INFLUENCE OF MILK AND STOCK DIETS ON THE INTESTINAL FLORA OF CECECTOMIZED AND NORMAL RATS

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by

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INTRODUCTION

This study was undertaken in an effort to determine certain factors that might possibly prove of value in the investigation of the intestinal flora and its nutritional significance. Work in this direction has been in progress for some time in the Department of Foods and Nutrition, School of Home Economics, Michigan State College.

The bacteriological study was undertaken with the hope of determining possible differences between the intestinal microflora of cecectomized rats and normal controls; and to observe how these floral differences might be affected by certain stock and milk diets. Only the numerically important organisms of the microflora of the two groups were considered.

REVIEW OF LITERATURE

The importance of the bacterial flora of the intestines probably was recognized for the first time in 1885 when Louis Pasteur suggested that animal life would be impossible without the cooperation of the microorganisms found in the digestive tract. Soon, thereafter, Hirstler (1886) and Winternitz (1892) found that certain foods gave rise to putrefactive products in the intestines, and later the alterable characteristic of the intestinal flora was demonstrated by Metchnikoff (1901). These studies suggested to subsequent investigators that the diet exerts a marked influence over the bacterial flora. With this thought in mind, studies on this problem were started by

Belonsky (1907), Tissier (1908), Herter and Hendall (1909) and Hull and Rettger (1917).

These and other early studies demonstrated that a predominance of proteolytic bacteria was induced when the diet consisted largely of meat or other protein food, and that an aciduric flora became dominant when the diet was high in carbohydrate (Torrey, 1919; Cannon, et al., 1920; Porter and Rettger, 1940; Winblad, 1941; Mitchell and Isbell, 1942).

Nath et al., (1948) found contrasting results with dextrin, but the majority of workers have discovered that of the many carbohydrates investigated, dextrin (Herter and Hendall, 1909; Cannon et al., 1920; Porter and Rettger, 1940) and lactose (Mitchell, 1927; Ruickshank, 1928) were most effective in producing a flora primarily aciduric in nature. Since these are the carbohydrates most incompletely digested, and reach the lower part of the digestive tract they probably provide a suitable substrate for the development of an aciduric type of flora.

Along with the diet studies, observations were made which brought attention to the possibility of bacterial synthesis of accessory food substances within the intestines. Among the early observations were those of Osborne and Mendel (1911), who noted that rats, maintained for long periods of time on isolated food stuffs, became coprophagists, and that the addition of a small amount of feces from a normally fed rat stopped the decline in growth of the deficient animals. Cooper (1914), however, was perhaps the first to suggest the

actual synthesis of a vitamin by the intestinal flora. About the same time Theiler, Green and Viljoen (1915) advanced a similar hypothesis. Steenlock, Bell and Nelson (1923) and Dutcher and Francis (1923) called attention to the fact that the ingestion of excreta by rats, subsisting on a so-called vitamin B free diet led to the cessation of vitamin B deficiency symptoms. Heller, McClory and Garlock (1925) confirmed these findings. These and similar studies, simulated a vast amount of research aimed at the determination of the factors involved in the probable synthesis of vitamins within the intestinal tract.

The synthetic activities of certain of the intestinal organisms were very difficult to determine, until a satisfactory bacteriostatic agent for the organisms was discovered.

The early use of drugs as modifying agents (Bouchard, 1887) was for the most part disappointing. In 1940 Marshall et al., reported the use of sulfonamides, and Black et al., (1941) and Mackenzie et al., (1941) immediately demonstrated their satisfactory bacteriostatic effects. Certain of these drugs have but a slight toxicity, are slowly absorbed from the intestinal tract and modify the flora by inhibiting the development of certain groups of organisms.

The feeding of the sulfonamides (e.g. sulfaguanidine, succinylsulfathiazole) was found by various workers to decrease the coliform count in the feces (White, 1942; Gant et al., 1942; Miller, 1944). Evanson et al., (1945) found that succinysulfathiazole depressed the lactobacillus as well as

the coliform groups, and that the lactobacilli showed a slower decrease in numbers, but a more permanent one.

Several workers discovered that, despite the decrease in numbers of organisms within certain groups, the total numbers or organisms seemed to remain fairly constant (Light et al., 1942; Gant et al., 1943; Miller, 1944; and Evanson et al., 1945). The reduction in numbers found in the lactobacilli and coliform groups in the studies of Evanson et al., (1945) was found, in part, to be compensated for by the increase in numbers of enterococci and yeastlike forms, and Gant et al., (1943) found that enterococci replaced the coliforms during the time of repressed Escherichia coli growth.

The inhibition of the growth of certain organisms by the use of sulfonamides has been found to be temporary. It appears that the organisms become resistant to the drug and often resume their original numbers, (Gant, et al., 1943; Miller, 1945) even though some strains may lose their ability to resynthesize vitamins.

In addition to depression of bacteriological growth, there is a reduction in the rate of growth of rats on synthetic diets receiving certain sulfonamides (Black et al., 1941; Mackenzie et al., 1941; Martin, 1942; Tepley et al., 1946). The retarding of growth and the signs of nutritional deficiency seen in rats fed purified diets containing the sulfonamides (Black et al., 1941; Welch and Wright, 1943; Miller, 1944) is thought to be due to the inhibitory action

of the drugs on the vitamin synthesizing bacteria of the intestines. Tepley et al., (1946) indicate some variance in this connection depending on the bacteria, drugs, and vitamins involved. Their studies revealed that phthalysulfathiazole produced a marked decrease in the concentration of niacin in the cecal contents, but the ceca increased in size so that the total amounts remained about the same. The folic acid content, however, was greatly decreased even on a total basis.

It appears that rats possess limited ability to synthesize members of the B-complex by means of the intestinal microorganisms. Neilson et al., 1942; Woolley, 1942: Light et al., 1942; Mitchell and Isbell, 1942; Taylor et al., 1942; Schweigert et al., 1945; Elvehjem, 1946 and Tepley et al., 1947, are among the many workers who have conducted experiments concerning this synthesis. Dam et al., 1941 and Day et al., 1943, studied the synthesis of vitamin K in the intestinal tract.

In view of the fact that diet is highly influential in the development of a certain kind of flora, it is not unexpected to find it of considerable importance in the bacterial synthesis of certain vitamins.

Recently Mitchell and Isbell (1942) and Taylor et al., (1942) have emphasized the marked effect diet has not only on the quality but also on the quantity of the intestinal flora, and point out that these factors in turn are reflected in the vitamin synthesis. Studies dealing with the effects

of the carbohydrates on the vitamin synthesis have been conducted extensively beginning with the early work of Hull and Rettger (1914) to the more recent investigations of Gall et al., (1948). Investigators point out that irrespective of the B vitamin being studied, the requirements for that vitamin are reduced when relatively insoluble carbohydrates like dextrin or starch are used in the diet; lactose greatly favors the production of some of the B vitamins, but glucose, sucrose, and other readily assimilated sugars are generally without appreciable effect in this respect (Fridericia et al., 1927; Cruickshank, 1928; Guerrant et al., 1934, a, b, 1935, 1937; Morgan et al., 1938; Schweigert et al., 1945; Sarma et al., 1946; Topley et al., 1947; Elvehjem and Krehl, 1947).

The fat component of the diet has received less attention than the carbohydrate portion, but considerable attention has been given to its effect on the intestinal flora. Among the recent studies on the role of fats, Boutwell (1943) demonstrated that when lactose is the sole carbohydrate, butterfat is superior to corn oil in affecting the growth of rats; Topley (1946) found that on "synthetic milk" diets butterfat resulted in greater vitamin synthesis than did corn oil, and Nath et al., (1948) found the aerobic and anaerobic plate counts as well as the numbers of coliforms to be decreased in the ceca of most of the rats fed sucrose diets containing a high level of corn oil. Mannering et al., (1944) demonstrated that riboflavin deficient rats survive for shorter periods of time when fed a high fat ration than when maintained on a high carbohydrate

diet. Elvehjem and Krehl (1947), however, have pointed out the effect of fat in modifying the requirement for some of the B vitamins.

In the studies of vitamin synthesis by the intestinal flora, workers have endeavored to establish the exact location of such synthesis in the intestinal tract. The cecum has been postulated by many to be the main site of vitamin synthesis (Tissier, 1908; Cannon et al., 1920; Guerrant et al., 1934; Griffith, 1935; Nielson et al., 1942; Taylor et al., 1942; Day et al., 1943; Schweigert et al., (1945). However, Day et al., (1943) state, that while the cecum is an important site of the synthesis of vitamin K, this vitamin can also be formed in other parts of the intestinal tract. Griffith (1935) found that coprophagy i proved the condition of cecectomized animals and concluded that vitamin synthesis occurs in other parts of the alimentary tract.

Since the cecum has been considered to be the site of much vitamin synthesis, several workers have conducted nutritional studies on rats deprived of their ceca (Griffith, 1935; Taylor et al., 1942; Day et al., 1943; Schweigert et al., 1945). Provided the diet was right, the cecum was found to make a contribution to the rat's supply of some of the B vitamins and it was of interest to observe, that as a whole, cecectomized animals grew as well as the controls provided the diet was adequate (Griffith, 1935; Taylor et al., 1942; Day et al., 1943). However, Taylor et al., (1942) found

that, on a diet such as the pyridoxin-deficient ration II of Conger and Elvehjem, the cecum adds very little to the vitamin sources already contained in the diet, and Schweigert et al., (1945) indicated that rats are not dependent on the cecum to any extent for the production or absorption of certain of the B complex vitamins when on a sucrose diet.

With the knowledge that bacterial vitamin synthesis occurs within the intestinal tract, the availability of these vitamins to the animal was to be determined. Among other workers, Guerrant et al., (1934) and Griffith (1935) indicated that although vitamin synthesis occurred in the intestinal tract, these vitamins did not appear available for absorption until the animals resorted to coprophagy. This seemed probable when the work of Abdel - Salam et al., (1938) showed that there is a synthesis of thiamin by a mixed cecal flora in vitro, although no diffusion of the synthesized vitamin B from the bacterial cells into the broth medium was detected; indicating that the vitamins may be kept within the bacterial cells making them unavailable for absorption. Mitchell et al., (1942), discovered, however, that in addition to thiamin; inositol, nicotinic acid and riboflavin are found in considerable quantities in bacterial cells and that pantothenic acid and folic acid diffuse to a greater extent, while biotin and pyridoxin apparently move freely from the cells into the surrounding medium. Mitchell et al., (1942) indicated that the vitamins that do escape into the medium are vitamins which can be absorbed

by body tissues. Schweigert et al., (1945) believed that intestinal absorption of some of the vitamins synthesized accounted for increased growth rates observed in some of their experimental animals. Mitchell et al., (1942) point out that the amount of vitamin absorption is a reflection of the amount of vitamin in the medium surrounding the bacteria and not the total quantity synthesized; and that the problem of the total quantity of absorption is complicated by the possible synthesis of vitamins by body tissues.

It appears probable that at least a part of the vitamins synthesized by the intestinal flora is absorbed by animal tissues and might thus partially or completely satisfy the requirements of a rat for particular vitamins, while the rest are not readily available to the animal unless coprophagy is practiced.

Along with the absorption, the anatomical segment where absorption occurs has been studied. Selye, (1943) found that in the small intestine, riboflavin is both absorbed and excreted, while in the cecum and colon injected riboflavin is destroyed with little if any absorption. The studies of Mitchell et al., (1942) and Schweigert et al., (1945) indicate the cecum to be the principal point of vitamin absorption. In all probability the kind of vitamins and the form in which the vitamins are held is an important factor in this respect.

It is known that diet is influential in establishing the nature of the flora and certain flora are believed to be synthesizers of particular vitamins, but the exact role of bacterial vitamin synthesis is still questionable.

It is possible that some of the organisms that occur in small numbers may directly or indirectly affect the floral products, and in their metabolic activities maintain or produce conditions helpful or harmful to bacterial vitamin synthesis and absorption. Considerable work indicates that coliform and lactobacilli groups are major influences, and these organisms are reported by Porter and Rettger (1940) to be the most prevalent in the intestinal tract. Mitchell et al., (1942) demonstrated some interesting points concerning the above groups of organisms as the result of feeding ground lean beef to support a proteolytic flora and ground lean beef containing 25% lactose to support an aciduric flora. It was observed that the acidophilic flora allowed the pantothenic acid produced to diffuse more freely into the surrounding medium than did the coliform flora. A similar situation existed with the folic acid but to a lesser extent. An opposite effect was demonstrated with pyridoxine and thiamin where the vitamin availability was favored by the coliform type of organisms.

In studies of this kind it is well to keep in mind, as Day et al., (1943) brought out, that variability is shown by individual organisms and the vitamins they produce.

Attempts have been made to find additional information in connection with the intestinal flora by attempting to raise animals aseptically. (Glimstedt, 1936; Lobund Reports, 1946) Although this will not offer final proof of what happens under normal conditions it will undoubtedly reveal many facts of prime importance concerning the intestinal flora.

Essentially the intestinal flora of man and animals are much alike. The white rat has been found to be the most suitable experimental animal for studies of this character and has therefore been used by the majority of investigators in this field. Although this review has dealt primarily with investigations involving rats many contributions to the study of the bacterial flora of the intestines have been made using other animals: monkeys, Waisman et al., (1943) McCall et al., (1946); mice, White (1941); dogs, Poth (1942); guinea pigs, Crelius and Rettger (1943); humans, Najjar et al., (1944); fowl, Luckey et al., (1946); Johansson et al., (1947); Couch et al., (1948).

As work advances in determining the nutritional role of the intestinal flora it is clear that before too many assumptions can be made, an overall picture is imperative, if indeed, the true picture can be seen at all under the multitude of complexities and variabilities produced by the biological processes that take place in the intestinal tract. This is evidenced in this particular review by the variety of results cited on the various problems discussed. In this connection, it is of interest to note that very recently Nath et al., (1948) found that a lactose diet induced a high coliform as well as a high lactobacilli count in the cecum of the rat. The effect of lactose in inducing a high aciduric flora has been known since early times, but its effect in maintaining a high coliform flora has not been reported in previous work.

As formerly stated, many workers indicate that conditions supporting a lactobacillus flora tend to discourage the growth of proteolytic flora and it is reported that this is due to the acid produced as a result of the metabolic activity of the lactobacilli.

Weinstein, Weiss and Gillispie (1938) found a connection between an acidophilic flora and a low pH value, but they were unable to ascertain any absolute correlation. Hath et al., (1948) state that since the proteolytic organisms are lactose fermenters there is no reason why they should be repressed by a lactose diet. In view of this statement, it is interesting to note the pH ranges for bacterial growth given by Porter (1946). It is observed that the optimum pH for the growth of Lactobacillus acidophilus is 5.8 - 6.6 and for E. coli 6-7, while the minimum pH values for growth are 4-4.6 for the L. acidophilus and 4.4 for the E. coli. Perhaps then, if further work does support the majority of previous studies and a lactose diet is found to favor the lactobacillus groups it is possible that the acid produced, due to growth of these organisms, may not be the only factor of primary importance in the depression of proteolytic organisms in such a diet. It is more than likely that the environment, supplying optimal conditions for the aciduric bacteria, causes population increases that depress coliform groups by virtue of their numbers.

Most of the recent work has pertained to those factors which would indicate the bacterial flora to be of nutritional value to the host. However, certain types of bacteria may be deleterious because they could possibly destroy or utilize vitamins and amino acids or produce toxic materials which may be absorbed and retard normal metabolism. Obviously studies on nutrition may have little meaning until more is known about the production and destruction of nutrients in the digestive tract.

EXPERIMENTAL METHODS

All experiments were conducted with weanling albino rats from the Sprague Dawley Colony, Madison, Wisconsin.

The rats were individually housed in raised, wire-meshed bottom cages to prevent coprophagy.

The milk diet used consisted of evaporated Carnation milk, diluted equally one to one with tap water, and supplemented by a mixture of iron phosphate and magnesium and copper sulphate. The stock diet consisted 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 this meal was moisted with corn oil.

In the choice of media it was considered important to have a set of media in which determinations could be made under the same conditions. Considering the large numbers of organisms to be estimated, a dilution count method was thought most practical, and was employed for all organisms so that, as nearly as possible, comparable results could be obtained.

It was considered necessary that one medium give a total count of the viable bacteria, and that the selective media allow estimation of the relative numbers of each of the individual groups of bacteria.

Evenson et al., (1946) reported a useful set of media which, for the most part, was found satisfactory in this study.

Liver infusion broth was used for estimating the total numbers of viable bacteria because it offered an adequate nitrogen and carbon nutrient complex for the diverse intestinal types, and has been found unsatisfactory only for the yeast-like organisms. Because of the difficulty in obtaining fresh dried liver when this experimental study was begun, dehydrated bacto liver powder was used in making the liver extract for this medium. Before dispensing the medium into the test tubes, approximately .25 gm portions of the dehydrated liver were placed in the bottom of the tubes in lieu of fresh dried liver chunks. This may have partially accounted for the fact that this medium was not found satisfactory for estimating

anaerobic growth, as it was reported to have been by Evanson et al., (1946). Readings of bacterial growth were based on turbidity of the medium.

Winblad's (1941) acetic acid medium, without the agar, was used for the estimation of numbers of lactobacilli. Kulp's tomato juice medium was used simultaneously with the acetic acid medium at the beginning of this study. As was noted by Evenson et al., (1946) the growth results in the two media were comparable and the use of the tomato juice medium was discontinued. Winblad's medium was also reported to support the growth of yeasts which could be detected by gas trapped in Durham tubes. Since considerable variance was exhibited by this method, and since the liver infusion medium used for total counts did not support the growth of yeasts, the determination of these organisms was not made in this particular study. Readings were based on the tubes of Winblad's medium showing turbidity.

The E C medium of Hajna and Perry (1943) was employed as a selective culture medium for the coliform group. It has been reported the bile salts mixture in this medium inhibits the growth of fecal streptococci and spore formers. Because enterococci have been reported tolerant to bile by other workers (Weissenbach, 1918; Evans, 1947) and because of what was considered as possibly low coliform counts at the beginning of this study, the lauryl sulfate tryptose broth of Mallmann and Darby was used in conjunction with the E C medium under the same set of conditions. The medium of

Mallmann and Darby consists of tryptose lactose broth plus the selective agent sodium lauryl sulfate in a dilution which has no toxic effect on the desired organisms. Both media were incubated in two series; one at 37° C. to detect the coliform bacteria as a group; the other, at 45° C. was used for *E. coli* determination. Readings were made on the basis of gas production.

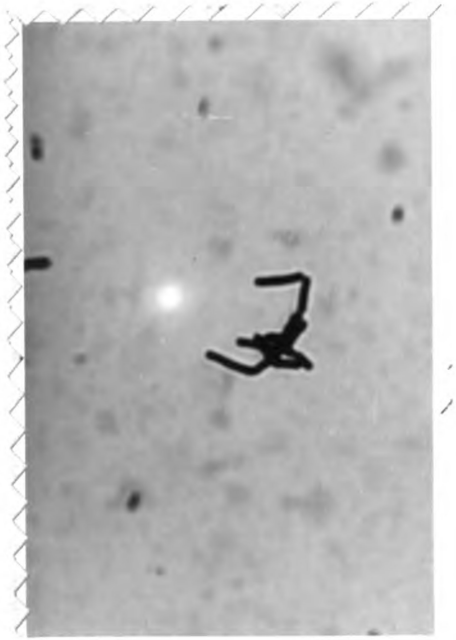
For the enumeration of the enterococci the S F medium of Hajna and Perry (1943) was employed. With this medium a selective temperature of incubation (45° C.) was used, and sodium azide was incorporated as an inhibitory agent. Turbidity and an acid reaction shown by the color change of the brom cresol purple were growth criteria. Since the selective temperature of 45° C. was reported by Evans (1947) to separate the enterococci from the other streptococci and 37° C. was reported as the optimum temperature for their growth, a set of tubes was incubated at 37° C. as well as at 45° C.

All media employed in this study were checked periodically by microscopic examination. Gram's stain was used. Figures 1-3 illustrate smears typical of those obtained throughout this study, and show that the groups of bacteria being determined in this study predominated in the selective media employed for the estimation of their numbers.

A group of 40 weanling rats were placed on the experimental diets. Twenty of these animals were fed the stock diet, and twenty others were fed the milk diet. Half of the animals in each diet group were cecectomized.

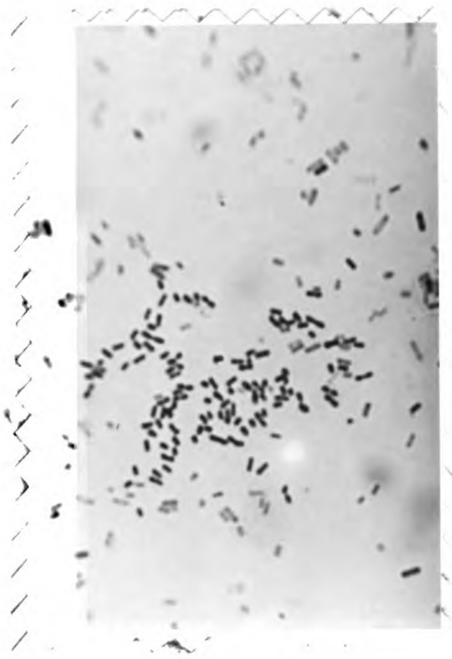
Smears made from bacterial cultures of the intestinal contents of the experimental rats. Microscopically the enterococci, lactobacilli, and coliforms were seen to be the predominating organisms growing in the selective media used for their determination.

Fig. 1



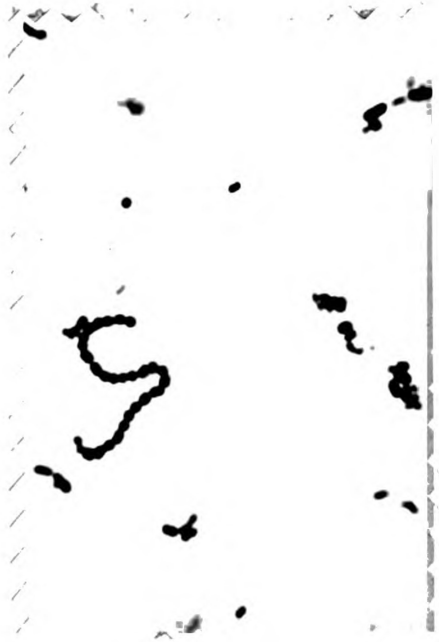
Lactobacilli
Winblad's medium 37° C.
10^{-6} dilution

Fig. 2



Coliforms
E C Medium 37° C.
10^{-4} dilution

Fig. 3



Enterococci
S F Medium 37° C.
10^{-6} dilution

The animals in this experimental work were subjected to cecectomy employing the method devised by Dr. Wade Brinker of the School of Veterinary Medicine, Michigan State College.

The experimental rats were anesthetized with sodium pentobarbital. The standard solution contained 1 grain per ml., and for immediate use .5 ml. was diluted with 9.5 ml. of distilled water. Injections of .1 ml. per 10 grams of body weight were made into the abdominal cavity. The operative area was clipped to remove the hair, scrubbed with soap and water, and swabbed with 70% ethyl alcohol. This area was draped with a sterile shroud.

A 1/2 inch incision was made through the skin, abdominal muscles and peritoneum. The incision was made on the left side starting on a level with the umbilicus and extended posteriorly. The cecum was located and drawn through the incision (Fig. 1). The serious attachment between the cecum and the intestine was severed, and two small straight mosquito forceps were placed at the base of the cecum where it joins the intestine (Fig. 2). The cecum was excised between the two forceps. The stump of cecum was swabbed with pure phenol and 70% ethyl alcohol.

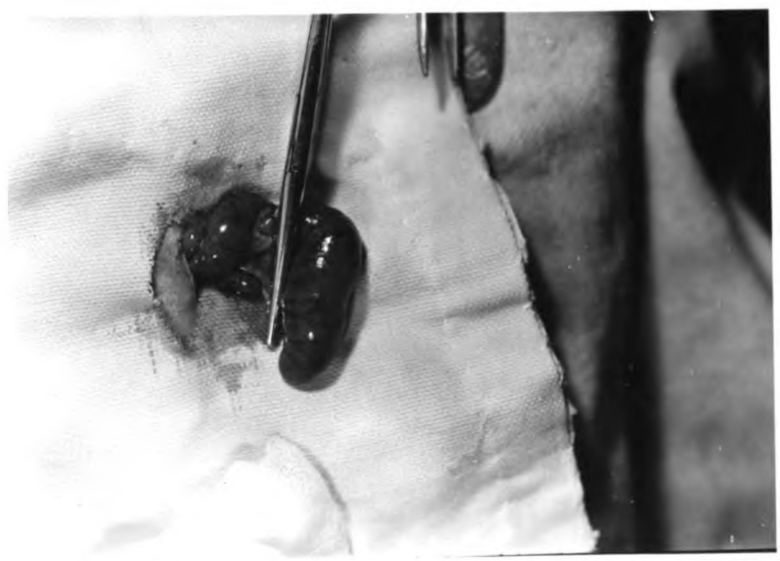
No. 100 cotton thread was used as suture material. The suturing was begun with a right angle Cushing stitch taken at the mesenteric border of the cecum close to the forceps; it was not tied. The needle was then carried over the forceps to the other side where another Cushing stitch was made. The suturing was continued by alternating from

Cecectomy

Figure 4.

Cecum Exposed

Figure 5



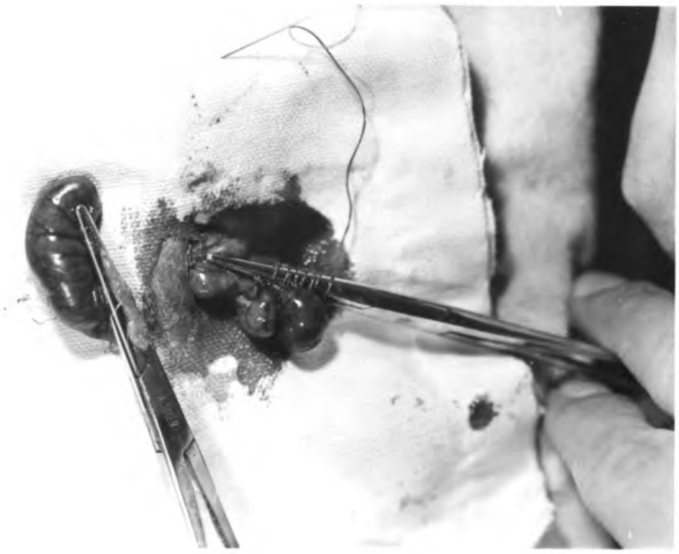
Forceps at base of cecum
Clamping off of the cecum.

one side of the forceps to the other (Fig. 3), covering the entire length of the stump. The stitches were applied approximately 1/8 inch and 1/16 inch from the edge of the forceps. Tension was applied in opposite directions to the loose ends of suture material and at the same time the forceps were opened and slowly retracted. After the forceps were withdrawn a short distance they were dipped into the lumen. This procedure aided in the inversion of the bowl edges. A second row of Cushing stitching was inserted to reinforce the inner row (Fig. 4). The abdominal muscle, peritoneum and skin were closed with interrupted cotton sutures.

Studies of the 40 experimental rats were made by Khanda (1947) previous to their autopsy. The bacteriological work in the present study was started by autopsying 1-2 animals at a time, and continued until the 40 experimental animals had been killed. Before autopsy the animals were allowed to fast 12 - 14 hours. Bacterial total counts and coliform determinations were made on the intestinal contents following autopsy (Study I).

Approximately .1 gram samples of the contents from the large intestine, small intestine, and the cecum were obtained. Each sample was transferred to a separate, previously weighed sterile test tube containing glass beads. The weights of the samples were determined, known dilutions (1:100) made by the addition of sterile distilled water, and a uniform suspension obtained after thorough shaking. The suspensions of material were further diluted in decimal series to 10^{11}.

Figure 6.



Excised cecum; Cushing stitching

Figure 7.

Operation Completed

Inoculations of media were made in triplicate, in 1 ml. amounts for each dilution (10^{3} - 10^{11}). All media were incubated for a period of 72 hours at a temperature of 45° C. or 37° C., as the medium called for. The counts were computed by a reference to the Dilution Count Tables of Buchanan and Fulmer (1923), and averages were obtained by geometric mean.

Approximately a year later, a second group of animals of the same number was put under identical experimental conditions, and enterococci and lactobacilli as well as coliform and total counts were determined (Study II).

The dilutions were made as previously, except that the entire contents were obtained from the three intestinal sections for the first dilution (1:100) instead of the .1 gram amounts. This was done with the possibility that a more representative picture of that section might be obtained.

RESULTS AND DISCUSSION

Tables 1 and 2 show the counts of the bacteria found in the various sections of the intestines in the 40 experimental rats used in Study II. The counts are expressed as the geometric mean of the individual bacterial counts obtained from 4 groups of animals. These 4 groups consisted of the 10 cecectomized and 10 control animals fed a stock diet, and the same number of cecectomized and control rats fed a milk diet. Differences are seen in the bacterial

Table 1.

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 Each of the Intestinal Sections (Study II).

Animal		Diet	Intestinal Section	Total and Group Counts*					
No.	Condition			37°C. Total	37°C. Coliform	45°C. E. coli	37°C. Streptococci	45°C. Streptococci	37°C. Lactobacilli
10	Control	Milk	Small	36,000,000	27,000	6,000	44,000	9,000	8,000,000
10	Control	Milk	Large	230,000,000	88,000	31,000	480,000	37,000	32,000,000
10	Control	Milk	Cecum	236,000,000	63,000	22,000	280,000	41,000	12,000,000
10	Cecec-tomized	Milk	Small	580,000,000	67,000	20,000	184,000	30,000	49,000,000
10	Cecec-tomized	Milk	Large	1,600,000,000	240,000	68,000	1,400,000	110,000	89,000,000
10	Control	Stock	Small	400,000,000	7,000	2,000	23,000	6,000	55,000,000
10	Control	Stock	Large	920,000,000	36,000	6,000	135,000	22,000	157,000,000
10	Control	Stock	Cecum	800,000,000	22,000	6,000	45,000	13,000	61,000,000
10	Cecec-tomized	Stock	Small	480,000,000	36,000	9,000	240,000	26,000	84,000,000
10	Cecec-tomized	Stock	Large	324,000,000	1,400,000	140,000	2,600,000	510,000	24,000,000

*Bacterial Counts expressed in Geometric Means

Table 2.

The Bacterial Counts Found in the Intestines of Cecectomized and Control Rats Fed a Stock and Milk Diet Arranged to Show How a Particular Intestinal Section is Effected by Each of the Experimental Diets (Study II).

Animal No.	Condition	Diet	Intestinal Section	Total and Group Counts* 37°C. Total	37°C. Coliform	45°C. E. coli	37°C. Streptococci	45°C. Streptococci	37°C. Lactobacilli
10	Control	Milk	Small	36,000,000	27,000	6,000	44,000	9,000	8,000,000
10	Control	Stock	Small	400,000,000	7,000	2,000	23,000	6,000	55,000,000
10	Control	Milk	Large	230,000,000	88,000	31,000	480,000	37,000	32,000,000
10	Control	Stock	Large	920,000,000	37,000	6,000	135,000	22,000	160,000,000
10	Control	Milk	Cecum	240,000,000	63,000	22,000	230,000	41,000	12,000,000
10	Control	Stock	Cecum	800,000,000	22,000	6,000	45,000	13,000	61,000,000
10	Cecectomized	Milk	Small	580,000,000	67,000	20,000	180,000	30,000	49,000,000
10	Cecectomized	Stock	Small	480,000,000	36,000	9,000	240,000	26,000	64,000,000
10	Cecectomized	Milk	Large	1,600,000,000	240,000	68,000	1,400,000	110,000	89,000,000
10	Cecectomized	Stock	Large	320,000,000	1,400,000	140,000	2,600,000	510,000	24,000,000

*Bacterial Counts Expressed in Geometric Mean

counts obtained from the intestinal sections of the cecemtomized and control animals fed the same and different diets, (Table 1) and the effects of these diets on the counts in a particular section of the intestines. (Table 2).

Tables 1 and 2 also show that, irrespective of the diet, the coliforms were the least numerous and the lactobacilli the most numerous organisms found in the intestinal tract. Lactobacilli counts have been seen to exceed coliform counts on other stock diets (Evenson et al., 1945).

The high lactobacilli counts seen on animals fed a milk diet was not unexpected. Milk, with its high lactose content has been known since the early studies of Hull and Kettger (1917) and Cruickshank (1938) to favor the development of an aciduric flora and depress coliform growth. The resultant aciduric flora seen in animals fed the stock diet was thought to be due to the combined influences of a number of factors. Certain ingredients in the stock diet were considered particularly important in this respect. Among these were: the milk incorporated into the stock feed, the mixture of grains which have been reported by Hull and Kettger (1917) to favor an aciduric flora, the help of certain inorganic elements in maintaining an aciduric growth (Eppricht et al., 1937), and the corn oil which has been seen to repress coliforms with little effect on lactobacilli (Torrey, 1919; Kath et al., 1948).

Tables 1 and 2 show that the enterococci were lower in numbers than the lactobacilli, but numerically exceeded

the coliforms. The writer believes that the enterococci found conditions favorable to their growth due to the environment produced by the diets used, and the fact that this diet also caused the predominating growth of lactobacilli and depressed coliforms. Evenson et al., (1945) found that depression of the coliform group and Gant et al., (1943) depression of E. coli resulted in an increase of enterococci; Osterlenk and Hunter (1946) observed that high total counts were more adverse to the growth of coliforms than to enterococci. Osterlenk and Hunter (1946) also observed in their studies that of 51 fecal samples examined, 37 per cent showed enterococci occurring in equal or in greater numbers than E. coli.

Tables 1 and 2 further show that the coliforms, other than E. coli dominated consistently. This has been seen previously by Evenson et al., (1945).

Table 3 shows the sum of the total and coliform counts obtained from the intestines of the experimental animals used in Study I and Study II. The counts, obtained a year apart, show similar results. Although the coliforms were found in greater numbers in Study I there was a close correlation in their counts in each study. The cecectomized animals showed higher numbers of coliforms than did the control animals; the differences were seen to be greater in the stock fed animals. In both studies the total counts also followed the same general trend. It was of particular interest to note that the cecectomized animals on a milk diet showed

Table 3.

The Sum of the Total Bacterial and Coliform Counts Found in the Intestines of Cecectomized and Control Rats Fed a Milk and Stock Diet (Study I and II).

Animals		Diet	Expt.	37° C.	37° C.
Number	Condition			Total Counts	Coliforms
10	Cecectomized	Milk	Study I	20,000,000,000	6,006,000
10	Control	Milk	Study I	587,000,000	1,700,000
10	Cecectomized	Stock	Study I	540,000,000	1,038,000
10	Control	Stock	Study I	2,711,000,000	146,000
10	Cecectomized	Milk	Study II	2,180,000,000	307,000
10	Control	Milk	Study II	502,000,000	178,000
10	Cecectomized	Stock	Study II	804,000,000	1,440,000
10	Control	Stock	Study II	2,120,000,000	65,000

*Bacterial counts expressed in Geometric Mean

showed higher total counts than the control animals on the same diet. Opposite results were seen in the animals fed the stock diet. The cecectomized animals on this diet showed lower total counts than the control animals. Dhanda (1947) found similar results in fecal samples obtained from the anus of the animals used in Study I.

The growth curves shown in Figure 8 reveal that cecectomy had no untoward effect on the growth rates of the cecectomized animals in Study I. Normal growth was also seen in the cecectomized animals in Study II. The animals were autopsied at approximately the same age and no growth curves were made, but the weights of all animals were found comparable. In the cecectomized animals, possibly the large numbers of bacteria, the increases of lactobacilli and coliforms in the milk fed group and the decided increase in the coliforms of the stock fed group may have provided the necessary elements for growth in the form of synthesized accessory food factors. Black et al., (1941); Gant et al., (1943); and Miller, (1945); among others believed E. coli to be particularly active in vitamin B production. It is well known that there are many important components in the B complex, and among them thiamin and niacin are important in carbohydrate metabolism, choline in fat metabolism, and riboflavin in biological oxidations.

The large intestines showed numbers of bacteria comparable to and often exceeding those in the ceca. This was noted most frequently in animals subsisting on the stock diet.

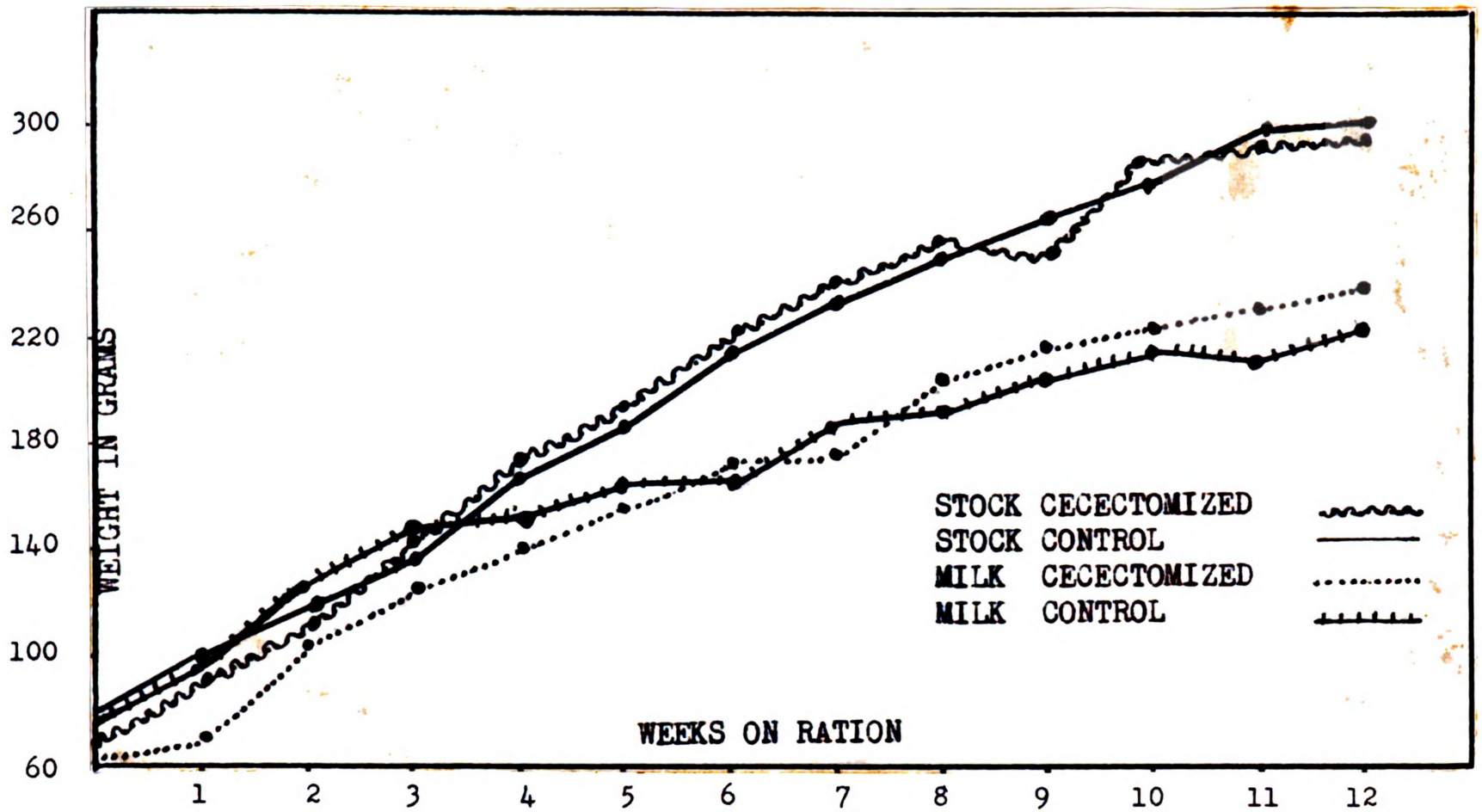


FIGURE 8. GROWTH OF NORMAL AND CECECTOMIZED RATS ON STOCK AND MILK DIETS (Study I)

Dhanda (1937) made similar observations in his studies. His examination of cecal contents and anal contents from rats at the time of cecectomy, revealed higher counts in the anal samples than in those obtained from the cecum. Tables 1 and 2 also show the high numbers of bacteria harbored by the small intestines in both the control and cecectomized animals. This again is more noticeable in the animals fed the stock diet. Porter and Hettger (1940) reported significant numbers of bacteria to be present in the small intestines.

Total and group counts obtained from the intestines of animals in Study II are shown in Table 4. The total counts and the lactobacilli counts are seen to correspond in trend. These corresponding results might indicate, that the lactobacilli counts influenced the total counts to a large extent. Perhaps, their rise in the cecectomized animals on the milk diet and their fall in the cecectomized animals on a stock diet could have been due to the fact that the composition of the milk, at first, offered a more readily available environment for the immediate growth of the lactobacilli than did the composition of the stock diet. The large amounts of indigestible material found in the stock diet may have had its effect in this respect.

Table 5 shows the sum of the bacterial total counts and the combined sums of the group counts obtained from the intestines of the animals used in Study II. A discrepancy between total counts and the sum of the group counts is seen in Study II. This kind of discrepancy was also seen in the studies of

Table 4.

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 (Study II).

	Animal		Total and Group Counts*					
No.	Condition	Diet	37° C. Total	37° C. Coliform	45° C. E. coli	37° C. Strepto-cocci	45° C. Strepto-cocci	37° C. Lacto-bacilli
10	Cecectomized	Milk	2,180,000,000	307,000	88,000	1,584,000	140,000	138,000,000
10	Control	Milk	502,000,000	178,000	59,000	804,000	87,000	52,000,000
10	Cecectomized	Stock	804,000,000	1,440,000	149,000	2,840,000	536,000	88,000,000
10	Control	Stock	2,120,000,000	65,000	14,000	203,000	41,000	273,000,000

*Bacterial Counts Expressed in Geometric Mean

Table 5.

The Sum of the Bacterial Total Counts and the Combined Sums of the Group Counts Obtained from the Intestines of Cecectomized and Control Animals Fed a Milk and Stock Diet. (Study II).

Number	Condition	Diet	Counts	
10	Cecectomized	Milk	Total	2,180,000,000
			Combined Groups	139,891,000
10	Cecectomized	Stock	Total	804,000,000
			Combined Groups	92,276,000
10	Control	Milk	Total	502,000,000
			Combined Groups	52,982,000
10	Control	Stock	Total	2,120,000,000
			Combined Groups	273,268,000

*Bacterial Counts Expressed in Geometric Mean

Evenson et al., (1945). In this study it is attributed in part to the necessity of using differential media, and the fact that many of the organisms believed present in the intestinal tract were not determined as groups, but may possibly have found a favorable environment in the liver infusion broth employed. The author believes that the organisms accounted for constitute the major portion of the organisms taken into consideration in this study.

A great many variables are necessarily encountered in study of this kind. In this work it was i possible to immediately examine the samples obtained at collection. They generally remained in their diluent from four to six hours before cultures could be made. Microscopic examinations of cultures were not made after each determination, however, the media were checked periodically throughout the study.

In both study I and II considerable variation was observed in the bacterial counts from animals which were under the same experimental conditions. White (1942), Miller (1947), and Nath et al., (1948) found this to be true in their studies.

In the cecectomized animals undoubtedly the absence of the physiological functions of the cecum, e.g., the absorption of water, influenced to a great extent the resultant bacterial flora found in these studies.

SUMMARY

A study was made of the intestinal flora found in cecectomized and control rats fed on stock and milk diets.

The cecectomized animals on a milk diet showed higher total counts than the control animals on the same diet. Opposite results were seen in the rats fed the stock diet; the cecectomized animals on this diet showed lower total counts than the control animals.

Total counts and lactobacillus counts corresponded in trend.

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.

Within the coliform group, organisms other than *E. coli* dominated consistently. Coliform counts were higher in cecectomized animals regardless of the diet.

Considerable variation was seen in the counts from individual animals under the same experimental conditions.

The large intestines showed counts comparable to and often larger than those found in the ceca. High numbers of bacteria were present in the small intestine of cecectomized and control animals.

Cecectomy did not retard the growth of the experimental animals.

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