THE ROLE OF AN INTESTINAL STREPTOCOCCUS ORGANISM IN THE NUTRITION OF RATS FED AN EVAPORATED MILK DIET

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THE ROLE OF AN INTESTINAL STREPTOCOCCUS ORGANISM IN THE NUTRITION OF RATS FED AN EVAPORATED MILK DIET

by

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The fundamental purpose of all nutrition research is to determine the adequate food needs of humans throughout their life span. Once it was thought that this objective could be realized simply by discovering the food nutrients necessary for growth, reproduction, and lactation. However, further studies revealed interrelationships between many of these factors. Varying the intake of one factor often changed the requirement for another factor. Still other investigations showed that some of the bacterial flora of the intestine contributed to the nutrition of the host by synthesizing some of the required factors, and by aiding in the breakdown of complex food materials. The bacterial flora has been shown to change with the diet, further complicating the requirements of the host.

In the main, previous workers, studying the relation of diet to the intestinal flora, have investigated the role of the lactobacilli, streptococci, and coliform organisms, as these organisms have been the most readily isolated and cultivated. Investigators have thus far reported the concentration of these organisms (differential counts) in the intestinal material, ignoring the high concentration of unidentified organisms which complete the total bacterial

population. Also these earlier studies revealed no apparent relationship between the total and differential counts. More information is needed relative to the unidentified organisms, as these may prove to be of vital nutritional importance.

The purpose of this study was to isolate and identify one of the organisms found in relatively large numbers in the intestinal tract of the milk-fed rat and to study its nutritional importance to this animal.

REVIEW OF LITERATURE

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The Effect of Diet on Intestinal Flora

Studies have been made on many species of animals, including horses, cows, sheep, cats, dogs, rats, mice, monkeys, and humans, demonstrating that diet does affect the intestinal flora. From these studies it appears that the alimentary bacterial flora of both man and animals is essentially alike.

Early studies revealed that in the intestinal flora of animals fed a high protein diet proteolytic or putrefactive organisms predominated, while in those fed a high carbohydrate diet, aciduric or fermentative organisms predominated (Porter and Rettger, 1940; Winblad, 1941; and Gall, et al., 1948a).

It was found that a variation in the kind of carbohydrate induced a change in the intestinal flora. Nath, et al. (1948) reported that the total aerobic and anaerobic plate counts, as well as the differential counts of coliforms and lactic-acid-producing organisms were higher in the ceca of rats on lactose diets than on dextrin or sucrose. It was found that ten times as many Lactobacillus acidophilus colonies were cultivated from the feces of rats fed a starch

diet than were cultivated from the same quantity of feces of rats fed a sucrose diet (Winblad, 1941). That a change in kind of carbohydrate does cause a change in the intestinal flora is supported by the work of Gall, et al. (1948a), who found that the cecal contents of rats on a dextrose diet always contained many large cocci, occurring in pairs and short chains. The ceca of animals on a dextrin diet contained many elongated cocci in pairs, tiny cocci in long chains, and highly curved rods.

Fredericia first described refection in 1926 as the synthesis of vitamin B by rats on vitamin B-deficient diets. Subsequent work reported by Kon and Porter (1947) showed that refection depended on the presence of undigested starch and starch-splitting organisms in the cecum of the rat. Heller, et al. (1925) presented evidence that a spore-forming organism was responsible for the synthesis of vitamin B. More recently, Gall, et al. (1948b) demonstrated that coliforms isolated from the alimentary tract of the mouse synthesized riboflavin, niacin, biotin, folic acid, and pantothenic acid in tubes of broth deficient only in each respective vitamin. The elongated cocci, characteristic of the flora found in mice fed a dextrin diet, were demonstrated in the same way to have synthesized all of these B vitamins, while the round cocci, found in animals fed a dextrose diet, were shown to have synthesized all of these vitamins except folic acid.

Other investigations have established the fact that vitamin K may also be synthesized by rats either in the cecum or other parts of the intestinal tract (Day, et al., 1943; Elvehjem, 1946).

Nath, et al. (1948) found that the total bacterial counts per gram of cecal material and the total weights of the ceca from animals receiving lactose as a source of carbohydrate were greater than the bacterial counts and weights of the ceca from rats receiving dextrin or sucrose. Nath, et al. (1948, p. 786) stated, "Thus the total numbers of organisms per cecum was higher in rats fed lactose than in those fed sucrose or dextrin."

The amount of protein in the diet seems to influence the intestinal flora. Wegner, et al. (1941) found that increasing the amount of nitrogen in the feed of a cow caused no bacterial synthesis of riboflavin, thiamin, pantothenic acid, biotin, and folic acid, and depressed the synthesis of niacin. Czackes and Guggenheim (1946) reported one-third as many bacteria per gram of fecal material from rats fed a high protein diet as from those fed a low protein diet.

Boutwell and coworkers (1943) investigated the effect of different fats on the growth of rats. They found that butterfat and lard induced superior growth to corn oil, coconut oil, cottonseed oil, soybean oil, peanut oil, and hydrogenated cottonseed oil when lactose was the carbohydrate

source. Butterfat and lard supported slightly better growth than oleomargarine of animal fat origin and decidedly better growth than oleomargarine of vegetable fat origin. Growth was the same for all oils and fats when fed with a mixed carbohydrate diet, including sucrose, starch, dextrin, dextrose, and lactose. The author suggests that these changes in growth may be caused by changes in the intestinal flora. When a high level of corn oil was added to a sucrose diet (Nath, et al., 1948), the total aerobic and anaerobic plate counts and the coliform counts on a differential medium were decreased in rat ceca. The ceca of rats fed butterfat diets weighed more and exhibited higher total bacterial counts than ceca of rats fed corn oil diets.

The Effect of Streptomycin on Intestinal Flora

Pratt and DuFrenoy (1949, p. 2) define an antibiotic as, "... a metabolic product of one micro-organism that is detrimental or inimical to the life activities of other micro-organisms, usually even when present in extremely low concentrations." These metabolic products may also be produced by higher plants and animals. The phenomenon is called antibiosis and was employed 30 or more centuries ago by the Chinese.

At the present time, many antibiotic substances have been identified and studied. Among them are penicillin,

streptomycin, streptothricin, aureomycin, chloromycetin, neomycin, tyrothricin, and terramycin. Even the acid produced by the lactic acid bacteria in the intestine can be considered an antibiotic, as it is antagonistic toward putrefactive bacteria.

Stokstad (1940) suggested three possible actions of antibiotics that reach the intestine after oral administration. First, they may prevent some organisms from competing with the animal for nutrients in the diet; second, certain types of organisms may be allowed to grow in the presence of the antibiotics, which, in turn, synthesize important nutrients used by the animal; or third, the antibiotics may suppress certain bacteria that normally produce toxic substances.

To date, most investigations of the effects of antibiotics on intestinal flora have been made with aureomycin and streptomycin. However, McGinnes (1950) reported that when terramycin, aureomycin, streptomycin, or penicillin was added to the feed of turkeys, their growth rate was greatly improved. Mortality and stunted growth of poults were reduced markedly and the feed efficiency was increased.

Streptomycin combats gram-negative, acid fast, and some gram-positive organisms *in vitro* and *in vivo*. It is produced by the fungus, *Actinomyces griseus*, which occurs universally. Streptomycin is water soluble, hydroscopic, and very stable in both solution and the dried state under

ordinary storage conditions. Reimann (1949, p. 460) states, "Streptomycin given orally is poorly absorbed and no toxic effects have been reported." Its mode of action is unknown. Youmans and Fisher (1949) report that streptomycin may act either as a bacteriostatic or a bactericidal agent *in vitro*. The predominance of either activity seems to depend, in part, on the time of contact with streptomycin, the organism involved, the concentration of streptomycin, the size of the inoculum, temperature, pH, and the age of the culture.

Smith and Robinson (1945) found that the coliform concentration of the feces of mice fed 30,000 units of streptomycin per kilogram of body weight decreased from a count of 100,000 to 100 bacteria per three milligrams of feces in 24 hours. This low coliform count was maintained throughout the three-week period with little fluctuation. When the diet was supplemented with 300,000 units of streptomycin per kilogram of body weight, coliforms disappeared from the feces within 24 hours. This higher dosage also eliminated all other gram-negative organisms, leaving only a small number of gram-positive spore-formers. The lower dosage caused a similar change, but with a less complete elimination of gram-negative organisms. Emerson and Smith (1945) found a marked diminution in the numbers of coliforms as well as in the total intestinal bacteria of rats fed streptomycin. They found doses as high as 580,000 and

875,000 units per kilogram caused symptoms similar to those observed in biotin-deficient rats.

By cultural methods Shaw and Dalldorf (1950) showed that after seven days, streptomycin (average dose: 5,500 units per mouse) inhibited all but the staphylococci and a few coliforms of the total flora; after twenty days (average dose: 11,250 units per mouse) this antibiotic inhibited all but staphylococci and spore-formers.

During oral administration of streptomycin to humans, bacteria sensitive to streptomycin, including Streptococcus faecalis, usually began to diminish twelve hours after the first dose and when at least 600 units of streptomycin per gram of feces were present (Weinstein, 1949). Growth of the organism was usually reestablished 24 to 48 hours after the last dose, or when the stool no longer contained bacteriostatic amounts of the drug. Smith and Robinson (1945) found normal counts were delayed six days after cessation of streptomycin supplements.

Pratt and DuFrenoy (1949) state that streptomycin-resistant strains of bacteria may develop with astonishing rapidity from cultures of organisms. Emerson and Smith (1945) found the bacterial population returned to normal in seven to twelve days, because of the development of streptomycin-fastness within the first 24 hours of therapy. Smith and Robinson (1945) assumed that no resistance developed in vivo as the organisms appearing in the feces before

and during therapy were isolated and tested for sensitivity to streptomycin. It was found that organisms most sensitive to streptomycin *in vitro* were eliminated first *in vivo* and those most resistant *in vitro* remained in the feces.

The ultimate purpose of studying the intestinal flora and its control through diet and antibiotics is to direct that flora to the greatest advantage of the host animal.

EXPERIMENTAL PROCEDURE

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Previous investigations conducted in these laboratories (McClure, 1949; Katainen, 1949) have demonstrated the great differences between the number of differentiated bacteria and the total number of bacteria in the intestine of both milk and stock-fed rats. It was hoped that one of the unidentified organisms in the intestine of a milk-fed rat, in which a characteristic flora had been established, could be isolated and identified and its nutritional importance to the animal studied.

White male rats[1] were fed, ad libitum, the basal diet of reconstituted evaporated milk fortified with iron, copper, and manganese. Water was given ad libitum. All animals were housed in wire bottomed raised cages to prevent coprophagy.

Preliminary examination of the intestinal flora of the milk-fed rat showed that a large coccus predominated the flora of the ileum and the cecum. This same coccus also appeared in the lower jejunum, but in much smaller numbers. It was decided that the lower jejunum was the best site for

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[1]Sprague-Dawley strain, Madison, Wisconsin

the isolation of this organism. As total counts were very low in this section, there seemed to be less chance for interference from other bacteria.

The isolation was made from the intestinal tract of a growing rat that had been on this basal diet for a sufficiently long time to establish a characteristic intestinal flora. The animal was chloroformed, the abdominal surface was swabbed with Roccal solution,[2] and the abdominal cavity opened with sterile[3] scissors and forceps. The cavity was flushed with Roccal,[2] and the organs were kept moist by intermittent flushing with this disinfectant. Sterile[3] scissors were used to make a transverse section of the lower jejunum, and its contents were sampled with a sterile platinum loop. The sample was transferred to double strength Bacto-S F medium (Difco)[4], and was incubated at 37°C. for 48 hours. This culture was plated with Bacto-TGE agar (Difco) in a concentration that allowed the growth of isolated colonies under the conditions of incubation described above. The isolated colonies were picked and inoculated into litmus

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[2]Roccal diluted 1:5000

[3]Sterilized by autoclaving 20 minutes at 15 pounds pressure and 121°C.

[4]Double strength S F medium contained 0.1% sodium azide to inhibit growth of gram-negative organisms but to allow growth of fecal streptococci.

milk and enriched tryptose medium.[5] The purified culture was carried on TGE agar slants and transferred bi-weekly.

The isolated culture was tested by the methods recommended by the Committee of Bacteriological Technic of the Society of American Bacteriologists (1946). It was identified as Streptococcus faecalis by the Bergey classification, 6th edition (Breed, et al., 1948).

The plan of studying the nutritional role of the isolated organism was altered because the nutritional requirements and reactions of S. faecalis have been studied extensively. Workers have reported that S. faecalis requires most of the essential growth factors (Bellamy and Gunsalus, 1944; Shankman, et al, 1947; and Sahyun, 1948). Investigations made to date, suggest that this organism could contribute to the nutrition of the milk-fed rat by converting lactose to lactic acid, and peptones to ammonia. Because the reactions and nutritional requirements of S. faecalis have been studied extensively, it was decided to determine the role this organism played in the well-being of the milk-fed rat by studying the effect which would be obtained if S. faecalis were removed from the intestinal tract. By the use of an antibiotic it was believed that this could be

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[5]Enriched tryptose medium contained 1% Bacto-tryptose, 0.5% Bacto-yeast extract, and 1% glucose and was adjusted to a pH of 7.0.

accomplished. If the use of an antibiotic resulted in a marked decrease in the number of S. faecalis present, without any detrimental effect on the animal, then it could be postulated that any other microorganism which replaced S. faecalis in the tract would have the ability to carry out the same nutritional function as S. faecalis.

Preliminary investigation verified other reports (Pratt and DuFrenoy, 1949) that penicillin, orally administered, is destroyed or absorbed in the intestine. No penicillin was present in the cecal contents of rats fed an average of ten units per milliliter of milk. This antibiotic was found to be ineffective in inhibiting this strain of S. faecalis in vitro, which agrees with reports of Herrell (1946) and Pratt and DuFrenoy (1949).

S. faecalis was inhibited by streptomycin both in vitro (Youmans and Fisher, 1949) and in vivo (Ravdin and Zintel, 1949). The results of other work underway in this laboratory, involving the effect of streptomycin on S. faecalis in the rumen of the cow, together with the results of a preliminary investigation carried out by the author agree with the published reports. Therefore streptomycin was chosen for use in this study.

In determining the correct dosage, it was found that 30,000 units per kilogram of body weight produced an average concentration of 160 units of streptomycin per milliliter of stomach contents and 450 units per milliliter of cecal

contents. This concentration was considered more than adequate to inhibit S. faecalis, as the range of sensitivity of this organism to streptomycin has been reported as 1.0 to 100.0 units per milliliter (Knop, 1946; Harrell, Herndon, Gillikin, and Aikawa, 1947; and Brooke, 1947). The paper disc method described by Loo, et al. (1945) was used to assay the streptomycin content, which was determined from a standard curve previously prepared (Figure 1., Appendix). The following variations of the method of Loo, et al. (1945) were used. Bacto-mycin assay agar was used for both the base and seeded media. Each paper disc was held with forceps so that it just touched the surface of the mixed solution being assayed and was saturated by capillary action. The disc was then touched to the side of the container to eliminate the excess amount clinging to the point of contact.

Two experimental groups of rats were placed on the basal milk diet supplemented with 30,000 units of streptomycin per kilogram of body weight daily for 30 days. Group I consisted of twelve 38 day old rats, housed in two cages. Two of these animals, one from each cage, were sacrificed every five days and the contents of the duodenum, upper and lower jejunum, upper and lower ileum, and cecum were sampled for total bacterial counts. Material was also obtained for culturing the flora of the lower jejunum in S F medium[4] and tryptose medium,[5] as described on page 12. Group II

consisted of seven 24 day old rats, one of which was sacrificed and sampled, as described, on each of the first five days. The two remaining animals were sacrificed and sampled at the end of the 30 day period.

Six 24 day old control animals received the basal milk diet supplemented with minerals for the 30 day period. These were sacrificed at the end of the experimental period and sampled by the same method used for the experimental animals.

Daily records were kept of the total weight of food consumed, the total weight of the experimental animals, and the streptomycin intake. Control animals were weighed on two consecutive days at five-day intervals. Fresh milk was given daily except on Sunday. Double rations were given on Saturday. The streptomycin[6] was added to the weighed milk by syringe and the mixture inverted 20 times in a glass jar to insure thorough mixing. The amount of streptomycin actually consumed was calculated from the amount of food eaten. The average intake for Group I was 26,074 units of streptomycin per kilogram and for Group II, 25,015 units per kilogram.

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[6]The crystalline streptomycin calcium chloride complex in a rubber-topped bottle was diluted to a concentration of 500,000 units/ml. with 0.5M sodium citrate.

Each rat was prepared for sampling as described on page 12 with the following changes in technique: The autopsy board was swabbed with Roccal,[2] and the instruments were immersed in the same disinfectant and returned to the Roccal[2] when not in use. Scissors were dried on a clean gauze to avoid diluting the intestinal contents with liquid adhering to the disinfected instruments. The outer skin of the abdominal cavity was removed with scissors, the peritoneum was removed, and the cavity flooded with Roccal.[2] Each section of the alimentary tract was clamped off with two small straight mosquito forceps. Prior to sampling, a transverse section was made of each segment followed by a longitudinal incision, which exposed the intestinal contents for easy sampling.

The duodenum included the pyloric sphincter and the point where it tapered to the even-bored jejunum. It was usually one to two inches long. The length of the ileum was considered approximately equivalent to the length of the duodenum and jejunum combined.

Samples taken from each section were transferred with a sterile platinum loop to sterile cotton-plugged 75 x 10 millimeter tubes, which had been dried (180°C. for 18 hours) to constant weight, and stored in a dessicator until used. The tubes containing the samples were wiped clean of finger marks and weighed immediately following the sampling of each animal. One milliliter of 7% formalin was pipetted

into each tube after weighing to serve as a preservative. Cork or rubber stoppers wrapped in cellophane were then substituted for the cotton plugs previously used and the samples were refrigerated until total bacterial counts were made.

The cecum of each animal was weighed before it was sampled.

The method of Simmons (1935) with adaptations by Bortree and Smith[7] was used in determining total bacterial counts. Crystal violet stain was used to stain the organisms and was prepared as follows: One milliliter of crystal violet-saturated alcohol solution diluted to 50 milliliters with distilled water was heated to simmering and filtered. The first dilution contained one part of sample in formalin to one part of stain in eight parts of distilled water. Higher dilutions were made when the total number of bacteria in the sample was too high to allow easy counting. Debris was worked out by carefully agitating the cover slip in a rotating motion, exercising care that contact with the solution was not broken.

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[7]Alfred L. Bortree, Veterinary Department, Pennsylvania State College, State College; and Clyde Smith, School of Veterinary Medicine, Michigan State College, East Lansing, personal communication

The organisms were counted under oil immersion.[8] A strongly lighted background showed up the violet stained organisms for easiest identification. A total of 200 squares were counted for each sample in the Petroff-Hausser chamber. Clumps of organisms were counted as one bacterium. The number of bacteria per gram of intestinal sample was calculated by the formula:

$$\frac{\text{Number of Bacteria} \times \text{Dilution Factor} \times 20{,}000{,}000^{(9)}}{\text{Number of Squares Counted} \times \text{Weight of Sample (Grams)}}$$

A sample was taken from the lower jejunum and kept in sterile physiological saline solution at the same time the sample was taken for total bacterial counts. A smear of this material was made immediately and gram stained for examination of the flora. Double strength S F medium[4] was inoculated with this sample in order to isolate S. faecalis and tryptose medium[5] was inoculated with this sample to grow possible substituting organisms. TGE agar plates were streaked with samples from tubes showing growth. After incubation for 48 hours, isolated colonies were picked and

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[8] A 1:1 dilution of cedar oil with xylene was used to prevent breaking the seal between cover slip and chamber with movement of the objective.

(9) Twenty million is a constant figure accounting for depth of chamber (1/50 mm.), square of sides (each sub-square 1/20 mm.), etc.

transferred into litmus milk and lactose broth fermentation tubes to observe their reaction in these media.

RESULTS AND DISCUSSION

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The organism isolated from the lower jejunum of a milk-fed rat and identified as Streptococcus faecalis had these morphological characteristics: It was a gram-positive coccus, spherical to oval in shape, and appeared mostly in clusters, frequently in pairs, and sometimes singly and in chains. Older cultures exhibited less tendency toward clustering. The size of this coccus ranged from 1 x 1 to 2 x 2 microns.

Gram stains of the flora from the lower jejunum[10] of milk-fed and of streptomycin-supplemented animals revealed no difference in types of organisms. The general flora consisted of tiny gram-negative and gram-positive cocci (they were so tiny the actual shape was not clear; medium-sized gram-positive oval cocci, occurring mostly in pairs; large gram-positive cocci, occurring singly and in pairs; large gram-positive yeast-like organisms; gram-positive rods, varying in length and shape and appearing mostly in chains; and single gram-positive slim rods. The significant difference between the flora of the milk-fed and streptomycin-

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[10]Suspended in 0.85% saline solution

supplemented rats was that the basal flora in the milk-fed rats was predominated by the large gram-positive cocci with only a small proportion of the other organisms present, while the large cocci appeared in a much lower proportion in the streptomycin-treated flora. In contrast, Porter and Rettger (1940) found very few cocci in any portion of the intestinal tract in rats fed 12 different diets. However, they found yeast-like organisms present in the stomach, duodenum, jejunum, upper ileum, lower ileum, and cecum. Shaw and Dalldorf (1950) reported these predominating types in the intestines of mice fed Purina Laboratory Chow: Lactobacilli, coliforms, enterococci, proteus, and spore-formers.

As already reported, S. faecalis was isolated from the lower jejunum of the milk-fed rat in double strength S F medium.[4] After animals were treated with streptomycin, two organisms could be isolated in double strength S F medium[4] (Table I): A large gram-positive cocci; and a rod that varied in length, sometimes assumed irregular shapes and occurred most frequently in chains. This rod was usually gram-positive but sometimes parts of the longer rods stained gram-negative. The large coccus did not always survive streptomycin therapy, while the rod always survived. The morphology of the large coccus was similar to the identified S. faecalis. However, neither the coccus nor the rod reacted with litmus milk or fermented lactose as did S. faecalis. Evidently these organisms were not duplicating the role of

TABLE I

ORGANISMS ISOLATED FROM LOWER JEJUNUM OF EACH RAT FED MILK WITH AND WITHOUT STREPTOMYCIN

Rat No.	Group	Treatment	No. of Days on Experiment	Organism Isolated from Lower Jejunum of Rat
13	II	Streptomycin	1	Spore-forming rod Large coccus
14	II	Streptomycin	2	Spore-forming rod Large coccus
15	II	Streptomycin	3	Spore-forming rod
16	II	Streptomycin	4	Spore-forming rod
17	II	Streptomycin	5	Spore-forming rod
1	I	Streptomycin	5	Spore-forming rod Large coccus
2	I	Streptomycin	5	Spore-forming rod Large coccus
3	I	Streptomycin	10	Spore-forming rod Large coccus
4	I	Streptomycin	10	Spore-forming rod Large coccus
5	I	Streptomycin	15	Spore-forming rod
6	I	Streptomycin	15	Spore-forming rod Large coccus Mycelia-like cells
7	I	Streptomycin	20	Spore-forming rod Large coccus
8	I	Streptomycin	20	Spore-forming rod Large coccus
9	I	Streptomycin	25	Spore-forming rod
10	I	Streptomycin	25	Spore-forming rod
11	I	Streptomycin	30	Spore-forming rod
12	I	Streptomycin	30	Spore-forming rod
18	II	Streptomycin	30	Spore-forming rod Large coccus
19	II	Streptomycin	30	Spore-forming rod Large coccus
20	Control	No Streptomycin	30	Large coccus
21	Control	No Streptomycin	30	Large coccus
22	Control	No Streptomycin	30	Large coccus
23	Control	No Streptomycin	30	Large coccus
24	Control	No Streptomycin	30	Large coccus
25	Control	No Streptomycin	30	Large coccus

the S. faecalis in the breakdown of lactose. Further investigation in this laboratory[11] revealed that after repeated culturing, the coccus reassumed all the characteristic reactions of S. faecalis. The rod was identified as Bacillus subtilis.[11] By cultural methods Shaw and Dalldorf (1950) found that the intestinal flora of mice, fed Purina Laboratory Chow supplemented for seven days with 5,500 units of streptomycin per animal, was predominated by staphylococci and a few coliforms; while the intestinal flora of mice, fed the same basal diet supplemented for 20 days with 11,250 units of streptomycin per mouse, was predominated by staphylococci and spore-formers.

The total number of bacteria per gram of intestinal contents at the sampled points in the control and streptomycin-supplemented rats were similar and are expressed as logarithmic numbers in Figure 1. In general, both the milk-fed and the streptomycin-supplemented rats showed an increase in the average number of bacteria from the duodenum to the cecum. The report of Porter and Rettger (1940) agrees with these findings. They reported low microbial counts from the stomach to the jejunum of the rat with a gradual increase in the number of bacteria in the upper and lower ileum, and the highest microbial counts in the cecum. It

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[11]Elbert S. Churchill, and Joseph Nichols, Department of Bacteriology and Public Health, Michigan State College, East Lansing

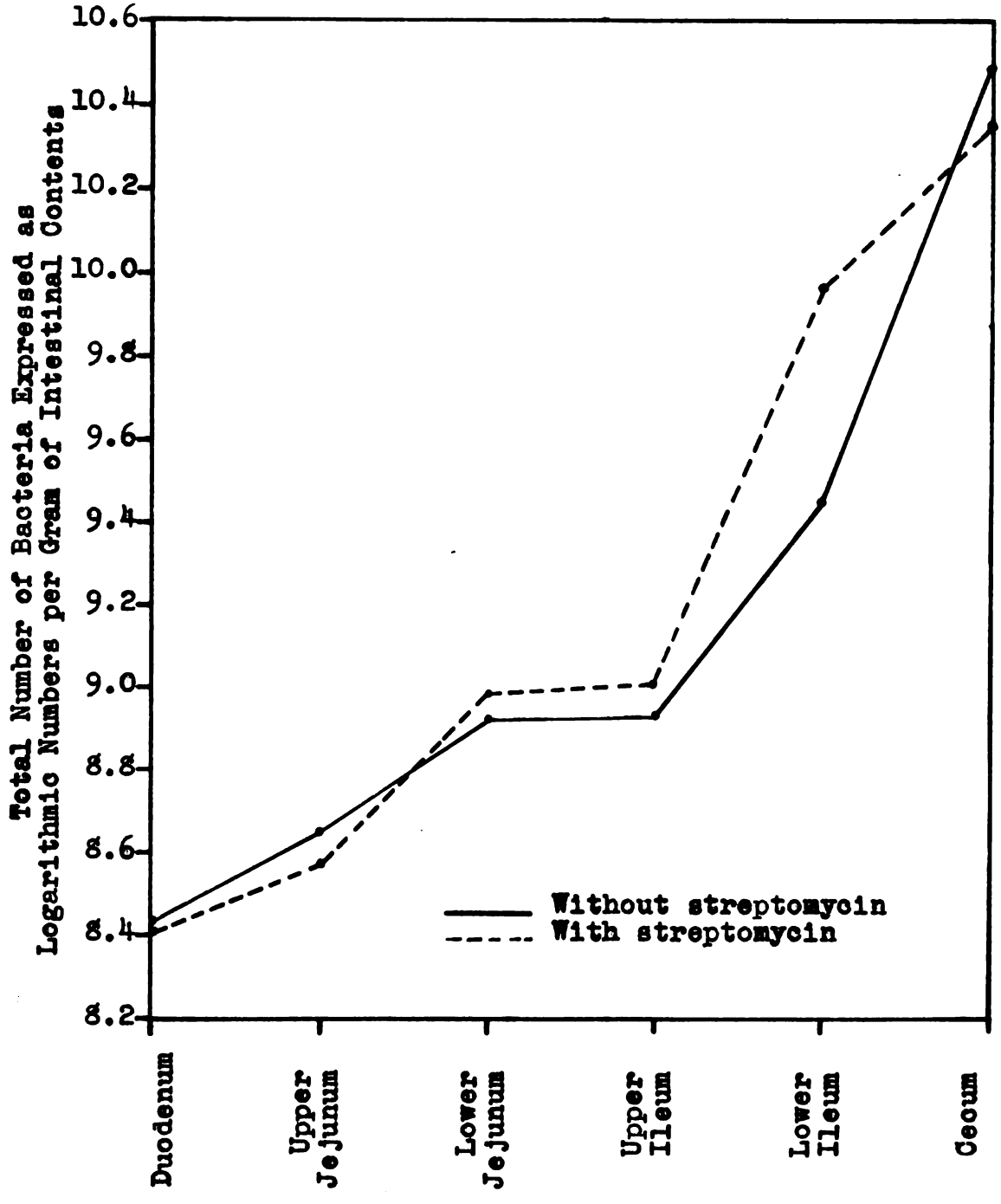

Figure 1. Average total bacteria expressed as logarithmic values per gram of intestinal contents in the duodenum, upper and lower jejunum, upper and lower ileum, and cecum of rats fed milk with and without streptomycin.

should be noted in Table II of the Appendix that individual variations, as well as group variations, increased as the total number of bacteria per gram of sample increased.

During streptomycin therapy, the greatest depression in number of bacteria occurred near the twentieth day in the duodenum, the fifteenth day in the lower jejunum, and the tenth day in the cecum (Figure 2.). After the twentieth day, there was a gradual increase in the average total number of bacteria per gram of fresh material in the duodenum and lower jejunum but a continued decrease in total bacteria in the cecal flora. Emerson and Smith (1945) reported that the bacterial population of rats returned to normal seven to twelve days after streptomycin therapy, because of the development of streptomycin fastness.

The average weight gained during the 30 day experimental period by the milk-fed and the two groups of streptomycin-supplemented rats is presented in Table II. Both groups receiving streptomycin gained more weight per rat (63.7 and 81.6 grams) than those not receiving the antibiotic (33.7 grams). It should be noted that only two animals were continued through the 30 day period in each experimental group, while six control animals were continued through the same period. The rats of Group I, beginning the experimental period at 38 days of age, gained less than the rats of Group II, beginning the experimental period at 24 days of age.

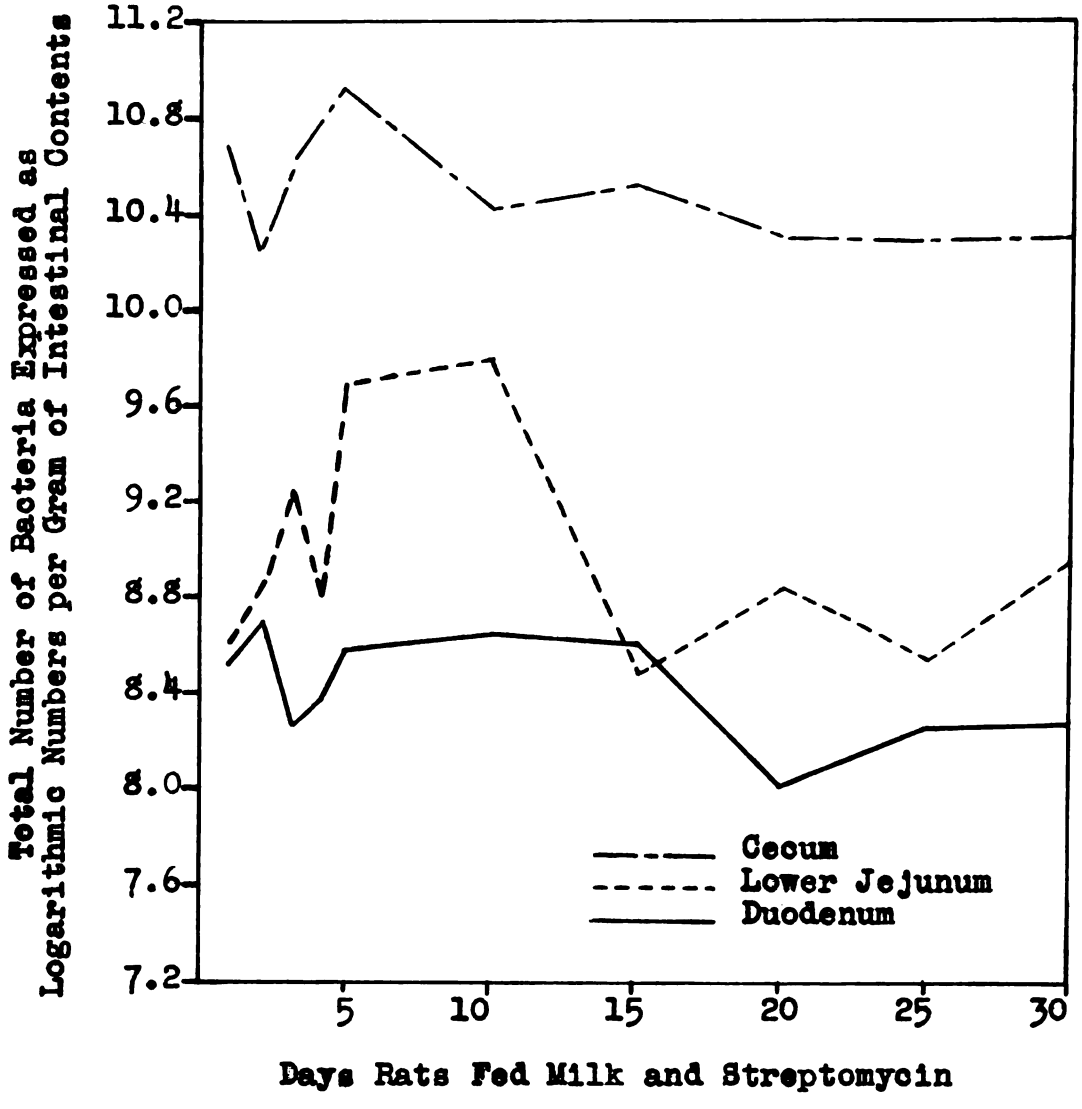

Figure 2. Relation of duration of treatment to the average total bacteria expressed as logarithmic values per gram of intestinal contents of the duodenum, lower jejunum, and cecum of rats fed milk supplemented with streptomycin.

TABLE II

AVERAGE WEIGHT GAINED BY RATS FED
MILK WITH AND WITHOUT STREPTOMYCIN

Group	Treatment	Number of Rats	Average Weight at Beginning of Period	Average Weight at End of 30 Days	Average Weight Gained
			grams	grams	grams
I	Streptomycin	2	93.3	157.0	81.6
II	Streptomycin	2	57.4	139.0	63.7
Control	No Streptomycin	6	60.0	93.7	33.7

Figure 3. shows the composite growth curves of the three groups of animals. The growth of the milk-fed control rats was slightly superior to that of the streptomycin-supplemented rats of the same age (Group II) for the first six days. After this initial period, the experimental animals grew more rapidly than those receiving only the basal diet. The older rats (Group I) grew at a more rapid rate than the control animals at the same age period, but at a lesser rate than the rats of Group II, which had received streptomycin for 14 days before reaching the comparable age of 38 days.

The mean weight of ceca of the control rats was 5.96 grams and of the streptomycin-supplemented rats, 7.50 grams. Only the cecal weights of the four experimental animals fed streptomycin for the full 30 day period were included in the average figure presented.

Since the inhibition of the characteristic functions of S. faecalis in the lower jejunum of the rat by streptomycin therapy did not cause a depression of the growth of the animals, it may be concluded that this organism did not contribute to the nutrition of the rat. On the contrary, the evidence suggests that the animals in which the characteristic reactions of S. faecalis were inhibited exhibited a more rapid rate of growth for the 30 day period. McGinnes (1950) reported similar growth improvement and in-

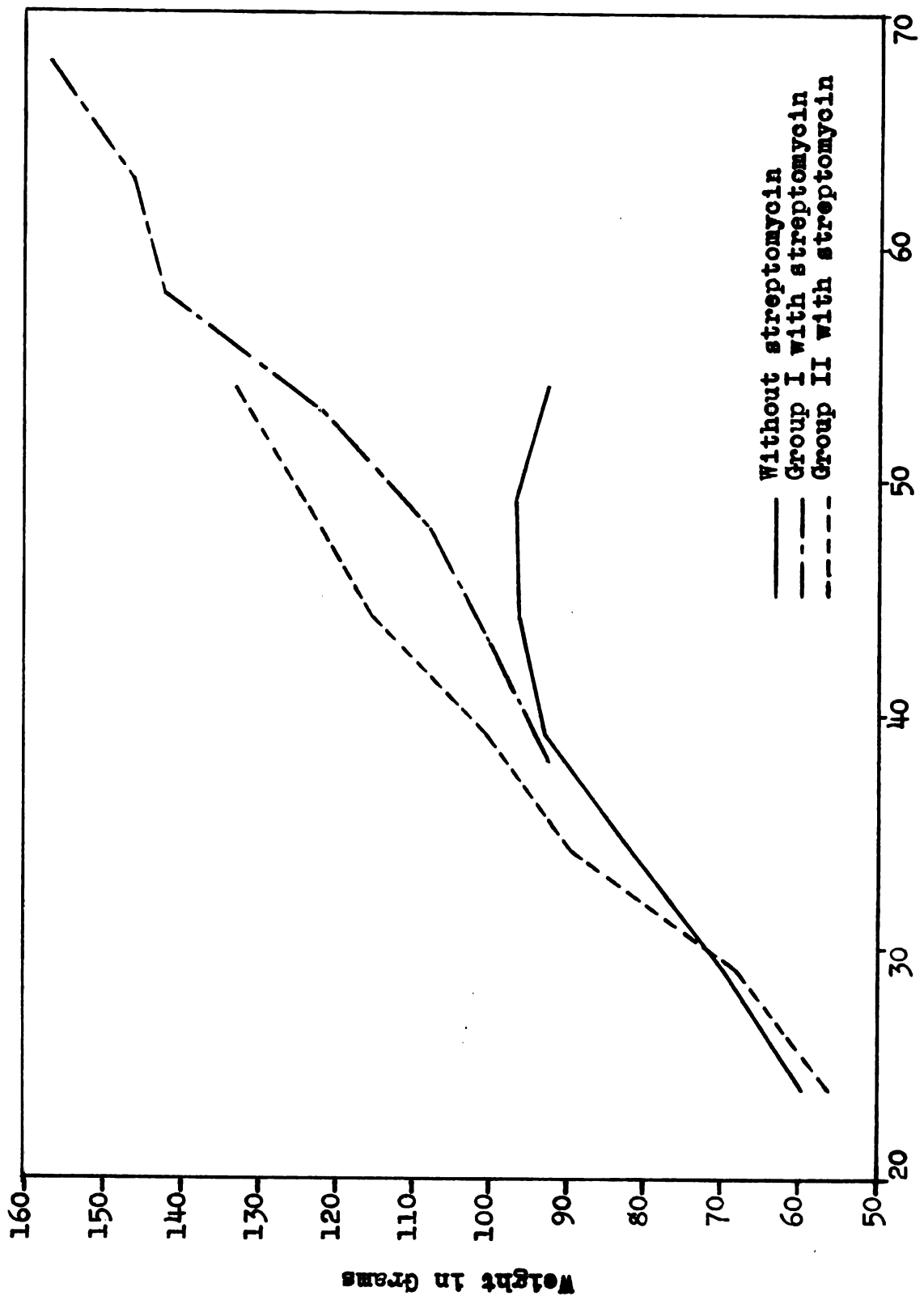

Figure 3. Composite growth curves of rats fed milk with and without streptomycin

creased feed efficiency in turkeys fed streptomycin-supplemented diets. This evidence suggests the possibility that S. faecalis may compete with the rat for nutrients in the intestinal tract. At any rate, it seems that this strain of S. faecalis does not improve the nutrition of milk-fed rats, as its absence does not depress growth, nor is it replaced by another organism or organisms that duplicate the reactions of S. faecalis.

This field of research requires much more investigation before the complete role of microorganisms in the nutrition of man can be fully understood. It seems desirable to identify all the microorganisms that can be identified in the intestinal tract, and study their possible contribution to the animal on different diets. Not only should the possible nutritional function of each microorganism be studied, but also the availability of any products of synthesis to the host animal.

SUMMARY

SUMMARY

A large coccus was isolated from the lower jejunum of milk-fed white male rats, and the organism was identified as Streptococcus faecalis.

Nineteen white male rats were fed the basal milk diet supplemented with an average daily oral dose of 25,545 units of streptomycin per kilogram of body weight. It was demonstrated that this antibiotic inhibited the characteristic functions of S. faecalis within the first 24 hours of therapy. This inhibitory effect was continued throughout the 30 day experimental period, but did not cause a depression of growth in the animals. On the contrary, the average weight gain (except for the first six days) was greater in the streptomycin-supplemented animals than in the six milk-fed animals.

The evidence suggests that this organism does not contribute to the nutrition of the rat, and may even be detrimental to the well-being of this animal by possibly competing with the host for nutrients.

It was also demonstrated that no other organism in the lower jejunum of streptomycin-supplemented rats replaced S. faecalis since the reactions of S. faecalis were not duplicated by other organisms.

It was found that the average total number of bacteria per gram of intestinal contents in the duodenum, upper and lower jejunum, upper and lower ileum, and cecum was similar in the streptomycin-supplemented and milk-fed rats, determined by the direct microscopic count method. Both groups showed an increase in the total number of bacteria from the duodenum to the cecum.

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APPENDIX

TABLE I

STREPTOMYCIN CONTENT OF THE CECUM OF EACH MILK-FED RAT SUPPLEMENTED ORALLY WITH AN AVERAGE OF 25,545 UNITS OF STREPTOMYCIN PER KILOGRAM OF BODY WEIGHT PER DAY AND DETERMINED BY METHOD OF LOO, ET. AL. (1945)

Group	No. of Days Fed Streptomycin	Units of Streptomycin per Ml. of Cecal Contents
I	5	208
I	5	180
I	10	280
I	10	275
I	15	188
I	15	275
I	20	215
I	20	250
I	25	280
I	25	275
I	30	275
I	30	260
II	2	190
II	3	156
II	4	220
II	5	295
II	30	-
II	30	-

TABLE II

TOTAL NUMBER OF BACTERIA EXPRESSED IN MILLIONS PER GRAM OF INTESTINAL SAMPLE FROM RATS FED MILK WITH AND WITHOUT STREPTOMYCIN DETERMINED BY THE DIRECT MICROSCOPIC COUNT METHOD

Group	Treatment	No. Days on Experiment	Total No. of Bacteria in Millions per Gram of Intestinal Sample					
			Duodenum	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum	Cecum
I	Streptomycin	5	224.4	192.3	5,746.4	9,190.6	36,324.7	103,586.0
I	Streptomycin	10	263.9	358.5	465.2	1,788.1	35,379.2	46,367.5
I	Streptomycin	10	802.9	1,068.7	87,790.9	4,045.5	12,479.2	16,769.3
I	Streptomycin	15	542.5	151.1	263.2	1,331.1	19,347.8	47,752.4
I	Streptomycin	15	302.7	789.9	348.0	1,281.2	24,039.6	24,498.1
I	Streptomycin	20	101.2	449.3	608.9	5,804.9	5,819.1	30,607.3
I	Streptomycin	20	111.7	149.1	830.0	1,222.8	17,666.0	14,669.1
I	Streptomycin	25	149.5	105.4	775.7	474.5	33,191.5	16,309.0
I	Streptomycin	25	228.6	256.4	177.4	280.6	14,956.0	26,110.1
I	Streptomycin	30	221.2	923.1	1,708.2	3,444.2	8,842.1	15,699.1
I	Streptomycin	30	69.3	63.6	687.2	71.3	6,492.1	15,595.1
II	Streptomycin	30	528.9	125.7	830.9	642.6	6,038.3	15,935.7
II	Streptomycin	30	230.0	414.2	660.1	659.0	15,755.1	44,326.4

TABLE II CONTINUED

Group	Treatment	No. Days on Experiment	Total No. of Bacteria in Millions per Gram of Intestinal Sample					
			Duodenum	Upper Jejunum	Lower Jejunum	Upper Ileum	Lower Ileum	Cecum
II	Streptomycin	1	360.8	654.0	419.3	528.2	6,512.5	49,635.0
II	Streptomycin	2	513.2	512.8	758.4	3,594.0	8,944.1	16,784.6
II	Streptomycin	3	198.0	1,010.5	1,879.3	794.9	30,000.0	42,689.1
II	Streptomycin	4	235.5	1,140.2	652.2	1,148.5	8,780.5	56,212.4
II	Streptomycin	5	616.1	503.1	3,947.4	3,763.6	66,022.7	71,860.5
Control	No Streptomycin	30	458.7	934.6	2,087.6	2,538.3	1,551.7	27,046.7
Control	No Streptomycin	30	138.9	118.1	666.7	588.2	667.7	27,651.5
Control	No Streptomycin	30	358.4	365.5	295.6	307.7	7,294.6	17,190.1
Control	No Streptomycin	30	183.7	234.4	717.6	534.5	4,865.9	36,087.0
Control	No Streptomycin	30	188.4	591.1	400.0	463.2	1,268.5	59,378.4
Control	No Streptomycin	30	295.6	489.8	898.4	755.0	1,189.8	21,971.8
Mean Total Bacteria in Millions per Gm. Sample of Six Rats Fed No Streptomycin for 30 Days			270.6	455.6	844.3	864.5	2,806.4	31,554.3
Mean Total Bacteria in Millions per Gm. Sample of Four Rats Fed Streptomycin for 30 Days			262.4	381.7	971.6	1,204.3	9,281.9	22,889.1

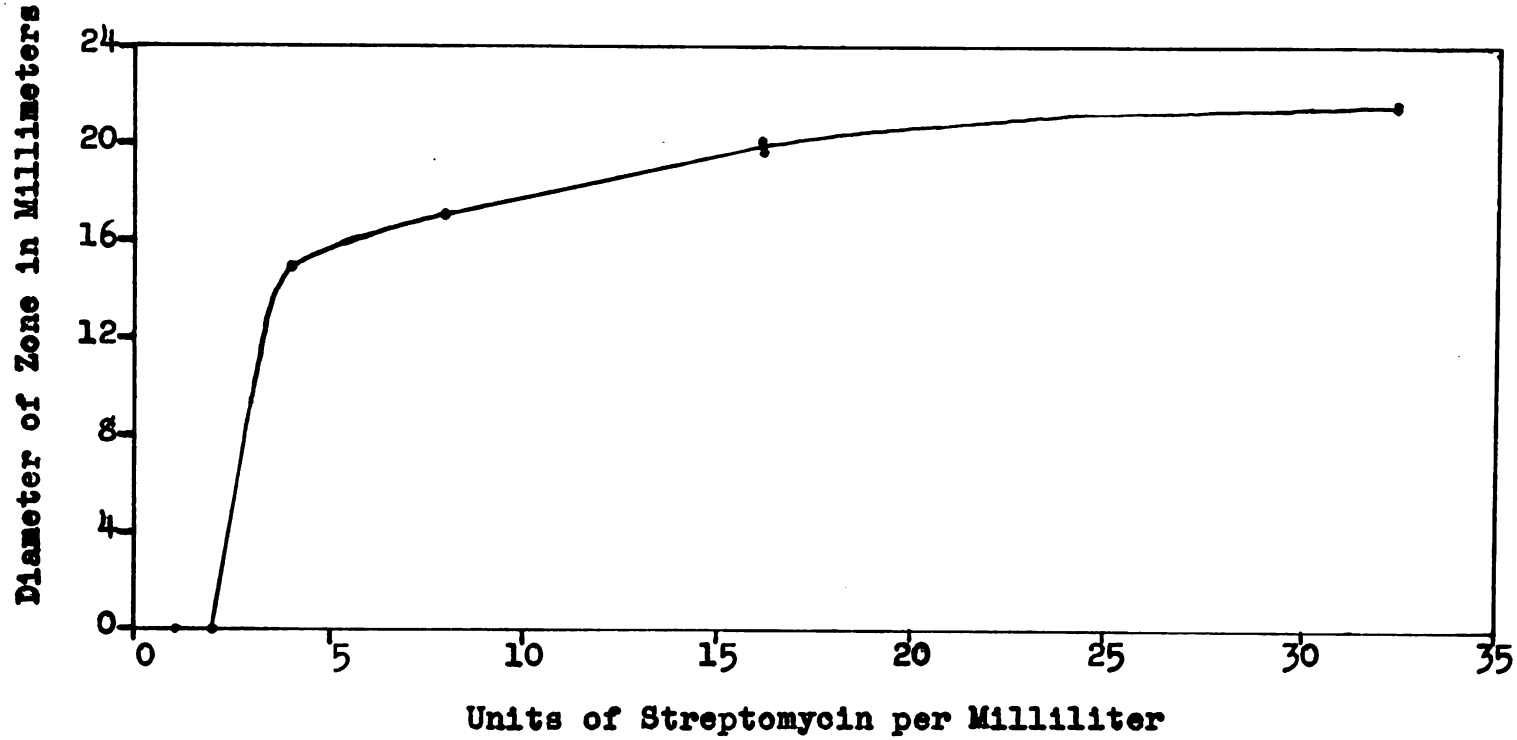

Figure 1. Standard curve of inhibition of Bacillus subtilis by streptomycin

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