RIBOFLAVIN AND THIAMINE CONTENT OF THE INTESTINAL TRACT OF CECECTOMIZED AND NON-CECECTOMIZED WHITE RATS

by

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TABLE OF CONTENTS

TABLES

FIGURES

INTRODUCTION

INTRODUCTION

Studies concerned with the synthesis of vitamins in the digestive tract of animals are becoming increasingly important. The problem is of considerable nutritional importance not only as a contribution to the nutrition of the animal but also as "... a complicating factor in the interpretation of the data obtained in feeding experiments" (Peterson and Peterson, 1945, p. 80).

Numerous investigators (Herter and Kendall, 1909; Porter and Rettger, 1940; Mitchell and Isbell, 1942) have demonstrated that the intestinal flora is influenced by diet. In a bacteriological study involving white rats, McClure (1949) found that the total numbers of intestinal bacteria were higher in cecectomized milk fed animals than in the corresponding controls whereas the reverse was observed in stock fed animals. From this interesting observation a question arose concerning the effect of diet and cecectomy on the intestinal synthesis of vitamins.

The purpose of this study was to investigate riboflavin and thiamine concentrations in certain portions of the intestinal tract of cecectomized and non-cecectomized white rats and to compare the vitamin concentrations with bacterial counts of the corresponding segments of the tract.

Since it has been shown that microorganisms are capable of synthesizing B vitamins (Thompson, 1942; Burkholder and McVeigh, 1942), it was believed that a study of this kind might present additional information to our knowledge of intestinal synthesis.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Introduction

"Pasteur, in 1885, suggested that animal life would be impossible without the co-operation of the micro-organisms found in the digestive tract" (Elvehjem, 1946). The influence of the intestinal flora upon the nutrition of the host has been studied by numerous investigators. Conversely, the growth stimulating activity of some of the vitamins for bacteria has also become important.

Peterson and Peterson (1945, p. 49) in discussing the relation of bacteria to vitamins, illustrate the merging of the fields of animal and microbiological nutrition. They advocate a mutual exchange of ideas to promote progress in both fields and "... predict that several of the compounds now known to be required by bacteria will some day become members of the vitamin family."

The Significance of Coprophagy

That the intestinal microorganisms are capable of synthesizing "anti-neuritic substances" was suggested by Cooper in 1914 when he found that alcoholic extracts of the feces of a rabbit fed white bread and cabbage cured polyneuritis in pigeons.

Osborne and Mendel (1913) noted improvement in the

performance of rats fed certain rations when they were given access to their feces as a supplement. As research in the vitamins and particularly in the synthesis of vitamins has progressed the significance of this early observation becomes interpretable.

The importance of preventing coprophagy in certain types of experimental work has since been emphasized by several investigators. Dutcher and Francis (1923) noted that when screens were introduced in the cages of rats receiving a ration deficient in vitamin B, a marked decrease in food consumption and in body weight resulted. That vitamin B was the factor supplemented by feces was the belief of Steenbock and his associates (1923). They found that either coprophagy or the addition of wheat germ to the diet resulted in an improved growth of rats maintained on a purified diet low in vitamin B.

Heller, McElroy, and Garlock (1925, p. 263) from observations made when feeding vitamin-free rations to rats, suggested that "... the spore bearing organisms present in the intestinal tract during the early part of the experiment synthesize and store vitamin B." Prolonged life, and in some cases growth, were benefits attributed by Roscoe (1931) to the feces consumed by rats receiving diets deficient in the vitamin B complex.

The Phenomenon of Refection

Refection refers to a condition in experimental animals whereby the animals are able to grow, thrive, and multiply on diets believed to be free of the undifferentiated vitamin B. The kind and amount of carbohydrate present in the diet plays an important role in the apparent synthesis of vitamins by the intestinal bacteria. The investigative work which the accidental discovery of refection stimulated in their laboratory has been described and discussed by Fridericia et al (1927). The effects of refection, which first arose spontaneously, could be transmitted to other young rats by adding to their vitamin B-free diet the feces of other refected rats. These feces were bulky and white and contained unusually large amounts of "vitamin B" and undigested starch.

Mendel and Vickery (1929) were unable to obtain refection in rats but numerous other investigators have confirmed Fridericia's original report (Roscoe, 1927; 1931; Kon and Watchorn, 1927; Parsons et al, 1933; Bliss and Green, 1936).

Fridericia and his co-workers noted that the cecal contents of refected rats resembled normal rats and differed from rats suffering from vitamin B deficiency. They proposed that "... the change in bacterial flora and in chemical processes in the coecum of refected rats may be correlated

with the production of vitamin B in the intestine of these rats" (1927, p. 91 - 92).

Other investigators have postulated the cecum to be the main site of vitamin synthesis by intestinal microorganisms (Porter and Rettger, 1940; Schweigert et al, 1945; Day et al, 1943; Taylor et al, 1942). In contrast, Griffith (1935) concluded that the cecum is not the principal source of bacterial synthesis.

The Role of Diet

These and other investigators studying synthesis have pointed out the important role which diet plays in this connection.

That the diet exerts a marked influence over the bacterial flora of the intestinal tract of animals has become a well-established fact. Herter and Kendall (1909) were among the early investigators to demonstrate the substitution of an acidophilic type of flora for a proteolytic type when the diet was changed from one dominantly protein to one high in carbohydrate. Other workers have since confirmed this finding (Hull and Rettger, 1917; Porter and Rettger, 1940; Mitchell and Isbell, 1942).

However, in studying the intestinal flora of dogs, Torrey (1919) found that the type of carbohydrate or protein food is also an important factor in determining the character of the intestinal flora.

The superiority of certain carbohydrates in promoting intestinal synthesis has been demonstrated repeatedly. Morgan et al (1938) found lactose superior to cornstarch and sucrose in the elaboration of B vitamins in the intestines of the white rat. Mannering et al (1944), Tepley et al (1947), and Guerrant et al (1935) found that dextrinized cornstarch caused the greatest synthesis of these vitamins while lactose appeared to occupy an intermediate position.

Refection, which has already been discussed, is obtained with diets having raw potato starch as the carbohydrate but the beneficial effect is lost when cooked potato starch is substituted for the raw starch.

Gall et al (1948a; 1948b) studied both the intestinal flora of mice fed different diets and the ability of the predominating flora to synthesize riboflavin, niacin, biotin, folic acid, and pantothenic acid in vitro. They have shown that there is a flora, characteristic of mice fed a dextrose diet, which is different from that of animals fed a dextrin diet. The coccus isolated from the animal maintained on the dextrin-containing diet was capable of existing in a synthetic broth lacking folic acid and at the same time was able to liberate this vitamin into the environment. In contrast, the coccus found in animals fed the dextrose-containing diet exhibited little or no growth in the deficient broth and liberated little if any folic acid.

The addition of agar or other fibre to the diet appears to favour growth of animals maintained on vitamin-free rations (Heller et al, 1925) and to possess a sparing effect on vitamin utilization (Guerrant and Dutcher, 1934). The latter workers believe the beneficial effect of fibre to be due to the production of more favorable conditions for the growth of microorganisms in the digestive tract. Nielson, Shull, and Peterson (1942), who found a natural stock diet to be far superior to synthetic diets in the intestinal synthesis of biotin in the rat, stated that the indigestible material contained in the stock diet probably favored bacterial growth.

The effect upon synthesis of varying amounts of fat and of different fats in the diet has been studied. Whipple and Church (1935) concluded that fat was essential for the production of the anti-beriberi factor in the gastro-intestinal tract of the vitamin B-deficient rat. Guerrant and Dutcher (1934) found that when increasing amounts of fat (Crisco) were added to the basal diet, the rats became depleted of their vitamin B reserve less rapidly but there was no effect on the vitamin G requirement. However, in further studies Guerrant et al (1937) did not find a more favorable production of the B vitamins in the digestive tract of the rat when 10 or 20 percent of Crisco was added to a vitamin B complex deficient diet containing sucrose as a source of carbohydrate. Nath and his co-workers (1948)

found that the addition of a high level of corn oil to a sucrose diet decreased the aerobic and anaerobic plate counts as well as the numbers of coliforms in the ceca of rats. Supplementation of this diet with reticulogen (Lilly liver extract) tended to counteract the inhibitory action of corn oil upon the growth of certain cecal microorganisms.

Czaczkes and Guggenheim (1946) studied the influence of high and low fat and high and low protein diets on the riboflavin metabolism of the rat. Rats kept on high protein and high fat diets required more riboflavin than rats on a normal diet but a low fat diet lessened the need for riboflavin. The differences in riboflavin requirement were shown to be due to differences in the amounts of riboflavin which were synthesized in the intestines. That high intakes of fat (25 to 40 percent) increased the riboflavin requirement of the rat has also been demonstrated by Mannering et al (1944).

Elvehjem and Krehl (1947) have discussed the divergence of opinion concerning the superiority of different fats in the diet. Boutwell et al (1943) showed butterfat to be superior to corn oil in effecting rat growth where lactose was used as the sole carbohydrate of the diet. With a mixture of carbohydrates, the growth response was equal for both fats. Tepley et al (1947) found increased niacin and folic acid synthesis in rats fed "synthetic milk" diets containing butterfat rather than corn oil.

Availability of Synthesized Vitamins

The investigations discussed in the preceding paragraphs have demonstrated the synthesis of vitamins by microorganisms in the intestinal tract of animals. However, the availability of the synthesized vitamins to the host has been questioned. Griffith (1935) showed that in order to utilize the B vitamins present in the intestinal contents rats must resort to coprophagy.

In refected rats, on the other hand, coprophagy, although it is practiced to some extent, is not necessary for the continuance of the beneficial effects of the condition.

The location of the vitamin production in the digestive tract may account for some of the differences observed. After studying the absorption of large concentrations of riboflavin in nephrectomized rats, Selye (1943) concluded that absorption takes place in the small intestine while little if any absorption occurs in the cecum and colon.

That synthesis occurs in voided feces kept at room temperature has been demonstrated. Nielson, Shull, and Peterson (1942) found a 55 percent increase within a period of two days in the biotin contents of the feces of rats fed a stock diet. Studies by Lamoreux and Schumacher (1940) revealed an increase in the riboflavin of the excreted feces of fowl.

Diffusion of the synthesized vitamin from the bacterial cell is another factor to consider in availability.

Abdel-Salaam and Leong (1938) grew in vitro a mixed flora taken from the rat cecum and were able to demonstrate synthesis of thiamine but the vitamin did not diffuse into the medium. Mitchell and Isbell (1942) also found a relatively small degree of diffusion of vitamins from the bacterial cells into the surrounding medium. Czaczkes and Guggenheim (1946) however, found that 90 percent of the riboflavin produced by the intestinal bacteria of rats was extracellular and therefore accessible to the rat organism.

Vitamin Excretion and Storage

In an attempt to explain the metabolism of vitamins, studies of their excretion in the urine and feces of animals have been conducted. Light and his co-workers (1938) found that rats fed a daily intake of 15 to 515 micrograms of thiamine excreted 25 to 30 percent of the ingested vitamin via the urine and 20 to 30 percent via the feces but the remainder was unaccounted for. They stated that:

> ...the explanation of this uniform distribution may lie in a fixed relation between the velocities of absorption, elimination (by urine), and destruction (utilization or detoxication). There is no evidence to indicate what may happen to the missing vitamin other than storage of a portion. (1938, p. 340).

Subsequent studies by these same workers (Schultz et al 1939) revealed that the tissue concentration was direct-

ly related to the intake when 10 to 65 micrograms were fed daily but that the tissue concentrations were not further increased with higher dosages. Leong (1937) had previously reported similar results in the storage of vitamin B_1.

Use of Sulfa Drugs in the Study of Vitamin Synthesis

In more recent experiments designed to demonstrate intestinal synthesis of vitamins, sulfa drugs have been employed to inhibit the bacterial action. Black, McKibbin, and Elvehjem (1941) found that 0.5 percent sulfaguanidine greatly reduced the growth rate of young rats on a purified basal ration but that liver extract counteracted the effect of the drug. Light et al (1942) demonstrated a similar reduction in growth which was prevented by feeding yeast or feces of rats kept on the same diet without the drug.

In studies with cecectomized rats, sulfasuxidine prevented vitamin K synthesis but para-aminobenzoic acid partially counteracted the effect of the drug (Day et al, 1943). Miller (1945) added succinylsulfathiazole and phthalylsulfathiazole to the highly purified diet of rats. Dietary deficiencies, lower fecal excretions of biotin, folic acid, and pantothenic acid, and a drop in the number of coliforms resulted.

Vitamin Synthesis in Ruminants

Although most of the work with intestinal synthesis has been conducted using rats as the experimental animals, studies of the synthesis of B-vitamins in the rumen of sheep

and cows have received attention.

At Pennsylvania State College, investigations were conducted on the rumen contents of a cow grown to maturity on a vitamin B complex-deficient diet. From this experiment Bechdel and his associates (1928) concluded that the vitamin B complex was produced in the rumen of the cow by bacterial fermentation. McElroy and Goss have done extensive work in this field and have demonstrated the production and utilization of thiamine, riboflavin, pyridoxine, pantothenic acid, and vitamin K in the rumen of sheep and cows (McElroy and Goss, 1939; 1940a; 1940b; 1941a; 1941b). Wegner et al (1941) studied the rumen synthesis of six members of the vitamin B complex.

Hunt et al (1941a; 1941b) studied the thiamine and riboflavin content of the rumen of cattle and found that an increase in the amount of carbohydrate (corn) in the ration produced an increase in the riboflavin content of the dried ingesta of the rumen. They did not obtain evidence of thiamine synthesis as judged by a comparison of the thiamine content of the feeds and the dried rumen contents but they did report evidence which suggested either that the thiamine was rapidly absorbed by the animal or that it was destroyed.

Vitamin Synthesis in Human Subjects

Experimental evidence of the microbiological synthesis of vitamins in human subjects has also been reported. Najjar and Holt (1943) measured the output of free and

combined thiamine in the feces of nine patients on a completely thiamine free synthetic diet. Those subjects which developed deficiency symptoms had almost no free thiamine in their stools, but those which remained free of symptoms had large quantities of free thiamine in the feces. That the fecal thiamine had its origin in the intestinal bacteria was demonstrated by the reduction in free thiamine in the feces of a subject given succinylsulfathiazole. Williamson and Parsons (1945) found the fecal excretions of thiamine in human subjects to be increased with a high fibre diet. Since urinary excretions were approximately equal to those obtained with a low fibre diet, limited availability of the synthesized thiamine was suggested.

Hathaway and Strom (1946), who compared the thiamine excretion of human subjects on synthetic and natural diets reported evidence suggesting that the natural diet was more favorable to bacterial synthesis of thiamine than the synthetic diet. Similar conclusions were obtained in riboflavin studies (Hathaway and Lobb, 1946). Excretion studies of normal adults by Denko et al (1946a; 1946b) implied intestinal synthesis of certain B complex vitamins with apparently slight absorption through the large intestine.

Problems Involved in the Study of Synthesis

Many problems have been encountered by the workers who have studied bacterial synthesis. Even where cultural studies of the predominant organisms are conducted, there is

the possibility "... that there might be other, unidentified bacteria which, though present in smaller numbers, might exert a strong influence on the nutrition of the animal "(Nath et al, 1948, p. 789). According to Schweigert et al (1945), the amount of any vitamin excreted does not measure the synthesis since the amounts absorbed or destroyed cannot be differentiated. These and other problems including the possible symbiotic relationships existing in a mixed flora have also been discussed by Mannering, Orsini, and Elvehjem (1944).

However, in spite of the many complicating factors involved, our knowledge of the role of bacteria in the nutrition of animals can be extended only through more extensive and more intensive research in this field.

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

Eighty weanling male albino rats of the Sprague-Dawley strain were used for this study. The initial weight of the control animals was 44 to 58 grams. Cecectomized animals weighed 58 to 78 grams at the time of the operation.

To reduce coprophagy to a minimum, the rats were housed individually in cages containing false bottoms made of galvanized wire screens (2 to 3 meshes to the inch).

Evaporated milk*, diluted with an equal volume of distilled water and supplemented by a solution containing iron pyrophosphate, manganese sulfate, and copper sulfate, was fed to 40 of the animals. The other 40 animals were fed a laboratory stock diet consisting of:

yellow corn meal	5,000 parts
linseed oil meal	1,600 parts
alfalfa meal	200 parts
casein	500 parts
wheat germ	1,000 parts
yeast	500 parts
powdered milk	500 parts
sodium chloride	50 parts
calcium carbonate	50 parts

As dispensed, 25 grams of corn oil was added to each 500 grams of stock diet.

Food and distilled water were given ad libitum and records were kept of food consumption. The animals were weighed at weekly intervals on two consecutive days. The average of the two weighings represented the weekly weight.

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* Pet Milk, Pet Milk Company, St. Louis, Missouri

Within each group one-half of the animals were cecectomized and one-half were maintained as controls. The cecectomy technique used in this study was devised by Dr. Wade Brinker of the School of Veterinary Medicine, Michigan State College. The method has been described in detail by McClure (1949).

The control animals were placed on their respective diets immediately and were maintained on that ration for 45 to 47 days before they were sacrificed. The animals to be cecectomized were fed the appropriate diets for 4 to 8 days before cecectomy and for 44 to 48 days following the operation.

Since preliminary trials had shown that there was insufficient material in the intestinal sections of one animal for thiamine and riboflavin assays by the methods to be used, pooled samples from four animals were used for each determination. Tables 1 and 2 illustrate this grouping of animals.

Food was withheld for 17 to 18 hours before the animals were chloroformed. The visceral cavity was opened up immediately and the intestinal tract removed. Cecal contents from the four rats in one group were transferred to a previously weighed beaker and mixed thoroughly with a glass spatula. Samples from the small intestine, and from the large intestine, were also combined for each group.

Table 1

Experimental Design Showing Subgroups of Animals Within Each Group of Milk-fed Animals

Milk Control Animals			Milk Cecectomized Animals		
Animals	Group	Studies in Addition to thiamine and riboflavin assays	Animals	Group	Studies in Addition to thiamine and riboflavin assays
1			21		
2	I	Bacterial Counts	22	VI	Bacterial Counts
3			23		
4			24		
5			25		
6	II		26	VII	
7			27		
8			28		
9			29		
10	III	Bacterial Counts	30	VIII	Bacterial Counts
11			31		
12			32		
13			33		
14	IV		34	IX	
15			35		
16			36		
17			37		
18	V	Balance Study	38	X	Balance Study
19			39		
20			40		

Table 2

Experimental Design Showing Subgroups of Animals Within Each Group of Stock-fed Animals

Stock Control Animals			Stock Cecectomized Animals		
Animals	Group	Studies in Addition to thiamine and riboflavin assays	Animals	Group	Studies in Addition to thiamine and riboflavin assays
101 102 103 104	XI	Bacterial Counts	121 122 123 124	XVI	Bacterial Counts
105 106* 107 108	XII		125 126 127 128	XVII	
109 110 111 112	XIII	Bacterial Counts	129 130 131 132**	XVIII	Bacterial Counts
113 114 115 116	XIV		133 134 135 136**	XIX	
117 118 119 120	XV	Balance Study	137 138 139** 140	XX	Balance Study

*Animal 106 escaped from its cage and was consequently discarded
**Animals 132, 136, and 139 died before the end of the experiment

For 8 of the 20 groups (Tables 1 and 2), samples of the contents from the cecum, large intestine, and small intestine were taken aseptically for bacteriological study. For this phase of the work, the method used by McClure (1949) was followed.

The material in each beaker was then weighed, transferred quantitatively with 0.2 N H_2SO_4 to a 100 ml. volumetric flask, and prepared for riboflavin and thiamine assays by enzymatic hydrolysis using polidase S enzyme. All determinations were made in duplicate.

The assay methods used were a modification of the thiochrome method for determination of thiamine in urine as developed by Mickelsen, Condiff and Keys (1945), and for riboflavin, the fluorometric procedure of Conner and Straub (1941) as modified by Keys (by communication). Readings were made with a fluorescence meter.* The linear relationship existing between the vitamin concentration of a solution and the instrument reading is shown for riboflavin in Figure 3 and for thiamine in Figure 4 (Appendix).

In addition to the thiamine and riboflavin concentration in the intestinal sections, the amount of these vitamins ingested and excreted by certain of the animals during a three-day period was measured. Tables 1 and 2 show the four groups of rats, representing a control and cecectomized group for each of the two diets, that were used for the balance study.

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*The Coleman Photofluorometer or the Lumetron Photofluorescence Meter was used.

For three days preceding autopsy, these animals were transferred to individual cages placed over large glass funnels in order that their excreta could be collected for assay. The urine was collected in a graduated cylinder containing 5 ml. of 1 N H_2SO_4. A plastic mesh screen placed under the cage prevented the feces from entering the urine-collecting cylinder. Brown paper was draped around the cages and funnels in order to prevent the destruction of riboflavin by light. The urine was transferred daily to a 100 ml. volumetric flask and stored in a refrigerator until the three-day sample was complete. At the time of urine collection, the funnel was washed once with 0.2 N H_2SO_4 and the washing added to the urine flask. Before the vitamin determinations were made, the urine samples were filtered.

The feces were collected once, at the end of the balance period and weighed. Feces and weighed samples of the diets were transferred quantitatively with 0.2 N H_2SO_4 to volumetric flasks and hydrolyzed with polidase S enzyme prior to thiamine and riboflavin determinations.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Intestinal Concentrations of Riboflavin and Thiamine

Table 3 shows the average concentrations of riboflavin and thiamine in the intestinal tract of the four groups of rats studied. The concentrations are expressed in micrograms per gram, moist weight, of the intestinal contents and represent an average of five determinations. Since the intestinal contents of four animals were pooled before assays were made, the final value is an average for 20 animals.

An examination of the riboflavin and thiamine values (Table 3) reveals that within each group the concentration is lowest in the small intestine, highest in the large intestine, and intermediate in the cecum. For example, concentrations of riboflavin in the small intestine, large intestine, and cecum of the milk control group were 8.75, 29.04, and 15.68 micrograms per gram respectively. The same general trend was found in the riboflavin and thiamine values of each group studied with one exception, namely the low riboflavin concentration in the cecum of the stock control group.

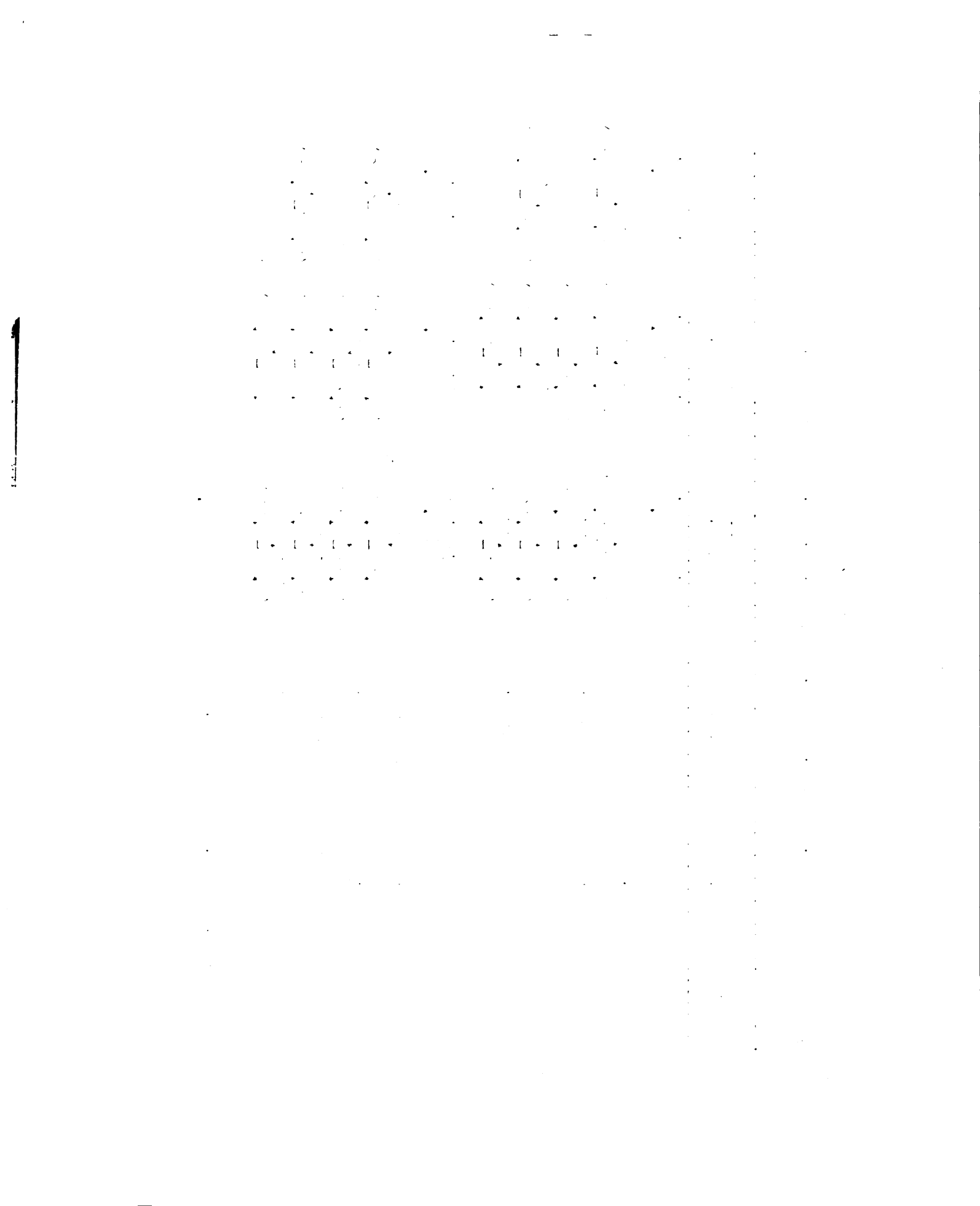
A difference is noted in the vitamin concentrations observed in animals on the two diets. The thiamine values

Table 3

Average Concentration of Riboflavin and Thiamine in the Intestinal Tract of Rats

Number of Rats	Diet	Condition	Small Intestine	Large Intestine	Cecum
			Riboflavin γ/gm.	Riboflavin γ/gm.	Riboflavin γ/gm.
20	Milk	Control	8.75 (7.06-11.87)*	29.04 (14.53-43.20)	15.68 (11.08-28.10)
20	Milk	Cecectomized	7.15 (4.46- 8.24)	15.74 (10.45-22.32)	
19	Stock	Control	7.42 (6.38-8.93)	10.98 (7.76-18.85)	5.73 (5.30- 6.17)
17	Stock	Cecectomized	6.20 (5.01-7.06)	8.05 (4.58-11.76)	
			Thiamine γ/gm.	Thiamine γ/gm.	Thiamine γ/gm.
20	Milk	Control	.77 (.34-1.06)	5.28 (2.29- 7.81)	2.39 (1.69-3.23)
20	Milk	Cecectomized	.42 (.33- .52)	3.05 (2.39- 5.04)	
19	Stock	Control	1.76 (1.58-2.35)	4.75 (3.52- 8.02)	2.78 (1.66-2.91)
17	Stock	Cecectomized	1.55 (1.30-2.11)	1.79 (1.26- 2.32)	

* Figures in parenthesis represent ranges.



in the small intestine are higher for the stock fed animals than for the milk fed animals. Differences in the large intestine and cecum are not marked.

The average daily intake of riboflavin was lower in the milk fed than in the stock fed animals (Tables 4 and 5). However, since the animals were fasted for 17 hours before autopsy, the influence which the dietary intake of riboflavin may have had on the concentration of the vitamin in the various intestinal sections should have been eliminated to a large extent. Riboflavin values in the small intestine lie within a close range in both groups of animals. Higher riboflavin values are found in the large intestine and cecum of the animals fed a milk diet than in the stock diet group. The high riboflavin content in the large intestine and cecum of the milk fed animals suggests a higher degree of intestinal synthesis for these rats than for the stock fed animals, if absorption or destruction of the vitamin is disregarded.

In both the milk fed and stock fed animals, the concentrations of thiamine and riboflavin were higher in the control group than in the corresponding cecectomized animals. As indicated previously, several investigators have expressed the belief that the cecum is the main site of intestinal synthesis. (Porter and Rettger, 1940; Schweigert et al, 1945; Taylor et al, 1942). The data presented in this study support the hypothesis that synthesis of riboflavin and

Table 4

Vitamin Content of Milk and Stock Diets

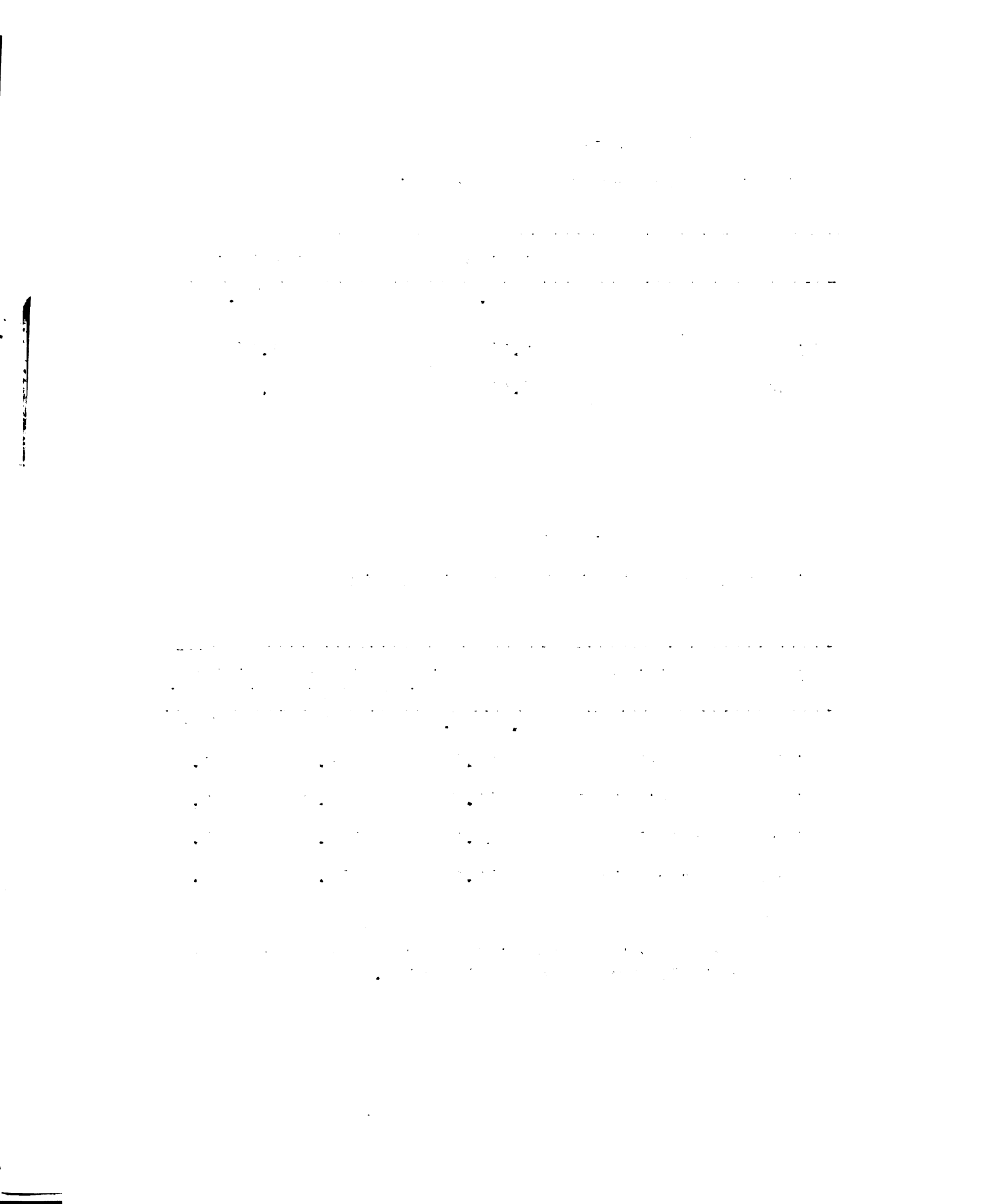
Diet	Thiamine	Riboflavin
	γ/gm.	γ/gm.
Milk	0.14	1.06
Stock	8.98	4.40

Table 5

Dietary Intake of Thiamine and Riboflavin

Diet	Condition	Average* Daily Food Consumption	Dietary Thiamine	Dietary Riboflavin
		gms.	γ/day	γ/day
Milk	Control	39.4	5.5	42.
Milk	Cecectomized	40.5	5.7	43.
Stock	Control	11.7	105.	51.
Stock	Cecectomized	13.3	119.	59.

* Average of the food intake of the total number of animals for a seven week period.



thiamine occurs in the cecum. However, it is difficult to compare this work with previous studies since the methods of investigation differ.

Bacterial Counts

Table 6 shows the counts of the bacteria found in the various sections of the intestines. The counts are expressed as the geometric means of bacterial counts obtained from the four groups of animals. However it was possible to have bacterial counts done on the intestinal contents of only 8 of the 20 groups. Thus the values obtained represent 8 cecectomized and 8 control animals fed the stock diet, and the same number of cecectomized and control rats fed a milk diet.

The bacterial counts obtained in this study were compared with those found in 40 rats maintained under similar experimental conditions (McClure, 1949). McClure (1949, p. 34) found that:

> Irrespective of the diet, the coliforms were the least numerous and the lactobacilli the most numerous organisms found in the intestinal tract. The enterococci were lower in numbers than the lactobacilli, but numerically exceeded the coliforms.

In this study the lactobacilli were again the most numerous organisms found in the intestinal tract of all groups. In the control animals, the coliforms were the least numerous organisms but in the cecectomized groups, the streptococci were the least numerous.

In McClure's study, the cecectomized milk fed animals showed higher total counts than the control animals on the

Table 6

The Bacterial Counts Found in the Intestines of Cecectomized and Control Rats Fed a Milk and Stock Diet Arranged to Show the Effect of a Particular Diet on the Bacterial Population of Each of the Intestinal Sections.

Condition	Diet	Intestinal Section	Total and Group Counts*					
			37°C. Total	45°C. E. Coli	37°C. Coliform	45°C. Streptococci	37°C. Streptococci	37°C. Lactobacilli
Control	Milk	Small	15,000,000	25,000	79,000	61,000	490,000	2,100,000
Control	Milk	Large	335,000,000	7,900	490,000	274,000	296,000	22,400,000
Control	Milk	Cecum	245,000,000	21,000	1,700,000	436,000	1,820,000	6,340,000
Cecectomized	Milk	Small	2,070,000	25,000	25,000	10,600	82,000	194,000
Cecectomized	Milk	Large	654,000,000	790,000	48,700,000	2,500,000	37,800,000	79,000,000
Control	Stock	Small	33,500,000	7,900	7,900	7,100	14,200	335,000
Control	Stock	Large	654,000,000	25,000	25,000	85,000	335,000	154,000,000
Control	Stock	Cecum	154,000,000	10,600	18,400	34,000	95,000	10,600,000
Cecectomized	Stock	Small	33,500,000	19,400	49,000	16,000	18,400	250,000
Cecectomized	Stock	Large	450,000,000	490,000	1,540,000	65,400	335,000	4,500,000

*Bacterial Counts expressed in Geometric Means

milk diet, whereas the reverse was observed in the stock fed animals. Table 7 summarizes the total bacterial counts obtained in this study. The results are in agreement with the data of McClure although the differences are not so marked. In both studies, the total counts and lactobacillus counts show the same general trend.

Other similarities in the two studies are the domination of the coliform group by E. coli, the higher coliform counts in cecectomized animals than in the controls regardless of diet, and the relatively high counts found in the small intestine.

When the variations in the counts of individual animals under the same experimental conditions are considered along with the relatively small number of animals used, the agreement found in the two sets of data is surprisingly good.

Comparison of Vitamin Concentrations and Bacterial Counts

When the average concentrations of riboflavin and thiamine in the small intestine, large intestine, and cecum (Table 3) are compared with the total bacterial counts in the corresponding intestinal sections (Table 6), a similar trend in values is found. The one exception to this similarity was mentioned previously, namely the riboflavin concentration in the cecum of the stock control group. The only bacterial group counts which did not follow the trend of the vitamin concentration were the coliforms and streptococci present in the milk control animals.

Table 7

The Sum of the Bacterial Total and Group Counts Found in the Intestines of the Cecectomized and Control Rats Fed a Milk and Stock Diet

Condition	Diet	Section	Total and Group Counts*					
			37°C. Total	45°C. E. Coli	37°C. Coliform	45°C. Strepto-cocci	37°C. Strepto-cocci	37°C. Lacto-bacilli
Control	Milk		595,000,000	54,000	2,300,000	770,000	2,600,000	31,000,000
Cecec-tomized	Milk		660,000,000	820,000	49,000,000	2,500,000	38,000,000	79,200,000
Control	Stock		842,000,000	44,000	51,000	126,000	444,000	165,000,000
Cecec-tomized	Stock		484,000,000	509,000	1,590,000	81,400	353,000	4,750,000

*Bacterial Counts Expressed in Geometric Means

However, in all of the milk animals and in some of the stock animals, the bacterial counts were lower in the control groups than in the corresponding cecectomized animals even though the vitamin concentration in each case was higher in the control than in the cecectomized group.

Demonstration, by other workers, of vitamin synthesis by intestinal microorganisms has been discussed in a previous section. Since the total bacterial counts were highest in those sections of the intestinal tract where the concentrations of riboflavin and thiamine were also the highest, there is a possibility that bacterial synthesis of these vitamins occurred.

Miller (1945) found that the addition of succinylsulfathiazole and phthalylsulfathiazole to the highly purified diet of rats resulted in decreased coliform organisms in the feces. Symptoms of dietary deficiency accompanied the drop in the number of organisms. Czaczkes and Guggenheim (1946), who studied the riboflavin metabolism of the rat, were able to show that the riboflavin content of the feces was a function of the numbers of intestinal bacteria and of the amount of riboflavin which they synthesized. The validity of this conclusion was strengthened by measurements of the amount of riboflavin that could be produced by the intestinal flora of rats.

The interesting and complex relationship existing between bacteria and vitamins has been reviewed by Peterson and Peterson (1945). During the seven year period preceding the publication of their paper, about 13 new compounds had been added to the list of accessory growth substances for bacteria. At that time, five of these additions, biotin, pantothenic acid, para-aminobenzoic acid, pyridoxine, and choline, had found a place in animal nutrition.

Campbell and Hucker (1944) studied the riboflavin requirements of a large number of strains belonging to the genus Lactobacillus and a few strains belonging to the genera Leuconostoc and Streptococcus. The effect of riboflavin upon the growth of Streptococci was found to be strain variable. Niven and Sherman (1944) tested 19 strains of streptococci and found only two that were able to grow well without added riboflavin. All grew well in the absence of added thiamine.

Roepke, Libby, and Small (1944) found E. coli sensitive to slight changes in the growth medium. Although E. coli usually grows well in a synthetic medium containing only inorganic salts and glucose, mutant strains appeared to have lost the ability to synthesize thiamine.

Studies on the synthesis of thiamine by four strains of an E. coli freshly isolated from human and animal sources, and a stock strain of the same organism were undertaken by Genung and Lee (1944). Among the results which they have reported is the observation that "... there is an optimum level of thiamin in the presence of which the organism will synthesize a maximum quantity of this vitamin" (1944, p. 435).

Burkholder and McVeigh (1942), studied the production of riboflavin, thiamine, nicotinic acid, and biotin by pure cultures of six species of common intestinal organisms including E. coli. Under the conditions of the experiment, the species of bacteria studied synthesized some of the B vitamins in greater amounts than were needed in their metabolism and the residues accumulated in the cultures.

These investigations demonstrate the many factors involved in the interpretation of data such as is being presented in this study.

Balance Study

In order to study the possibility of intestinal synthesis of thiamine and riboflavin and to investigate their metabolism within the body of the rat, a three-day balance study was conducted with four animals from each group. The results obtained are presented in Tables 8 and 9. The same data are shown graphically in Figure 1.

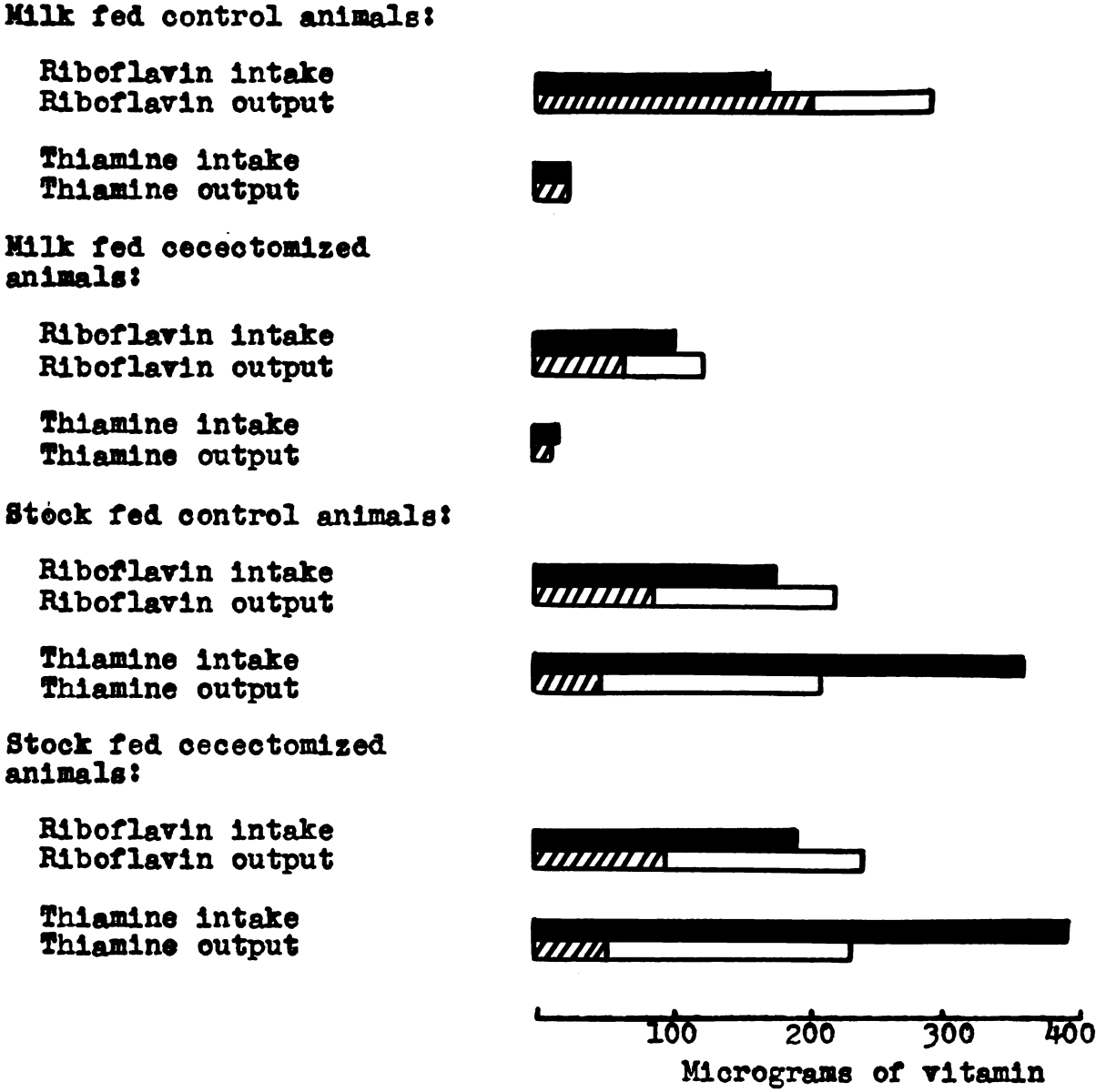
Table 8

The Dietary Intake and Excretion of Riboflavin of Animals Fed Milk and Stock Diets

Number of rats	Diet	Condition	Average Riboflavin Intake per Rat for 3-day Period	Average Riboflavin Excreted per Rat for 3-day Period			
				Feces	Urine	Total	Output / Intake
			γ	γ	γ	γ	%
4	Milk	Control	174	198	94	292	168
4	Milk	Cecectomized	99	61	59	120	121
4	Stock	Control	178	86	136	222	125
3	Stock	Cecectomized	192	96	146	242	126

Figure 1

Intake and Output of Riboflavin and Thiamine of Animals Fed Milk and Stock Diets



It appears that riboflavin was synthesized in the intestinal tract of the control group of the milk fed animals. The average intake of riboflavin for the three-day period was 174 micrograms. For the same period, the average total excretion was 292 micrograms.

The large quantity of riboflavin in the feces, 198 micrograms, as compared with the urine, 94 micrograms, suggests several possible explanations. Firstly, the synthesized riboflavin may have been held within the bacterial cell, thereby rendering it unavailable for use in the metabolism of the animals. Secondly, the riboflavin may have been synthesized in the lower part of the intestinal tract where absorption is probably limited, if at all possible. Lastly, the riboflavin may have been synthesized in the feces after excretion, since the feces were collected only once during the three-day period. Lamoreux and Schumacher (1940) have demonstrated an approximate 100 percent increase in riboflavin in the feces of fowl held at room temperature for 24 hours and a 300 percent increase or more when they were held one week.

The excretion of riboflavin, 121 micrograms, was greater than the intake, 99 micrograms, in the cecectomized milk fed animals also.

A comparison of the riboflavin balance of the control and cecectomized milk fed animals suggests that the cecum was an important site of intestinal synthesis in the control

animals, but that the riboflavin was in an unavailable form. However, the possibility of synthesis in the excreted feces cannot be disregarded.

Intake and output of thiamine were equal in the milk control animals but most of the vitamin was excreted in the feces (Table 9). No apparent synthesis occurred in the cecectomized group.

Apparently riboflavin was synthesized in both the control and cecectomized animals fed the stock diet (Table 8). With dietary intakes of 178 and 192 micrograms respectively for the control and cecectomized groups, the excretions were 222 and 242 micrograms. In these animals a greater percentage of the eliminated riboflavin was voided in urine than was excreted in feces.

The thiamine intake for the stock diet animals was much greater than the output (Table 9). However, synthesis of this vitamin is not necessarily precluded. One of the results reported by Genung and Lee (1944, p. 435) in their study of E. coli was that "... the stock strain in the presence of a relatively large amount of thiamin tends to use the vitamin rather than synthesize it". Further investigations with thiamine and with other vitamins may reveal that under certain conditions other bacteria and strains of E. coli isolated from the intestinal tract will also display a similar tendency to utilize rather than to produce vitamins.

Growth of Animals

Figure 2 and Table 10 show the growth curves and weight gains of the four groups of rats. The animals receiving the stock diet grew better than those on the milk diet, but there were no striking differences in the control and cecectomized animals on one ration. These results are in agreement with those obtained by Dhanda (1947) and McClure (1949).

Figure 2

Composite Growth Curves of Animals

Fed Milk and Stock Diets

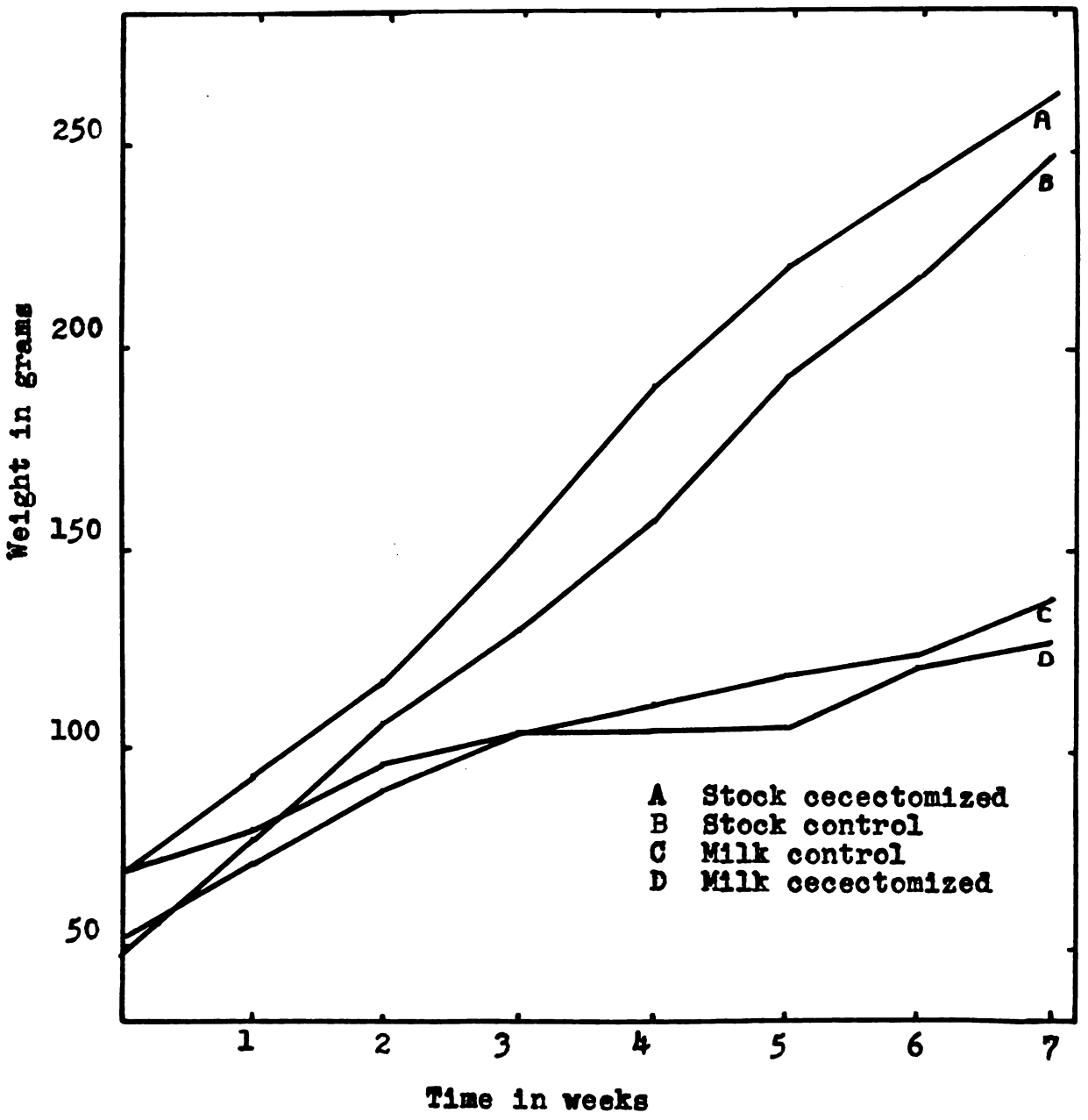


Table 10

Average Weight Gains of Rats Fed Milk and Stock Diets

Number of rats	Diet	Condition	Weight at Beginning of Feeding Period	Weight at end of 7 weeks	Weight Gain
			gms.	gms.	gms.
20	Milk	Control	51.9	137.6	85.6
20	Milk	Cecectomized	68.8	127.6	58.8
19	Stock	Control	49.2	249.0	199.8
17	Stock	Cecectomized	69.1	265.0	195.9

SUMMARY

SUMMARY

Eighty young albino rats were divided into two groups; one group was fed a laboratory stock diet and the other group was fed a milk diet. Within each group, one-half of the animals were cecectomized.

After a feeding period of about seven weeks, the animals were sacrificed and thiamine and riboflavin determinations made on the contents of the cecum, large intestine, and small intestine. Intestinal contents from four animals were pooled for each determination.

In certain cases, bacterial counts were also made on the intestinal contents.

With the exception of one group of animals, the concentration of thiamine and riboflavin within each group was found to be lowest in the small intestine, highest in the large intestine, and intermediate in the cecum. In all cases, the vitamin concentrations were higher in the control group than in the corresponding cecectomized animals.

The lactobacilli were the most numerous organisms found in the intestinal tract of all groups of animals. The coliforms and streptococci, respectively, were the least numerous organisms in the control and cecectomized groups. Total counts were lower in the cecectomized

stock fed animals than in the controls but little difference was noted in the milk fed animals.

The total bacterial counts were highest in those sections of the intestinal tract where the concentration of thiamine and riboflavin were the highest.

A three day balance study was conducted with four animals from each group. The total excretion of riboflavin was higher than the intake for each group of animals but the greatest increase appeared in the milk control animals. In this group the thiamine excretion equalled the intake, but in all others, the amount excreted was lower than the intake.

The possibility of intestinal synthesis of thiamine and riboflavin in these animals is discussed.

Animals receiving the stock diet grew better than those on the milk diet, but cecectomy had no effect on growth.

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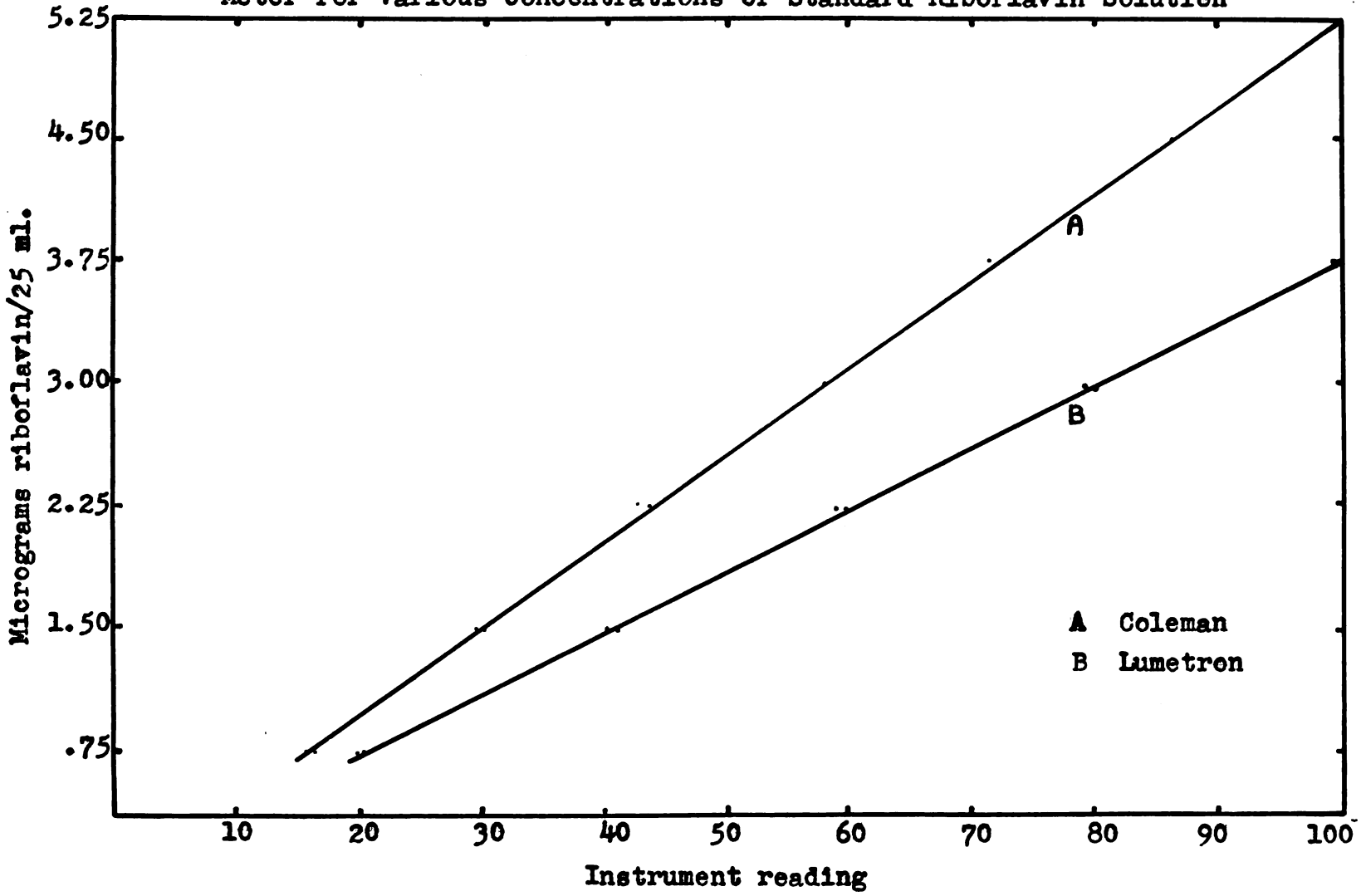
APPENDIX

Figure 3

Scale Readings on Coleman Photofluorometer and Lumetron Photofluorescence Meter for Various Concentrations of Standard Riboflavin Solution



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