as compared with the nitrogen intake for periods one and two.

TABLE 7

The average retentions of nitrogen by six women with active tuberculosis

Diet Periods	Nitrogen Intake			Nitrogen Retention
	Gm. per 24 hr.	Gm. per kg. body weight	Gm. per square meter body surface	Gm./24 hr.
Preliminary study: Usual diet	10.10 ± 0.56* (9.00 - 12.27)	0.18 ± 0.012 (0.14 - 0.21)	6.26 ± 0.34 (5.35 - 7.22)	0.65 ± 0.44 (-0.46 - 1.88)
Period 1: 300 gm. milk daily	8.90 ± 0.38 (7.57 - 9.88)	0.16 ± 0.004 (0.14 - 0.17)	5.50 ± 0.15 (5.01 - 5.90)	1.15 ± 0.20 (0.51 - 1.78)
Period 2: 300 gm. milk and 5 mg. riboflavin daily	8.85 ± 0.49 (6.55 - 10.04)	0.15 ± 0.008 (0.12 - 0.18)	5.47 ± 0.25 (4.34 - 5.91)	0.57 ± 0.38 (-1.16 - 1.28)
Period 3: 1200 gm. milk daily	13.00 ± 0.59 (10.48 - 14.31)	0.23 ± 0.008 (0.20 - 0.26)	8.04 ± 0.25 (6.94 - 8.53)	1.95 ± 0.29 (1.01 - 3.21)
Period 4: 1200 gm. milk and 5 mg. riboflavin daily	13.62 ± 0.51 (12.27 - 15.38)	0.24 ± 0.008 (0.20 - 0.26)	8.43 ± 0.20 (7.70 - 9.05)	1.48 ± 0.40 (0.17 - 2.53)

* Standard error of the mean

The differences in nitrogen intakes for periods one and two were not statistically significant ('t' test, Fisher, 1938); neither were the differences between the nitrogen intakes of periods three and four significant.

The menstrual losses of nitrogen by the subjects were not calculated with the total output but are summarized in Table 8. The average daily losses ranged from 0.015 to 0.065 gm. nitrogen.

The nitrogen retention data expressed as gm. per 24 hours were treated by analysis of variance. A summary of the analysis is given in Table 9. Of the total 23 degrees of freedom which were available, there were three degrees of freedom for differences between means of periods, four degrees of freedom for individual differences and 15 degrees for the interaction (error term) of periods and individuals. Differences between the means of periods were significant ($^{F}0.05$). The three degrees of freedom for period differences were further divided with one degree of freedom for a comparison of periods one and three against periods two and four, and one degree of freedom for a comparison of periods one and two against periods three and four. There was not a significant difference in nitrogen retention when periods one and three were compared with periods two and four. This was essentially a comparison on constant nitrogen intake of nitrogen retention at moderate riboflavin intakes with nitrogen retentions at high riboflavin intakes. Since the differences were not significant, it can be assumed that for these experimental conditions, variations in riboflavin intake did not influence the retention of nitrogen. Therefore the nitrogen metabolism

TABLE 8

Menstrual losses of nitrogen by six women with active tuberculosis

Menstrual Period	Subject					
	SW	H	G	B	F	DW
	gm./24 hr.	gm./24 hr.	gm./24 hr.	gm./24 hr.	gm./24 hr.	gm./24 hr.
1	1.01	0.43	0.40	1.26	1.10	2.09
2	0.57	Lost	0.44	1.52	2.00	2.47
3	0.88	0.60	0.39	1.37	1.69	1.81
4	0.60		0.24	1.04	0.70	
Total Nitrogen loss	3.06		1.47	5.19	5.49	6.37
Avg. loss per day, gm.	0.031		0.015	0.053	0.056	0.065

TABLE 9

Analysis of variance of nitrogen retention data

Source of variation	F value obtained	Predicted F 0.05	Predicted F 0.01
Between means of periods	3.65	3.29	5.42
Comparison of periods 1, 3 vs 2,4	3.00	4.54	8.68
Comparison of periods 1, 2 vs 3, 4	7.92	4.54	8.68

data may be considered independently of period differences in riboflavin intake.

The nitrogen intakes for the various periods also are expressed in Table 7 as gm. per kg. of body weight and as gm. per square meter of body surface. Nitrogen retentions expressed as gm. per 24 hours have been plotted against nitrogen intake expressed as gm. per kg. of body weight in the scatter diagram shown in Figure 2. A regression line was drawn for these points from the predicting equation calculated according to the equation, $Y = a + bx$. The intercept of the line at equilibrium occurred at an intake of 0.11 gm. per kg. of body weight. The average body weight of the subjects was 57.5 kg.; therefore the average requirement for equilibrium by these subjects would be 39.5 gm. of protein daily. For a 56 kg. woman, this corresponds to an intake of 38.5 gm. of protein.

The surface area for each individual was calculated according to the formula of DuBois (1924):

$$\text{Log surface area} = (\text{Log weight [kg.]} \times 0.425) + (\text{Log height [cm.]} \times 0.725) + 1.8564$$

This equation was developed from a limited number of experimental observations and therefore its validity can be readily criticized. However it does express a weight-height relationship and it has been widely used as an expression of total surface area.

In Figure 3, the nitrogen retentions expressed as gm. per 24 hours have been plotted against nitrogen intakes expressed as gm. per square meter. An intake of 4.16 gm. per square meter would be predicted from these data for nitrogen equilibrium. Since the average body surface area for the six patients was 1.62 square meters, this would be equiva-

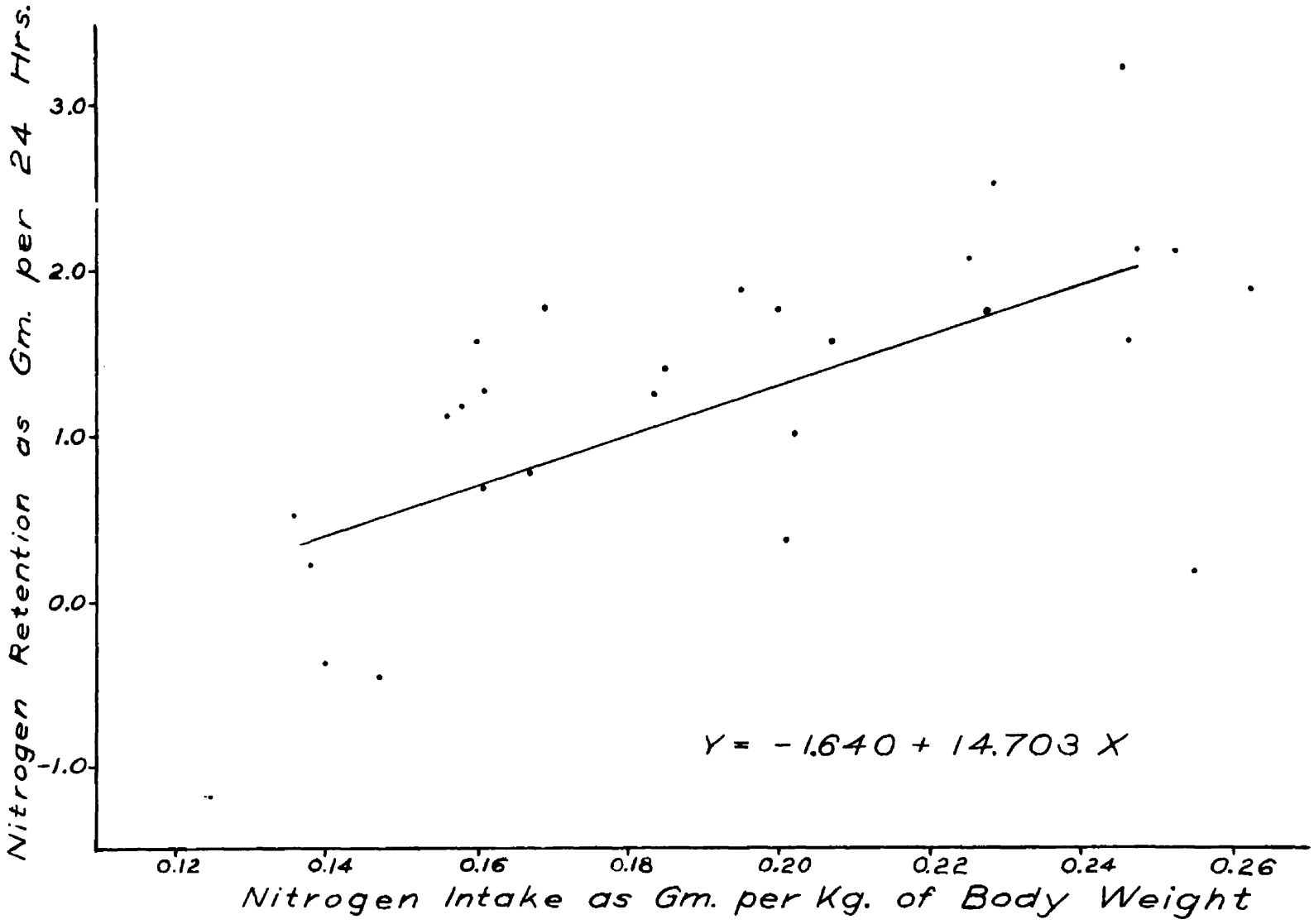

Fig. 2 Regression of nitrogen balance upon intake expressed as grams per kilogram of body weight.

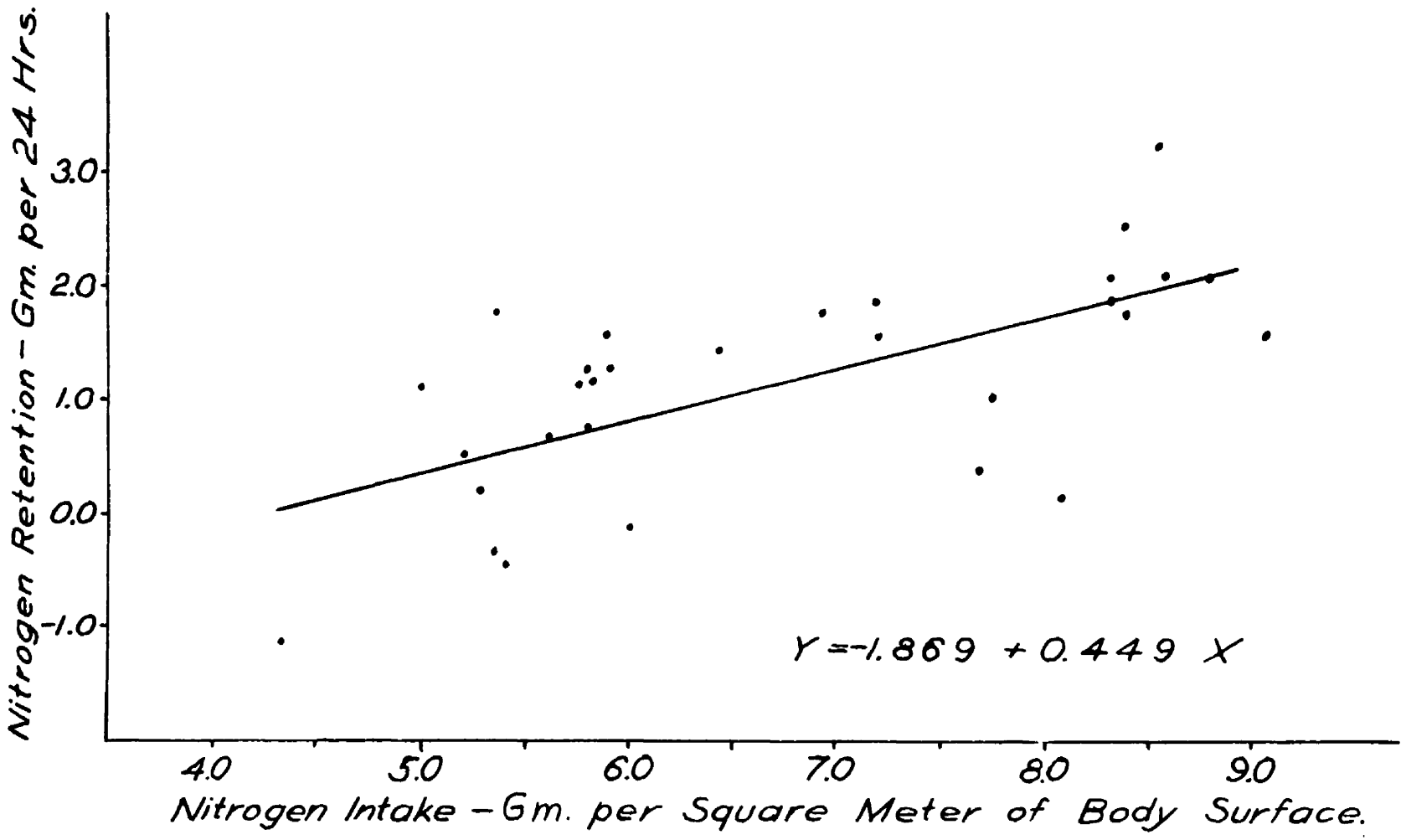

Fig. 3 Regression of nitrogen retention upon intake expressed as gm. per square meter of body surface.

lent to an intake of 42 gm. of protein daily.

There is little difference in the predicted values for nitrogen equilibrium when the intake was expressed as gm. per kg. of body weight or when the intake was expressed as gm. per square meter of body surface. The dispersion of the individual data about the regression lines also is similar. This is not in accord with Hegsted (1948) who considered that nitrogen retention data had a closer relationship to nitrogen intake expressed as gms. per square meter of body surface than as kg. of body weight. Bricker (1945) assumed that the nitrogen retention of an individual was related to his basal metabolism and therefore expressed nitrogen metabolism as a function of basal calories. Since basal metabolism values were not obtained for the tuberculous women, this comparison could not be made.

To obtain a comparison of these data with data for healthy women, the individual values were plotted about a regression line which was drawn from the predicting equation reported by McKay (1942) from studies with mid-western college women. The distribution is shown in Figure 4 and it is apparent that the values fall quite uniformly about the regression line. Thus the relationship of nitrogen intake and retention of these subjects was similar to that for apparently healthy college women. Both groups were retaining nitrogen at the higher dietary intakes. These data confirm the observations of McCann (1922) that the nitrogen required for equilibrium is essentially the same for tuberculous as for non-tuberculous individuals.

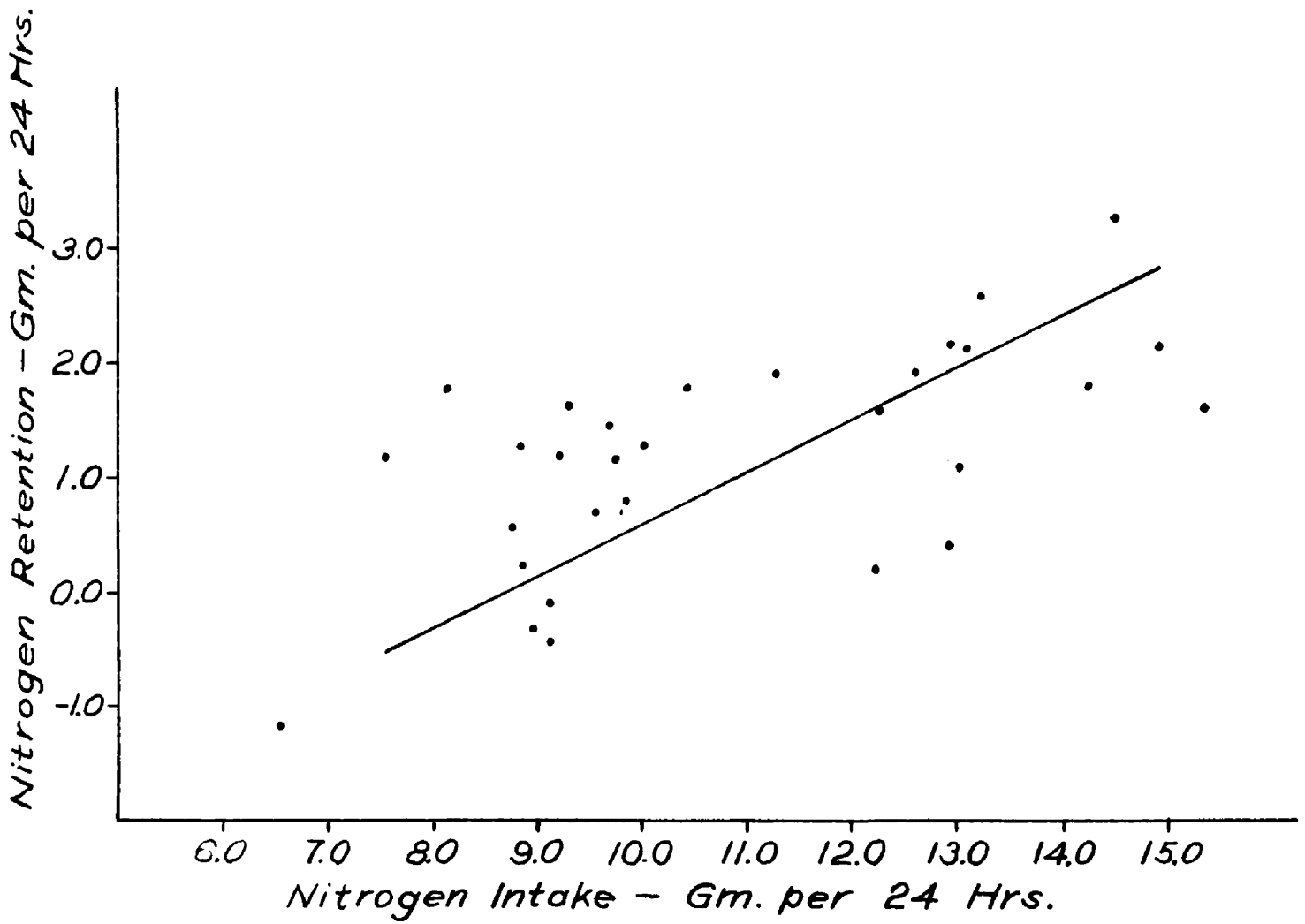

Fig. 4 Distribution of nitrogen retention data for tuberculous women about regression line predicted by McKay (1942) from values for healthy women.

If these data were compared with those of the studies of Bricker (1945) or of Hegsted (1948), the apparent requirement for nitrogen equilibrium by tuberculous women would exceed that of healthy individuals. However the experimental conditions of the study compare more closely to the conditions described by McKay than for the Illinois or Harvard studies, and the range of nitrogen intakes for these subjects was comparable to those reported by McKay. Therefore the assumption that the nitrogen metabolism of these subjects was similar to that of apparently healthy college women seems justified.

The ages of the subjects in this study were higher than for the subjects of McKay's study; however there is no evidence that age differences between 20 and 40 influence nitrogen metabolism. The change in nitrogen intake and retention relationship reported by Ohlson (1948) was for women who had completed the menopause.

Miller (1945) found that there was an increase in urinary nitrogen excretion when healthy young men were confined to bed rest. However, bed rest apparently did not influence the nitrogen metabolism of the tuberculous patients since these data compared closely with values for active individuals. This is in agreement with Howard (1946) who observed that patients debilitated by chronic illness or undernutrition were in positive nitrogen balance under conditions of bed rest and diet which resulted in nitrogen loss for healthy vigorous persons.

Estimated nitrogen requirement of tuberculous women.

The physiological significance of the retention of nitrogen at higher intakes by the adult has not been adequately defined. It is recognized that the animal body possesses the ability of adaptation to different planes of nutrition (Mitchell, 1944) but it also is recognized that certain advantages in terms of prolonged life and vitality of the individual occur at higher planes of nutrition (Sherman, 1946). For the tuberculous individual, an intake of protein adequate for tissue repair and for retention of nitrogen seems desirable. McCann (1922) estimated that an intake of 60 to 90 gm. of protein was adequate. All of the subjects in this series were at equilibrium or storing nitrogen at an intake of 60 gm. protein. It is suggested that a dietary intake of 80 gm. of protein would provide an adequate margin of safety to permit nitrogen retention by the individuals and also would constitute a practical recommendation from the standpoint of diet planning.

Riboflavin metabolism of tuberculous women.

The mean values for the riboflavin intake and excretion of the six patients are given in Table 10. The standard deviation of the mean and the range of values are included. The subjects had received varying amounts of riboflavin supplement prior to the investigation; since the subjects were studied on their usual routines during the preliminary period, the range of intakes was wide and the standard deviation was large. Subject SW received 1.83 mg. of riboflavin, RH, 4.21 mg. of riboflavin and each of the other four subjects received

TABLE 10

Average intakes and excretions of riboflavin

of six women with active tuberculosis

Diet Period	Riboflavin Intake			Urinary Excretion of riboflavin		Fecal Excretion of Riboflavin
	Mg. per 24 hr.	Mg. per Kg. Body Weight	Mg. per 1000 Cal.	Mg. per 24 hr.	Test Dose Response	
					%	mg./24 hr.
Preliminary Study: Usual diet	7.21 ± 1.36 (1.83 - 9.37)	0.126 ± 0.024 (0.035 - 0.192)	3.75 ± 0.79 (0.99 - 5.44)	4.83 ± 1.17 (0.60 - 7.24)	60 ± 7 (23 - 74)	0.94 ± 0.06 (0.78 - 1.07)
Per.1. 300 gm. milk daily	1.35 ± 0.06 (1.18 - 1.47)	0.024 ± 0.0004 (0.022 - 0.025)	0.75 ± 0.04 (0.63 - 0.87)	0.31 ± 0.04 (0.20 - 0.45)	39 ± 5 (26 - 62)	0.68 ± 0.10 (0.27 - 0.99)
Per.2. 300 gm. milk and 5 mg. riboflavin daily	6.41 ± 0.08 (6.21 - 6.68)	0.113 ± 0.006 (0.099 - 0.137)	3.91 ± 0.19 (3.35 - 4.61)	3.53 ± 0.49 (1.08 - 4.29)	65 ± 6 (41 - 82)	0.68 ± 0.08 (0.37 - 1.00)
Per.3. 1200 gm. milk daily	2.60 ± 0.09 (2.23 - 2.89)	0.046 ± 0.002 (0.043 - 0.054)	1.24 ± 0.06 (1.09 - 1.43)	1.08 ± 0.07 (0.88 - 1.29)	55 ± 3 (42 - 64)	0.83 ± 0.07 (0.60 - 1.00)
Per.4. 1200 gm. milk and 5 mg. riboflavin daily	7.79 ± 0.05 (7.56 - 7.93)	0.137 ± 0.006 (0.122 - 0.161)	3.67 ± 0.22 (2.89 - 4.21)	5.62 ± 0.63 (3.47 - 7.08)	72 ± 7 (43 - 88)	0.87 ± 0.08 (0.61 - 1.14)

from 9.22 to 9.37 mg. daily. These subjects received 7.5 mg. riboflavin from vitamin supplements. Previous studies in the laboratory (Brewer, 1946) indicated that the urinary excretion of riboflavin adjusted to a constant value after six days on a controlled intake. Therefore it may be assumed that fourteen days of dietary adjustment for each experimental period were adequate for adjustment to the riboflavin intake for that period.

The range of riboflavin intake for the four experimental periods was narrow and the standard deviations were small. The mean intake for period one was 1.35 mg. and the mean excretion of riboflavin in the urine was 23 percent. For period three, the dietary riboflavin was 2.60 mg. and the daily excretion represented 42 percent of this amount. Periods two and four were periods of high riboflavin intakes. For period two with a daily average intake of 6.41 mg., the percentage excretion in the urine was 55 and for period four, with a daily average intake of 7.79 mg., 72 percent of the intake was excreted in the urine.

A close relationship of riboflavin excretion to intake expressed as mg. per kg. of body weight is shown in the graph in Figure 5. In comparison there is a wider dispersion of values about the regression of riboflavin excretion upon intake expressed as mg. per 1000 calories which is shown in Figure 6. The thiamine requirement of an individual usually is expressed in terms of his caloric intake; this practice was at first used in respect to riboflavin intakes. However the distribution of values in Figures 5 and 6 suggest that the riboflavin requirement is not as closely related to

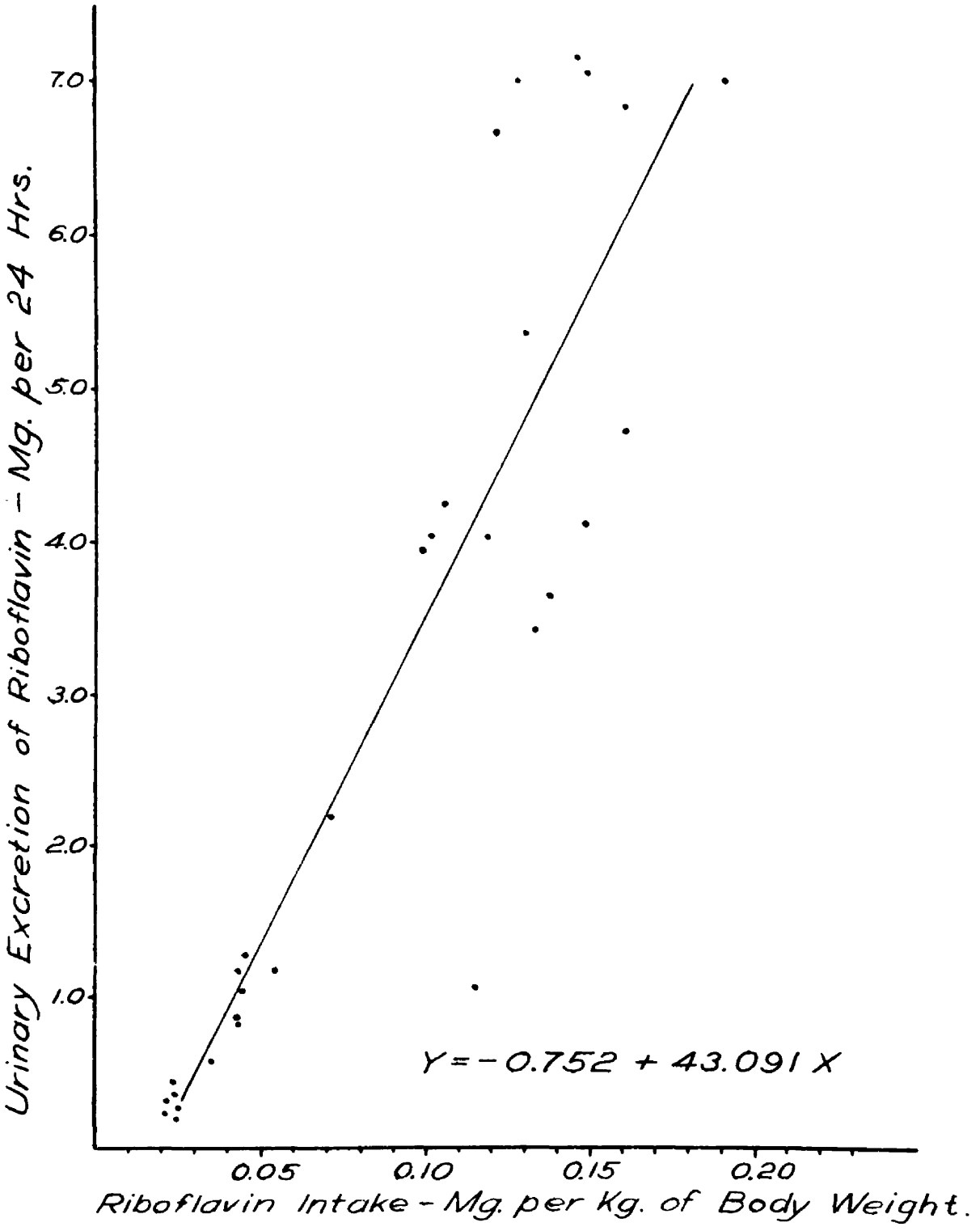

Fig. 5 Regression of urinary riboflavin excretion on riboflavin intake expressed as mg. per kilogram body weight.

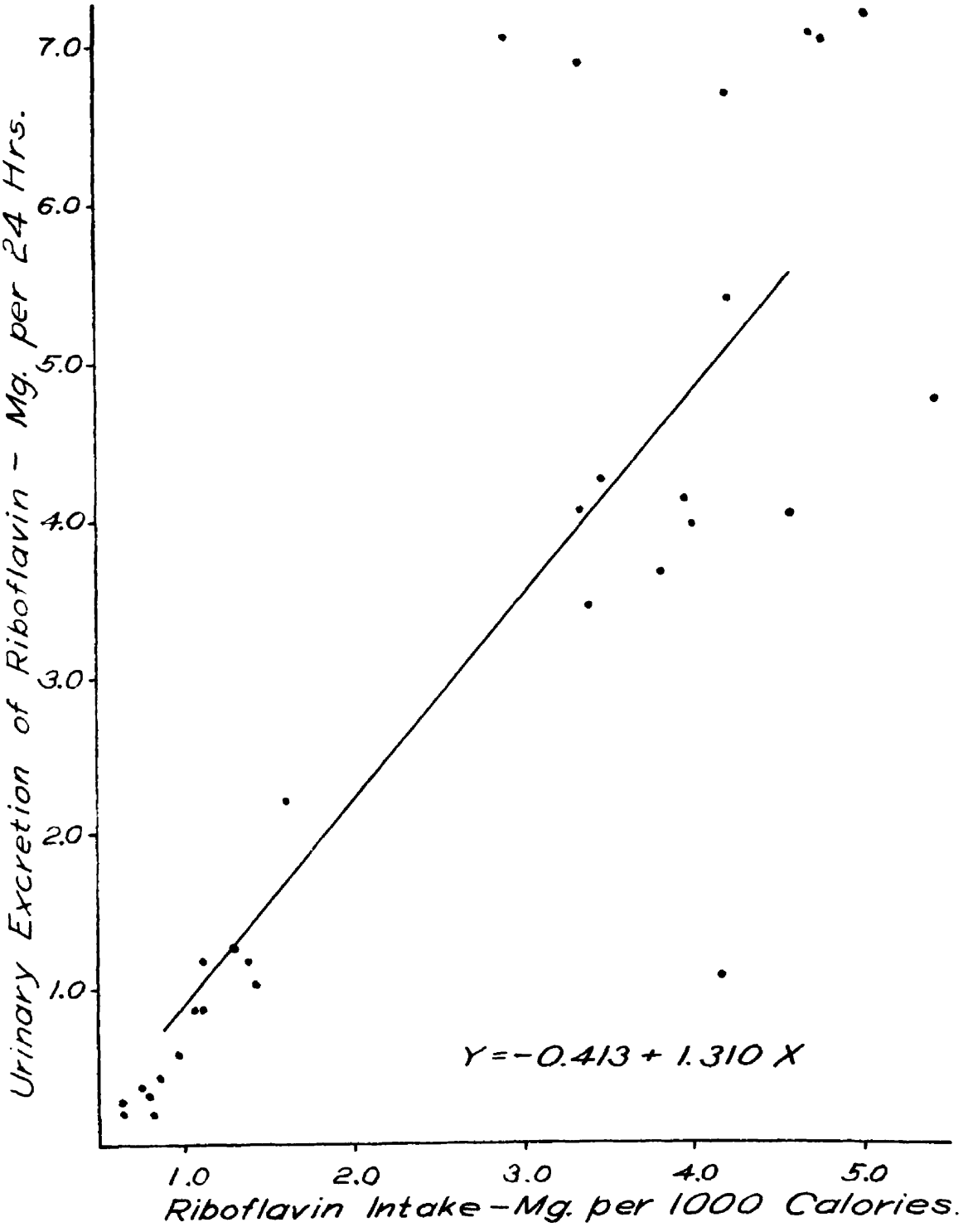

Fig. 6 Regression of urinary riboflavin excretion upon riboflavin intake expressed as mg. per 1000 calories.

the caloric intake of the individual as to the total body mass. This was recognized in the last revision of the recommended dietary allowances made by the Foods and Nutrition Board of the National Research Council (1948); recommendations for riboflavin intake were made irrespective of the activity of the individual whereas in earlier recommendations, the suggested allowance varied with the degree of physical activity.

A comparison of daily urinary excretions of riboflavin by the six tuberculous patients with those of healthy women is shown in Figure 7. The regression lines were drawn from predicting equations which were reported previously (Brewer, 1946) and which were calculated from riboflavin intake and excretion data from the literature and from studies in our laboratory. The two predicting equations were calculated from riboflavin intakes of 0.5 mg. to 2.0 mg. and from riboflavin intakes of 1.3 mg. and above. The scatter of the individual data for the tuberculous patients has been superimposed on the graph. These values do not vary from the patterns established for healthy women as preliminary studies indicated, and it is apparent that the relationship of riboflavin intake and excretion for these patients is similar to that for women free from the disease.

The average test dose responses were 39 and 55 percent for periods one and three and 65 and 72 percent for periods two and four (Table 10). Comparative values for measurements of test dose response under similar conditions are available for healthy women only at the lower riboflavin intakes of periods one and three. From data previously published for nine college women with a dietary intake of 1.6 mg. riboflavin, the

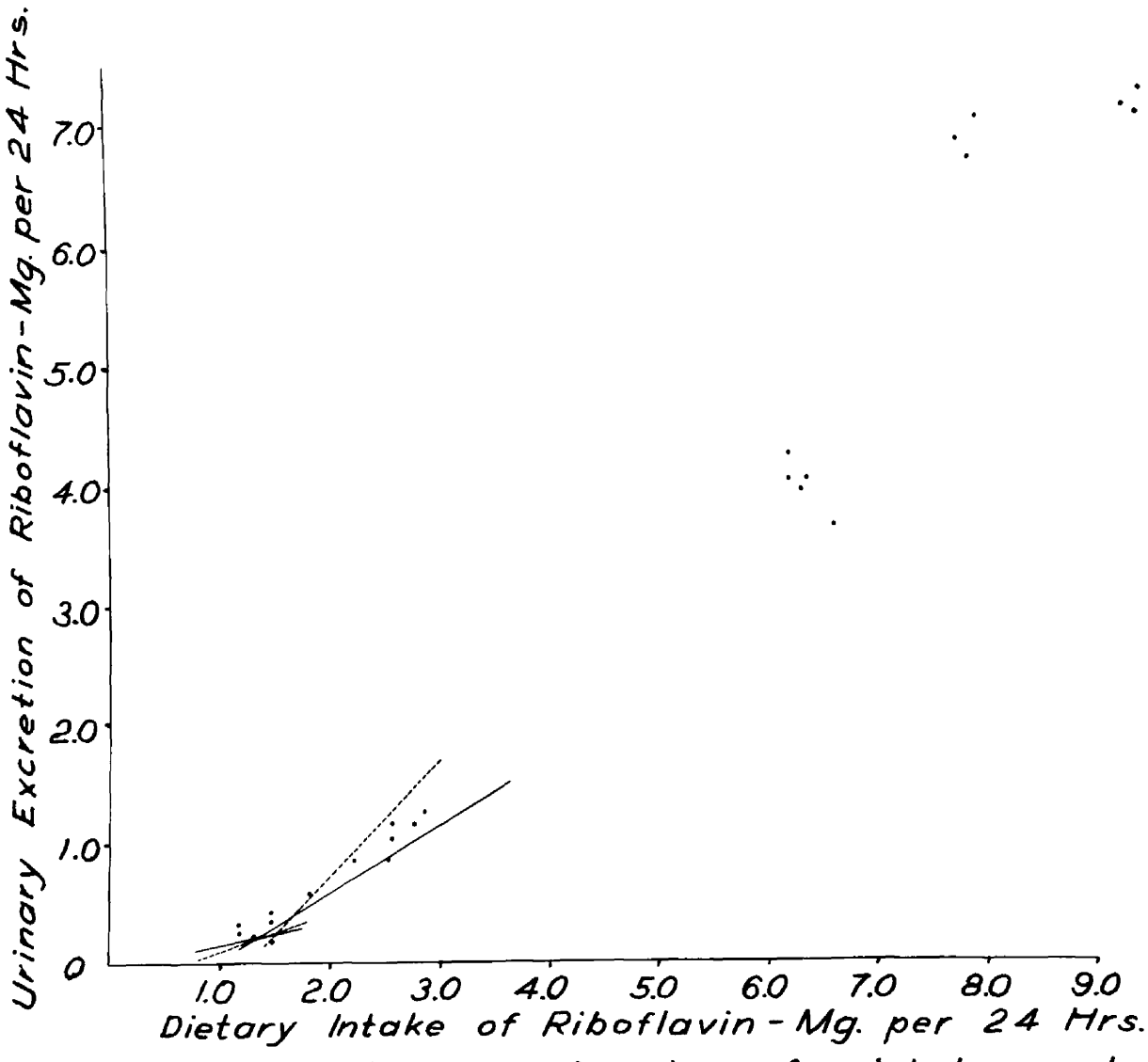

Fig. 7 Distribution of values for intake and urinary excretion of riboflavin by tuberculous women about regression lines predicted by Brewer (1946) from values for healthy women.

excretion of a three mg. test dose was 31 percent with a range of 27 to 38; for a dietary intake of 2.73 mg., the percent excretion of four women was 56 with a range of 51 to 60. Thus values for tuberculous subjects in periods one and three were similar to those for healthy women who had been receiving slightly higher riboflavin intakes.

The values given in Table 10 for percent urinary excretion of riboflavin following a three mg. test dose were calculated according to the equation:

$$\% \text{ test dose} = \text{Excretion after test dose (24 hr.)} \div (\text{Riboflavin in diet} + 3 \text{ mg.}) \times 100$$

This represents the relationship of the excretion of riboflavin in the urine following the test dose to the total riboflavin intake for the day. This procedure for calculating the test dose response was used for riboflavin studies with college women in this laboratory. Therefore the two series were comparable. Another procedure for calculation of percent excretion of the test dose is based upon the increment in urinary excretion which occurs following a test dose of riboflavin and is calculated according to the equation:

$$\% \text{ test dose} = \text{Excretion after test dose (24 hr.)} - \text{basal excretion (Avg. for 24 hr.)} \div 3 \text{ mg.} \times 100$$

This method of calculation has been used in this laboratory for survey studies which did not include food collections and analyses. This method of calculation also has been used by Melnick (1945) in

vitamin bioassay procedures. In Melnick's study, a basal diet was fed previous to administration of the test dose so that basal urinary excretions were constant preceding the test dose. In the present study, the variations of basal urinary excretion of riboflavin were greater among subjects in periods of high riboflavin intake than for periods of moderate riboflavin intake.

A comparison of values calculated by the two methods is given in Table 11. In each case the standard error is higher when the calculation is based upon the increment in excretion than when the calculation is based upon the total day's intake of riboflavin. The widest variations were obtained for periods three and four. The magnitude of the values calculated by the two methods is of the same degree and the two methods apparently would yield similar information.

The fecal excretion of riboflavin was relatively constant for five of the six subjects within each period. Wide variations were observed for subject DW. The average fecal excretions were higher for periods of increased milk intake than at restricted milk intakes. For periods one and two, the average fecal excretion was 0.68 mg. and for periods three and four, the average excretion was 0.85 mg. per 24 hours. These data do not indicate the source of the extra fecal riboflavin for periods three and four. The hypothesis that greater intestinal synthesis occurred during the periods of increased milk intake seems acceptable in view of various reports in the literature. Moreover the increase in excretion for the last two periods apparently was unrelated to riboflavin intake since the riboflavin supplements were given during periods two and four. Since the fecal riboflavin values were related to the character of

TABLE 11

Comparison of two procedures for calculation

of percent urinary excretion of test dose of riboflavin

	Percent Urinary Excretion of 3 mg. Test Dose of Riboflavin							
	Period 1		Period 2		Period 3		Period 4	
Subject	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
	%	%	%	%	%	%	%	%
SW	41	42	79	72	80	59	-	-
H	47	36	61	70	52	47	38	43
G	41	30	94	41	55	42	65	72
B	23	26	99	75	74	60	64	81
F	69	62	121	82	82	64	100	88
DW	46	40	37	52	66	55	52	77
Mean	44.5	39.3	81.8	65.3	68.2	54.5	63.8	72.2
Standard error	6.0	5.1	12.1	6.3	5.2	3.4	9.4	7.1

(1) % of test dose $= \frac{\text{Excretion after test dose (24 hrs.) - basal excretion}}{\text{3 Mg. riboflavin}} \times 100$

(2) % of test dose $= \frac{\text{Excretion after test dose (24 hrs.)}}{\text{Dietary intake of riboflavin + 3 mg.}} \times 100$

the diet and since urinary riboflavin values were directly related to the dietary intake of riboflavin, it would seem that absorption of synthesized riboflavin did not occur in measurable amounts under conditions of the study.

The range of fecal values for these subjects was similar to the range of values obtained for six college women on a diet containing 1.06 mg. riboflavin daily (Table 12). These women were subjects of nitrogen metabolism research in progress concurrently with this study; the riboflavin metabolism was studied for one five-day balance period to obtain fecal excretion values for comparison with the tuberculous subjects. The range of fecal excretions for the six women was 0.45 to 1.20 mg. per 24 hours. The women were given an adequate diet of mixed foods which supplied approximately 40 gm. of protein daily. The test dose responses for the women were much lower than for the tuberculous patients; however the dietary intake also was lower than the intakes studied for tuberculous women.

Interrelationship of nitrogen and riboflavin metabolism

Metabolic studies with college women reported by Oldham and coworkers (1947) indicated an inverse relationship of nitrogen balance and riboflavin excretion. The data presented here were examined to determine whether this relationship also could be demonstrated in tuberculosis. Analysis of covariance was used to determine the correlation coefficients for nitrogen retention and riboflavin excretion. A summary of the covariance analysis for nitrogen retention with daily riboflavin excretions is given in Table 13 and the covariance analyses for nitrogen retention with test dose responses is given in Table 14. In either case

TABLE 12

Daily intakes and excretions of riboflavin by five college women on a controlled diet with restricted nitrogen intake

Riboflavin Intake and Excretion	Subject					
	Gr	Ha	Be	Ri	Ro	Avg.
(a) Intake						
1. Dietary riboflavin, mg. per day	1.06	1.06	1.06	1.06	1.06	1.06
(b) Excretion						
1. Urinary riboflavin, mg. per day	0.05	0.18	0.17	0.05	0.07	0.10
2. Fecal riboflavin, mg. per day	0.62	0.64	1.20	0.71	0.45	0.72
3. Urinary riboflavin, after test dose*, mg. per day	0.46	0.56	1.23	0.61	0.31	0.63
4. Percent of test dose excreted	11.2	13.8	30.4	14.9	7.5	15.6

* Test dose, 3 mg. riboflavin

TABLE 13

Analysis of covariance of nitrogen retentions and urinary excretions of riboflavin; values for six tuberculous women

Source of Variation	Degrees of Freedom	Correlation Coefficient	Regression
Total	23		
Between means of periods	3	-0.184	-0.770
Within periods	20	-0.487*	-0.595

* Probability, < 0.05

TABLE 14

Analysis of covariance of nitrogen retentions and urinary excretions of riboflavin following a test dose; values for six tuberculous women

Source of variation	Degrees of Freedom	Correlation Coefficient	Regression
Total	22		
Between means of periods	3	-0.244	-6.319
Within periods	19	-0.543*	-9.002

* Probability, < 0.02

the correlation coefficients for within periods are the values of interest; the effects of variations in intakes were removed from the total correlation by isolation of the correlation for between means of periods. The analyses indicate that for these data, riboflavin excretion, whether measured by daily excretion or following a test dose was negatively correlated with nitrogen balance and that the relationship was statistically significant.

Since riboflavin frequently occurs in living tissue in the bound form with protein as flavo-protein, it would be expected that within certain limits, an interrelationship of the two nutrients would exist. In this study, the relationship has been demonstrated at a much higher plane of riboflavin nutrition than in the study reported by Oldham in which the nitrogen intake was increased from five gm. to 20 gm. and the riboflavin intake was controlled at intakes of 0.86 to 1.52 mg.

It is difficult to explain why the inverse relationship between nitrogen retantion and riboflavin excretion could be demonstrated by Oldham and in the present study whereas Hagedorn (1945) and Mickelsen (1945) were unable to observe such a relationship among their subjects. The correlation of nitrogen retention and riboflavin excretion within periods for this study cannot be explained by variations of riboflavin intake among subjects within periods since the range of intakes was narrow and the standard deviations were small.

It would seem that the dietary requirement for riboflavin is variable, depending upon the protein content of the diet. If the protein intake is low, the apparent riboflavin requirement is correspondingly increased. Therefore if the tuberculous individual is

losing nitrogen or if the retention of nitrogen is low there may be impaired utilization of riboflavin.

Estimated riboflavin requirement for tuberculous women

The values for the percentage excretion of a test dose by the tuberculous women were lower for period one than for the other three periods (Table 10). The mean percent response of the test dose for period one was 39 and the range of values was from 26 to 62 percent. When the intake of riboflavin was 2.6 mg. daily, the test dose response ranged from 42 to 64 percent. The lower limit of this range was similar to that for the two periods of high riboflavin intake. For period one, the average daily excretion of riboflavin in the urine was 23 percent of the intake. When the intake was increased to 2.6 mg. in period three, the percentage excretion was 42 percent. There were no apparent signs of clinical deficiency of riboflavin at an intake of 1.35 mg. daily, but the lower test dose response and urinary excretion of riboflavin at this intake suggest that this amount was not adequate for tissue saturation. There was no apparent advantage from intakes above 2.6 mg. riboflavin daily. This estimate of the requirement may be high but there were no intakes between 1.35 and 2.6 mg. to establish a lower value.

The data for nitrogen and riboflavin metabolism for these subjects have indicated that for women with moderately advanced, active tuberculosis, dietary intakes of 80 gm. of protein and 2.6 mg. of riboflavin are adequate. If the day's diet includes a quart of milk and generous amounts of cereals and meat, these requirements are easily met.

Nutritive value of sanatorium diet

In Table 15, average intakes of several essential nutrients have been summarized from the five periods of the study to demonstrate the influence of added milk intake on the various substances. The dietary supplements provided throughout the study were not included with these values. The average nutritive value of the diets of the subjects preceding the experimental periods was higher for all nutrients except vitamin A than for the first two periods of restricted milk intake but not as high as for the last two periods during which the intake of milk was increased to 1200 gm. daily. Additions of 900 gm. of milk to the diet during periods three and four resulted in a marked increase in intake of all nutrients except thiamine and ascorbic acid. The calcium intake was increased from 0.64 to 1.66 gm. daily and the phosphorus intake was increased from 0.96 to 1.83 gm. daily. The average increase of the vitamin A content of the diet should not be attributed entirely to milk since there was a wide variation in the vitamin A intake for period three in comparison with period four. The hospital diet served to these subjects over the fourteen weeks period typified the dietary practices of this sanatorium. The data presented in Table 15 indicate that the milk consumption of patients of the sanatorium is important in determining the nutritional adequacy of the patient's diet.

TABLE 15

The average nutritive value of diets of six women with active tuberculosis

Diet Period	Calories*	Nitrogen	Calcium*	Phosphorus	Thiamine	Riboflavin	Vitamin A*	Ascorbic Acid*
		gm./24 hr.	gm./24 hr.	gm./24 hr.	mg./24 hr.	mg./24 hr.	I.U.	mg./24 hr.
Preliminary: Usual diet	1996	10.10	1.02	1.30	1.02	1.80	4947	94
Per.1. 300 gm. milk daily	1802	8.90	0.66	0.96	0.66	1.35	5300	81
Per.2. 300 gm. milk daily	1659	8.85	0.62	0.95	0.76	1.41	4666	75
Mean, Per. 1 & 2	1731	8.88	0.64	0.96	0.71	1.38	4983	78
Per.3. 1200 gm. milk daily	2117	13.00	1.66	1.84	0.80	2.60	6102	79
Per.4. 1200 gm. milk daily	2168	13.62	1.65	1.82	0.70	2.79	4581	81
Mean, Per. 3 & 4	2143	13.31	1.66	1.83	0.75	2.70	5342	80

* By calculation. (Donelson and Leichsenring, 1945)

SUMMARY AND CONCLUSIONS

The riboflavin and nitrogen metabolism of six women with moderately advanced, active pulmonary tuberculosis was studied. The subjects were non-ambulatory patients of the Ingham county sanatorium and the regular hospital diet was served throughout the experiment. Ages of the patients ranged from 25 to 39 years. The riboflavin and nitrogen contents of the diets were varied by adjustment of the milk intake. The milk intake for periods one and two was restricted to 300 gm. daily; for periods three and four, the intake was increased to 1200 gm. milk per day. Daily supplements of five mg. riboflavin were given during periods two and four. Fourteen days of dietary adjustment preceded each seven day balance period during which intakes and excretions of riboflavin were determined.

The average nitrogen intake of the subjects on the usual hospital routine was 10.1 gm. per day. When the milk intake was restricted to 300 gm. daily, the nitrogen intake averaged 8.90 gm.; the average nitrogen retention was 1.15 gm. during period one and 0.57 gm. during period two. The nitrogen intake for periods three and four were 13.00 and 13.62 gm. respectively. The mean retentions at these intakes were 1.95 gm. nitrogen for period three and 1.48 gm. nitrogen for period four.

The relationship between nitrogen intake and retention for the tuberculous women was similar to that reported for healthy, active college women.

The urinary excretion of riboflavin was directly related to the

dietary intake. The riboflavin intakes for the four successive experimental periods were 1.35, 6.41, 2.60 and 7.79 mg. daily; the urinary excretion of riboflavin at the corresponding intakes were 0.31, 3.53, 1.08 and 5.62 mg. The percent test dose responses for the four periods were 39, 65, 55 and 72, respectively.

The fecal excretion of riboflavin was related to the amount of milk in the diet rather than to the dietary intake of riboflavin. The average excretion for periods one and two was 0.68 mg. and 0.85 mg. for periods three and four.

The riboflavin metabolism of the tuberculous patients also was similar to that of healthy college women.

The riboflavin content of the diet did not influence the nitrogen retention but there was a significant inverse relationship between the urinary excretion of riboflavin and nitrogen retention.

It is estimated that a diet adequate in calorie value and supplying 80 gm. protein and 2.6 mg. riboflavin would meet the requirements of these patients for these nutrients.

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APPENDIX

Patient histories.

Subject SW: Patient's discharge summary.

Date of Admission: 6-3-46

Date of Discharge: 7-13-48

The patient was admitted to Ingham Sanatorium as a refer from Genesee County. She had been x-rayed in May of 1946 because of an attack of pleurisy and at the time of admission she complained of fatigue of one month's duration with right chest pain.

X-ray examination revealed that in the right lung there were infiltrations beneath the 2nd anterior rib and extending to within the circle of the 1st rib. In the left lung, there were infiltrations scattered throughout the 2nd anterior intercostal space. She was classified as active moderately advanced pulmonary tuberculosis, II-B. The sputum was positive for tubercle bacilli by culture and guinea pig inoculation and continued to be consistently positive until November 1947. Since that time it has been negative.

A right pneumothorax was established 11-11-46 followed by a right closed pneumonolysis on 12-10-46 when one cord and one band-like adhesion suspending the apex were cut. Following the initiation of the pneumothorax the sputum continued to be positive. She was bronchoscoped on 5-6-47 and again on 9-20-47. A normal bronchial tree was found both times except for some reddening of the right stem bronchus and mucoid discharge coming from the right upper lobe. Because of the persistently positive sputum, streptomycin, one gram per day, was begun in January, 1948, and continued for 120 days. Sputum examination six weeks following the initiation of streptomycin therapy was negative and has been consistently negative since that time.

Subject H. Patient's discharge summary.

Date of Admission: 2-12-47

Date of Discharge: 1-21-49

This 24 year old housewife and ex-servicewoman was re-admitted to the sanatorium because of the persistence of positive sputum. She had previously been treated at this hospital, between the dates of 6-18-46 and 8-4-46, by bed rest and the initiation of a right pneumothorax. At the time of her return after five months absence, x-ray examination revealed that there had been some increase in the infiltrations in the right, upper lobe. She was classified as active moderately advanced pulmonary tuberculosis, II-B. The pneumothorax which had been initiated at the time of the first admission had completely re-expanded. The pneumothorax was reinitiated but it was now noted that there were several adhesions suspending the apex of the lung which required a closed intrapleural pneumonolysis done on 3-6-47.

Since that time she has been maintained on bed rest and there has been a gradual continued clearing of the infiltrations in the right upper lobe so that at the present time there is very little density remaining. The last examined specimen was a gastric which was negative for tubercle bacilli by culture and guinea pig inoculation.

Discharge weight, 129 pounds. This represents a gain of 15 pounds over her first admission weight. During her stay she received streptomycin, one gram per day for 125 days, beginning 1-7-48.

Subject G. Patient's discharge summary.

Date of Admission: 12-9-46

Date of Discharge: 11-3-48

This patient was readmitted to the sanatorium because of finding of positive sputum and marked deterioration in her general physical condition. There was no definite x-ray change. She had previously been treated at Ingham Sanatorium between the dates of 4-21-44 and 3-19-46.

Following her return to the sanatorium she was placed on bed rest. She immediately began to gain weight. Her general condition rapidly improved. The sputum became negative on concentrate but remained positive on culture and guinea pig inoculation. Following streptomycin therapy the sputum became negative by all methods of investigation and has remained so. There has been no change in the x-ray appearance of this patient's chest. She has gained approximately 25 pounds in weight.

Subject B. Patient's discharge summary.

Date of Admission: 9-19-46

Date of Discharge: 5-18-49

This 33 year old housewife was admitted to the sanatorium as a refer from the Ingham County Health Department. An original diagnosis of pulmonary tuberculosis was made in Morgan Heights Sanatorium in Marquette May, 1933. Originally the disease occupied the left upper pulmonary lobe and was minimal in extent. She entered Pinecrest Sanatorium, Powers, Michigan for treatment in May 1938 where she was on bed rest for six months. She was then treated by left pneumothorax until January 1943 when a pneumothorax was also started on the right side. There had been very little follow up of this case since her discharge from Powers sanatorium in 1943. The sputum was found to be positive in June 1946.

On admission here, she complained of only a small amount of expectoration and ease of fatigue. X-ray examination revealed, in the right lung, scattered areas of infiltration extending from the level of the 3rd anterior rib to the apex with a rather well circumscribed area of infiltration at the level of the 4th anterior rib in the middle lung field. On the left, there was considerable retraction of the mediastinal structures to the left and there was a thickened pleura over the outer field. She was classified as moderately advanced pulmonary tuberculosis, II-B. The sputum was positive by culture and guinea pig inoculation of the seven-day pooled sputum material.

Over nearly a two year period of bed rest, there was no appreciable change in the x-ray appearance. Bronchographic examination revealed a normal bronchial tree except for lack of filling of the middle lobe which

was obstructed. Decortication of the left lung was carried out on 7-6-48 and after a period of two months, right middle lobectomy was done. At the time of operation a tuberculoma approximately three cm. in diameter surrounding the middle lobe bronchus was found. There was a direct, small communication to the middle lobe bronchus. Beginning at this time the patient received streptomycin one gram per day for a period of four months. Since the lobectomy the patient has been negative repeatedly by all methods of investigation.

Discharge weight was 137 pounds. This represents a gain of 17 pounds over admission weight.

Subject F. Patient's interval summary.

This 35 year old white woman was seen as a referral case on 11-29-45 at which time a single 4 x 5 inch projection showed infiltrations in the right 2nd anterior intercostal space and scattered throughout the left lung. She was admitted to the sanatorium January 2, 1946, and her sputum was found to be routinely positive for tubercle bacilli. She gave a history of having had a severe cold the preceding February which became somewhat better in April and May but a cough persisted and shortly thereafter she began to notice fatigue. Physical examination revealed a normal developed, poorly nourished, white woman appearing chronically ill. She weighed 123 pounds and her height was 63 inches. X-ray examination showed: right lung, scattered infiltrations at the level of the 1st, 2nd and 3rd anterior intercostal spaces; left lung, dense infiltrations within the circle of the 1st rib and less dense infiltrations scattered throughout most of the entire lung. The diagnosis and classification was moderately advanced, bilateral pulmonary tuberculosis, II-B.

The patient was placed on strict bed rest and because of the wheezing type of respiration and a history of occasional hoarseness, bronchoscopy was done at which time the larynx was found to be normal but the mucous membrane of the trachea was somewhat reddened and edematous. The right bronchus appeared normal. The left bronchus was markedly reddened and edematous and the lumen was so stricted that the bronchoscope could be passed only a short distance into the stem bronchus. A laryngoscopy on 7-2-46 showed a fairly marked ulceration of both cords but especially the posterior third of the right cord. Because of the fairly extensive involvement, cauterization of the cords was not indicated and the treatment prescribed was absolute silence.

Because of the obliterative pleuritis over the right lung the pneumothorax was abandoned 11-17-47. Beginning May 16, 1947 the patient was placed on two grams of streptomycin daily which was continued for 85 days. Since her admission to the sanatorium there has been a gradual improvement in the x-ray picture of her disease and the sputum is now positive to culture and guinea pig inoculation only. Her laryngitis is apparently completely healed and the patient's clinical appearance has much improved. She now weighs 135 pounds constituting a gain of 12 pounds since admission. Because of the continued positive sputum she has once again been placed on streptomycin therapy following a determination of sensitivity to the drug by the organisms.

Subject DW. Patient's interval summary.

This 24 year old housewife was first seen as a referral case on April 22, 1938. She complained of cough and chest pain for the past six weeks and an occasional hemoptysis for the past three weeks. There was no knowledge of weight loss. Physical examination at that time disclosed scattered rales throughout both lung fields. An x-ray film dated 4-22-38 showed wide spread bilateral pulmonary tuberculosis involving the right lung down to the anterior rib and throughout the left lung with a large cavity beneath the 1st rib on the left. Admission was advised and the patient was admitted 4-22-38. Following admission, a complete evaluation of her case showed far advanced, bilateral cavernous pulmonary tuberculosis as above described and it was decided that the treatment of choice was bilateral pneumothorax. The bilateral pneumothoraces were continued in the sanatorium through 4-8-39 at which time the patient left against medical advice. Her pneumothorax refills were continued in the Outpatient Department through July 18, 1941, after which time the patient failed to return to the sanatorium for her usual treatments. During this period there had been gradual improvement in the pulmonary tuberculosis.

The patient was not seen again until 10-30-47 at which time she was admitted to the sanatorium following an interpretation of a film. Physical examination at this time showed an undernourished, adult white woman appearing older than her stated age and chronically ill. There was a harsh systolic murmur heard best at the level of the 2nd anterior intercostal space at the sternal border. The blood pressure was systolic 88, diastolic 60. There was a respiratory lag on the right with decreased tactile fremitus and the right chest was dull to percussion posteriorly. Decreased breath sounds were heard over the right chest posteriorly but no rales

could be heard at this time.

A thoracentesis done 11-10-47 yielded 25 cc. of a very thick purulent material. 200,000 units of penicillin and saline were instilled in the pleural cavity at this time. She was seen at intervals thereafter for aspiration and sterilization of this empyema cavity on the right. Laboratory examination at this time showed the sputum to be positive by culture and guinea pig inoculation. The gastric samples were positive on slide examination. Examination of the pleural fluid did not reveal the presence of organisms. The fecal material was positive for tubercle bacilli on slide examination.

An evaluation of the patient's status indicated that arrest of her tuberculosis would best be produced by decortication and re-expansion of the right lung followed by thoracoplasty over the left upper lobe. The former procedure was done 9-28-48 and a satisfactory expansion of the right lung effected. On 11-9-48, the first stage left thoracoplasty was done. Following this procedure, the patient decided to postpone her second stage until her condition seemed more suitable. Inasmuch as a single stage had failed to close the cavity in the upper lobe of the left lung and the patient being in suitable condition, a second posterior stage completing a six rib thoracoplasty was done 2-8-49.

She was placed on streptomycin therapy at the time of the first operation 9-29-48 and this was continued $\frac{1}{2}$ gram daily for 78 days through 12-15-48. She was again started on streptomycin at the time of the second stage. There has been a gain of 37 pounds over admission weight and her sputum has been negative on two occasions by culture and guinea pig inoculation.

TABLE 1

Variations in temperatures of six women with active tuberculosis

Period	SW	H	G	B	F	DW
Prelim. per. Avg.,14 days						98.7 ± 0.3
Avg., 7-day balance	98.6 ± 0.03*	98.5 ± 0.2	98.5 ± 0.2	98.6 ± 0.2	98.5 ± 0.1	98.6 ± 0.2
Test day	98.6	98.6	98.8	98.4	98.4	98.8
Per. 1, Avg., 14 days	98.4 ± 0.3	98.6 ± 0.1	98.6 ± 0.1	99.1 ± 0.6	98.8 ± 0.3	98.8 ± 0.3
Avg., 7-day balance	98.4 ± 0.2	98.3 ± 0.2	98.5 ± 0.2	98.5 ± 0.5	98.8 ± 0.3	98.7 ± 0.1
Test day	98.2	98.2	98.6	98.2	98.0	98.6
Per. 2, Avg., 14 days	98.4 ± 0.2	98.1 ± 0.3	98.5 ± 0.3	98.7 ± 0.2	98.7 ± 0.2	98.4 ± 0.4
Avg., 7-day balance	98.9 ± 0.2	98.5 ± 0.2	98.3 ± 0.2	98.7 ± 0.3	98.7 ± 0.2	99.5 ± 0.2
Test day	98.8	98.2	98.0	98.4	98.6	98.6
Per. 3, Avg., 14 days	98.6 ± 0.2	98.3 ± 0.2	98.4 ± 0.3	98.8 ± 0.2	98.4 ± 0.2	98.6 ± 0.3
Avg., 7-day balance	98.5 ± 0.2	98.4 ± 0.2	98.2 ± 0.3	98.7 ± 0.2	98.6 ± 0.1	98.7 ± 0.2
Test day	98.6	98.4	97.8	99.0	98.6	99.2
Per. 4, Avg., 14 days	98.6 ± 0.3	98.5 ± 0.2		98.8 ± 0.4	98.5 ± 0.2	98.7 ± 0.3
Avg., 7-day balance	98.4 ± 0.2	98.2 ± 0.3		98.8 ± 0.2	98.7 ± 0.2	98.7 ± 0.3
Test day	98.4	98.8		98.6	98.4	98.4

*Mean deviation

TABLE ii

Record of Streptomycin administration

Patient	Dates	Amount	Notes
		Gm./ day	
SW	1-14-48 to 5-14-48	1.0	Follows diet study
H	1-7- 48 to 5-10-48	1.0	Follows diet study
G	2-4- 48 to 6-10-48	1.0	Extends over Period 4 of diet study
B	10-3- 47 to 2- 1-48	1.0	Preceded diet study
F	5-16-47 to 8- 8-47	2.0	Preceded diet study
DW	9-28-48 to	0.5	Follows diet study

Comment: Of these patients, only F experienced unpleasant reactions to streptomycin, i.e., ringing in ears, dizziness, deafness. There may have been persistent dizziness after cessation of streptomycin.

TABLE iii

Records of sputum tests for six tuberculous women

Subject and Date	No. of days	Slide	Culture	Guinea Pig
Subject SW				
8- 4-47 to 8-10-47	7	0	+	+
11-13-47 to 11-19-47	7	0	+	+
2-25-48 to 3- 2-48	7	0	0	0
Subject H				
6- 8-47 to 6-15-47	7	0	+	+
12-10-47 to 12-16-47	7	0	+	+
3-12-48 to 3-19-47	7	0	0	0
Subject B				
10-15-47 to 10-22-47	7	0	+	+
12- 3-47 to 12-10-47	7	0	0	0
2-18-48 to 2-25-48	7	0	+	+
4-21-48 to 4-28-48	7	0	+	+
8- 8-48 to 8-15-48	7	0	0	0

TABLE iii

Records of sputum tests for six tuberculous women

Subject and Date	No. of days	Slide	Culture	Guinea Pig
Subject G				
9-10-47 to 9-17-47	7	0	+	+
12-11-47 to 12-19-47	7	0	+	+
3-31-48 to 4- 7-48	7	0	0	(G.p. died)
4-28-48 to 5- 5-48	7	0	0	0
Subject F				
11-16-47 to 11-23-47	7	0	Contaminated	+
2-18-48 to 2-25-48	7	0	+	+
5-12-48 to 5-19-48	7	0	+	+
8- 1-48 to 8- 8-48	7	0	0	0
Subject DW				
11-19-47 to 11-26-47	7	0	+	+
2-11-48 to 2-17-48	7	0	+	+
5- 5-48 to 5-12-48	7	0	+	+
8- 2-48 to 8- 9-48	7	0	0	0

TABLE iv

The intake and retention of nitrogen by six women

with active tuberculosis; individual data; preliminary period

Subject and Diet Period	Nitrogen Intake	Urinary Nitrogen	Fecal Nitrogen	Nitrogen Retention
	gm. per 24 hr.	gm. per 24 hr.	gm. per 24 hr.	gm. per 24 hr.
Preliminary period: Usual diet				
SW	9.709	6.888	1.416	1.405
H	12.268	9.451	1.245	1.572
G	11.319	8.550	0.890	1.879
B	9.182	8.639	1.002	- 0.459
F	8.995	8.159	1.214	- 0.378
DW	9.123	7.946	1.300	- 0.123

TABLE v

The intake and retention of nitrogen by six women

with active tuberculosis; individual data; periods 1 and 2

Subject and Diet Period	Nitrogen Intake	Urinary Nitrogen	Fecal Nitrogen	Nitrogen Retention
	gm. per 24 hr.	gm. per 24 hr.	gm. per 24 hr.	gm. per 24 hr.
Period 1. 300 gm. milk daily				
SW	7.569	5.305	1.140	1.124
H	9.875	7.895	1.204	0.776
G	9.314	6.583	1.135	1.596
B	9.773	7.501	1.139	1.133
F	8.749	7.071	1.164	0.514
DW	8.140	5.757	0.606	1.777
Period 2. 300 gm. milk and 5 mg.riboflavin daily				
SW	6.547	6.749	0.961	- 1.163
H	9.556	7.929	0.951	0.676
G	9.213	7.269	0.781	1.163
B	10.042	7.578	1.181	1.283
F	8.890	7.595	1.093	0.202
DW	8.848	6.753	0.859	1.236

TABLE vi

The intake and retention of nitrogen by six women

with active tuberculosis; individual data; periods 3 and 4

Subject and Diet Period	Nitrogen Intake	Urinary Nitrogen	Fecal Nitrogen	Nitrogen Retention
	gm. per 24 hr.	gm. per 24 hr.	gm. per 24 hr.	gm. per 24 hr.
Period 3. 1200 gms. milk daily				
SW	10.476	7.232	1.494	1.750
H	14.506	10.254	1.046	3.206
G	13.121	10.135	0.892	2.094
B	14.275	11.018	1.513	1.744
F	13.009	10.711	1.293	1.005
DW	12.620	9.708	1.036	1.876
Period 4. 1200 gms. milk and 5 mg.riboflavin daily				
SW	12.968	9.278	1.565	2.125
H	14.939	11.720	1.119	2.100
G	13.238	9.736	0.970	2.532
B	15.381	12.661	1.125	1.595
F	12.930	11.445	1.117	0.368
DW	12.268	10.819	1.282	0.167

TABLE vii

The intake and excretion of riboflavin by six women

with active tuberculosis; individual data; preliminary period

Diet Periods Subject	Riboflavin Intake	Urinary Riboflavin	Fecal Riboflavin	Test Dose Response
	Mg./24 hrs.	Mg./24 hrs.	Mg./24 hrs.	%
SW	1.832	0.597	1.069	62
H	4.213	2.200	1.065	63
G	9.373	4.770	0.887	23
B	9.218	7.105	0.776	72
F	9.360	7.242	1.032	74
DW	9.271	7.072	0.790	63

TABLE viii

The intake and excretion of riboflavin by six women

with active tuberculosis; individual data; periods 3 and 4

Diet Periods Subject	Riboflavin Intake	Urinary Riboflavin	Fecal Riboflavin	Test Dose Response
	Mg./24 hrs.	Mg./24 hrs.	Mg./24 hrs.	%
Period 3.				
SW	2.230	0.884	0.997	59
H	2.542	0.877	0.901	47
G	2.582	1.049	0.602	42
B	2.885	1.288	0.975	60
F	2.777	1.196	0.902	64
DW	2.579	1.196	0.627	55
Period 4.				
SW	7.805	4.151	1.140	-
H	7.879	3.468	1.034	43
G	7.562	5.403	0.609	72
B	7.927	7.078	0.889	81
F	7.846	6.740	0.770	88
DW	7.745	6.909	0.769	77

TABLE ix

The intake and excretion of riboflavin by six women

with active tuberculosis; individual data; periods 1 and 2

Diet Periods Subject	Riboflavin Intake	Urinary Riboflavin	Fecal Riboflavin	Test Dose Response
	Mg./24 hrs.	Mg./24 hrs.	Mg./24 hrs.	%
Period 1.				
SW	1.178	0.345	0.698	42
H	1.320	0.230	0.986	36
G	1.470	0.200	0.564	30
B	1.470	0.361	0.841	26
F	1.457	0.446	0.733	62
DW	1.194	0.261	0.269	40
Period 2.				
SW	6.212	4.059	0.727	72
H	6.221	4.289	0.696	70
G	6.677	1.084	0.636	41
B	6.383	4.080	0.996	75
F	6.335	3.991	0.674	82
DW	6.625	3.677	0.370	52