THE INTESTINAL FLORA OF THE GUINEA PIG[1]

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In reviewing the earlier work on intestinal flora one notes that the animals which served as subjects were, with few exceptions, omnivorous. The fact that the guinea pig is herbivorous appeared to the present writers to be ample reason for engaging in the present study. Furthermore, the guinea pig is one of the most commonly used experimental animals, and it would seem that a knowledge of its intestinal flora should be of considerable value to investigators. Such an assumption is already being borne out by requests for information about the intestinal flora of so-called normal guinea pigs.

Baumatz (1925) observed only a few bacterial species in the guinea pig, members of the subtilis group and coliform organisms being the most frequent. Baker and Martin (1937–1938) investigated the rôle of bacteria in the breakdown of ingested cellulose. The search was limited to a study of stained smears. Reference was made by them to "*B. caviae*," a large gram-positive sporulating aerobe, first described by Schussing in 1921.

In recent years some attention has been given to a study of bacteria occurring in the different levels or segments of the digestive tract of animals, with results that were more or less conflicting. Porter and Rettger (1940) found the intestinal flora of the white rat relatively simple. The upper segments harbored acidophilus-like forms as the dominant organism. The numbers of bacteria were decidedly larger in the lower sections, *Escherichia coli* becoming more and more common. Contrary to much prevailing opinion, the stomach and upper reaches of the intestine of the albino rat contained appreciable numbers of viable bacteria, under the dietary regimens to which the animals were subjected. Alteration of the flora could be brought about by certain changes of diet.

EXPERIMENTAL

Stock guinea pigs were obtained from three different animal breeders within a few miles of New Haven. As soon as the animals were received at the laboratory they were placed on a stock diet of oats, lettuce and water. A cage was prepared for each animal, which facilitated the collection of fecal samples. Clean but not sterile paper lined the dropping pans under the cages. Feed cups were suspended by wires from the tops of the cages, thus preventing the animals from contaminating the feed with their excreta.

The choice of the proper media for the cultivation of the intestinal bacteria was based upon the experiences of Porter and Rettger (1940) and upon the results of a comparative study of several media not employed by them.

[1] This paper covers in part the dissertation submitted to the Graduate School of Yale University by the senior author as part requirement for the degree of Doctor of Philosophy.

Levine's eosin-methylene-blue agar was selected as the most satisfactory plating medium for the enumeration and differentiation of the coliform bacteria. The medium was plated with appropriate dilutions of intestinal contents or fecal suspensions, 0.5 or 1.0 ml. of the diluted material being used. The seeded plates were dried under sterile clay tops, then incubated for 48 hours at 37°C. and examined for coliform organisms.

A blood-agar medium—beef-infusion base containing 1% neopeptone and 3% defibrinated beef blood—was used for the cultivation of enterococci. Inoculation and incubation of the plates were carried out in the same manner as with the eosin-methylene-blue agar.

The deep-tube cysteine soft-agar medium of Valley (1929) minus the carbohydrate was chosen for the study of anaerobes, because of the ease and rapidity with which the examination could be carried out. The presence of sporulating anaerobes was determined by inoculating 1.0 ml. of the original suspension into the melted soft-agar tubes, heating the tubes at 80°C. for ten minutes, and incubating at 37°C. in an anaerobic jar. The tubes were examined at the end of three days, and again after one week's incubation.

Kulp's tomato agar medium, as modified by Rettger, Levy, Weinstein and Weiss (1935), gave the highest colony counts of all of the media used. The Petri plates were prepared from 1:10 to 1:1,000 dilutions of the test material. Duplicate sets of tomato agar plates were made; one was incubated under increased carbon dioxide tension and the other anaerobically, the method of Weiss and Spaulding (1937) being employed.

The sacrificed animals were placed on an operating board and the ventral surface of the animal sponged with 5% liquor cresolis compositus. The skin was cut along the midline and pinned back; the abdomen was opened and sections of the intestine clamped off with sterile hemostats. The segments were removed, one at a time, and the contents stripped into sterile Petri dishes.

In every instance the segment labeled "duodenum-jejunum" in this report was a section five inches long taken ten inches posterior to the stomach; the "ileum" was the four-to-eight-inch stretch of gut lying immediately anterior to the ileocecal valve; the segment from the colon was usually removed from the large intestine at a point three to four inches below the cecum; and the sample from the "rectum" was the most posteriorly formed fecal mass. The stomach and cecum were opened in approximately the mid portion, and some of the contents removed with a sterile pipette.

The pH of the stomach and intestinal contents was determined routinely by the colorimetric method, using brom-thymol-blue as the indicator, and known buffer solutions. Once in each experiment the pH determinations were checked against readings obtained with a Beckman glass electrode pH meter.

A loop of material from the original suspensions was spread on a slide and stained by a modified gram technic. The data obtained by this method are presented under the heading "Direct Microscopic Examination."

The composition of the experimental diets was as follows:

1. Normal stock diet consisting of oats in unlimited amounts and one daily feeding of washed lettuce weighing from 40 to 50 grams.

2. The balanced scorbutic ration of Dann and Cowgill (1935) fed in unlimited amounts and augmented by 1.0 ml. of orange juice per 100 grams of body weight per day. The diet was supplemented with water.
3. Cracked lentils given in unlimited amounts and the usual portion of lettuce fed daily.
4. Vegetable diet consisting of mangel-wurzel, about 108 grams of root, supplemented with 40 to 50 grams of lettuce daily.
5. Starvation diet augmented only by water.

A technic was devised by means of which a fine catheter (number 5 or smaller), of the kind used in cystoscopy, could be introduced into the stomach of the guinea pig and the material injected by a syringe attached to the outer end of the catheter.

The flora of the stomach is undoubtedly influenced by the ingested food and its flora. Information was sought regarding the numbers and types of bacteria and fungi present in the normal stock diet of lettuce and oats. A summary is presented in table 1 of the data gathered over a period of two years during which the feeding experiments were conducted.

Examination of fecal flora

The fecal flora of animals is a variable which can be determined only by the examination of a large number of samples taken over a considerable period of time. For this reason the intestinal flora of the guinea pigs was followed by daily examination of fecal specimens over a period of one week.

Cultural studies. The viable bacterial counts varied greatly from animal to animal, and from day to day in the same animal; however, there was a general trend in the kinds and numbers of microorganisms present in the feces.

Coliforms appeared only rarely on the standard Levine eosin-methylene-blue agar plates. The total colony count on this medium varied between 380 and 2,800 per 100 mg. of specimen. As compared with those obtained from man or from dogs, the figures are insignificant, even if they represented *Escherichia coli* alone. The bacterial growth on the plates was for the most part composed of subtilis-like and actinomyces-like colonies. Gram-negative *non-lactose* fermenting organisms appeared to the extent of about 50% of the total count.

Inasmuch as the enterococci are found in relatively large numbers in the feces of man and the dog, enterococci were anticipated in the guinea pig. The blood agar plates were used to bring out this flora and other organisms that have a preference for blood-containing media. Here again the total colony counts were very low, ranging between 1,500 and 9,400 colonies per 100 mg. of feces. Cocci were not observed in any of the plates.

Yeasts and yeast-like organisms were often present in guinea pig feces, but, with rare exceptions, the plate counts never ran higher than 200,000 per 100 mg. sample. It is interesting to note, on the other hand, that in a few of the animals a yeast-like flora was present to the exclusion of nearly all bacteria; the observations on these animals were fairly consistent in several daily examinations.

Tomato agar plates incubated anaerobically and under increased carbon dioxide tension showed a high degree of uniformity in the colony types. A few of

what appeared to be grain types of lacto-bacilli were seen, but fully 90% of the flora usually manifested itself in the form of small rough colonies having an average diameter of 100 micra. The colony counts varied from 2.6 to 800 million per 100 mg. stool. A very unusual anaerobic coccus form was observed also on the tomato agar plates, namely *Sarcina maxima* of Smit (1933), or a closely related organism. This anaerobe was of exogenous origin, but in a few instances

TABLE 1

Microbic flora of lettuce and oats

Average counts per gram of test material

TEST MATERIAL	COLI-FORMS	COCCI	LACTO BACILLI	GRAM + SPORULATING RODS		
				Aerobic	Anaerobic	Sarcina
Lettuce T	0	0	0	1,000	0	0
Lettuce B	0	0	0	63,000	10	65
Oats	1,100	0	390	1,200	3	0

T = top, and B bottom of lettuce.

The time required for the passage of food through the intestine of the experimental animal was determined by using gelatin capsules containing 100 mg. of finely divided animal charcoal. One capsule along with a small leaf of lettuce was administered to each of 8 guinea pigs. One-half hour later the animals received the regular ration of lettuce. Stool specimens were collected at 2, 6 and 10-hour intervals.

TABLE 2

Time for passage of labeled food through the intestine of guinea pigs

TIME	GUINEA PIG								
	1	2	3	4	5	6	7	8	C
hrs.									
2	−	−	−	−	−	−	−	−	−
6	−	+	+	+	++	+	+	++	−
10	++	++	++	++	++	++	++	++	−

No charcoal particles visible microscopically or macroscopically, −; microscopically visible, +; Feces visibly colored, ++.

C = control animal.

At the end of six hours two of the guinea pigs voided stools that were darkened visibly, and by the eleventh hour all of the animals has passed charcoal-containing feces. It was concluded from this that in guinea pigs receiving a stock diet the time for passage of labeled food is between six and ten hours.

it was definitely established in the guinea pigs' digestive tract, and was eliminated in very large numbers.

Sporulating anaerobes were present in such small numbers as to be practically negligible, namely 15 to 50 colonies per 100 mg. of test material. What appeared to be *Clostridium sporogenes* was the most common member seen.

Direct microscopic examination: This indicated a much higher bacterial population than did the plating method; however, it may be assumed that the cells

observed were not all viable, and that the direct cell counts to a large extent represent dead and partially autolysed bacteria. Many gram-positive and gram-negative rods were observed on the slides, but the gram-positive rods rarely appeared as solidly stained cells. Rod-shaped bacteria and coccus-like organisms were the predominant forms; fusiform and spiral-shaped rods were next in order of frequency; the remaining stained material on the slides was made up almost entirely of gram-negative debris.

Examination of stomach and intestinal flora

Cultural. The flora of different segments of the intestine of guinea pigs on diets of lettuce and oats, scorbutic ration, and vegetables was not markedly different in the lower intestine from that found in the feces of animals held on the stock diet of oats and lettuce. In the small intestine there was considerable variation in the numbers and types of bacteria present.

The stomach, duodenum-jejunum and ileum contained few if any coliform bacteria or enterococci. Yeast-like organisms were present in small numbers in some of the animals in each of the three groups maintained on the different diets. Gram-positive non-spore-forming rods were the predominating flora of the upper segments of the intestine when there were significant numbers of bacteria present. It must be pointed out, however, that in the stomach and duodenum-jejunum the gram-positive rods were, on the whole, members of the grain types of lactobacilli, and not the small gram-positive rough colony form encountered in the fecal flora in the previous experiments. One animal carried on the vegetable diet contained large numbers of the small colony fecal form in the stomach, but it is quite possible that this was due to reinfection, since none of the other animals showed large numbers in the stomach. In the ileum there was at times a distinct fecal flora, but usually there was a transition flora with an increase in the numbers of the small-colony-producing rough form of lactobacillus. The genus *Clostridium* was present in the small intestine and stomach in such small numbers as to be practically negligible.

In the cecum and large intestine of guinea pigs fed the stock diet and the scorbutic ration coliform organisms were found in two animals, while the others showed no coli-like flora in these sections in dilutions as low as 1:10. Enterococci were found in the cecum and large intestine of three of the animals kept on the scorbutic ration, varying in numbers between 10,000 and 10,000,000 colonies per 100 mg. of contents. The colonies appearing on the blood agar plates, exclusive of the cocci, were composed of gram-positive sporulating cells, gram-negative non-lactose-fermenting rods, lactobacilli and a few actinomyces-like rods.

In animals harboring yeast-like organisms these were distributed throughout the digestive tract. The total numbers of colonies counted on tomato agar plates were, however, fewer than 50,000 per 100 mg. sample, barring a few exceptions. One guinea pig subsisting on the stock diet had a predominant yeast flora at all levels to the point of exclusion of practically all bacteria.

In the lower intestinal segments of the guinea pig fed the stock diet, scorbutic

ration and vegetable diet, the rough lactobacillus-like form appeared in large numbers on the tomato agar plates, namely from 10 to 50 million colonies per 100 mg. of intestinal contents. This organism constituted 90% of the total cultivable flora in the lower segments of the intestine, which is in strong contrast to the findings in the stomach and small intestine, the flora of which was definitely related to the flora of the feed.

Little if any variation in the numbers of the anaerobic sporulating organisms was noted in the animals held on different diets. When present in the intestine at all, the spore-forming anaerobes were cultivated in numbers fewer than 100 colonies per 100 mg. specimen.

Direct microscopic examination

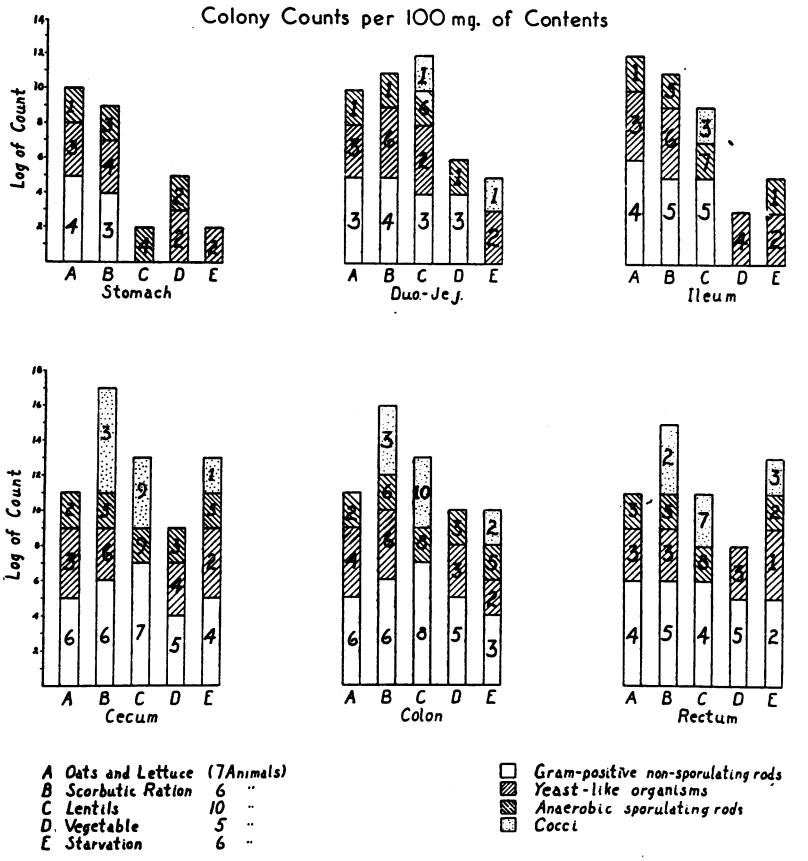
Gram-positive rods and cocci formed the predominating flora of the upper digestive tract. In many of the stained preparations the cells appeared to be undergoing autolysis with varying degrees of swelling and granulation. The guinea pigs fed the vegetable diet differed from the others in that the predominating flora was made up of gram-positive and gram-negative cocci in all of the segments studied. The animals fed the stock diet and the scorbutic ration yielded gram-positive rods and cocci in almost equal numbers; in some instances the rods were less numerous than the cocci. In material from the lower segments gram-negative rods, spirilla and fusiform bacteria occurred frequently. The total number of organisms appeared to be greatest in the colon.

In this investigation lentils were found to be the highest protein-containing food that was acceptable to the guinea pigs. The fecal flora at the beginning of the lentil feeding experiment and at the end of one week had not changed from that of normal guinea pigs on the stock diet of oats and lettuce. At the end of two weeks several marked differences were noted. The color of the feces was altered from a greenish brown to almost a "pure" black; the consistency changed from that of a well-formed easily crushed stool to one that was very sticky and in some cases without form, and there was a definite "fecal" odor to the feces.

In the lentil feeding experiment the most striking change in the viable intestinal flora of the guniea pigs occurred in the numbers of cocci. In the stomach and upper stretch of intestine only one animal showed any cocci, while in the cecum and large intestine relatively high colony counts of cocci were obtained, varying from 5,000 to 20 million colonies per 100 mg. of test material. The rough (fecal type) gram-positive small-colony-producing bacterium was observed in the small intestine in relatively small numbers, with the exception of one guinea pig. In the cecum and large intestine this lactobacillus was apparently suppressed in six animals, and was present in the usual numbers in four. In the summary of results (see chart) it will be noted that from two to five animals failed to yield any gram-positive non-sporulating rods in the segments under discussion. At the same time there were relatively large numbers of cocci present which produced a zone of inhibition for the small colony form.

The direct microscopic examination of the stomach and intestinal contents

of guinea pigs kept on a diet of lentils showed the flora to be predominantly gram-positive rods and cocci. The frequency of occurrence was greatest in the lower segments, cecum and colon.



STOMACH AND INTESTINAL FLORA OF GUINEA PIGS ON VARIOUS DIETS

This chart is an attempt to present in one series of graphs the distribution of four different groups of bacteria observed in the digestive tract. The figures in the columns indicate the number of guinea pigs yielding the bacterial counts plotted. For example, the stomach bar "A" shows that of seven animals fed on oats and lettuce diet four had 100,000 gram-positive non-sporulating rods per 100 mg. of test material in the stomach; three had 1,000 yeasts; and one of the seven had 100 spore-forming anaerobes.

The original tabulated results are too extensive to include in this report. For a more complete summary, readers are referred to the tables in the senior author's Ph.D. dissertation, on file in the Sterling Library of Yale University.

The starvation diet was maintained for four days; examination at the end of that period showed the intestinal flora to be unchanged as to kinds of microorganisms present, but greatly reduced in numbers in a majority of the animals used. The gram-positive small-colony (fecal) form was affected more than the other types, being largely or entirely suppressed.

Direct microscopic examination of stained smears of intestinal contents of

the starvation-diet guinea pigs revealed cocci as the predominating flora of the cecum and large intestine. The upper segments appeared to be relatively free from bacteria.

BRIEF SUMMARY

The data reported here show that the intestinal flora of the guinea pigs was relatively simple and contained few bacterial types. The predominating cultivable flora was a lactobacillus apparently closely related to *Lactobacillus acidophilus* and *L. bifidus*. It constituted about 80% of the total cultivable flora. Yeast-like organisms were present in the intestine, irrespective of the diet, and in some instances they were present to the exclusion of practically all bacteria.

It will be noted that three common fecal organisms, the coliforms, enterococci and sporulating anaerobes, were not demonstrable as part of the cultivable intestinal flora. These organisms, if they were present at all, were there presumably as exogenous organisms, and not as part of the guinea pig intestinal flora.

Among the organisms observed in the guinea pigs two are of particular interest. They are (1) the small gram-positive non-sporulating bacillary form of the intestinal lactobacillus type, and (2) the large anaerobic highly aerogenic sarcina. Both frequently appeared in the digestive tract in large numbers, and gave definite indications of having undergone extensive multiplication there. As the former is obviously a member of the *Lactobacillus* genus it is referred to here as an intestinal lactobacillus.

THE INTESTINAL LACTOBACILLUS

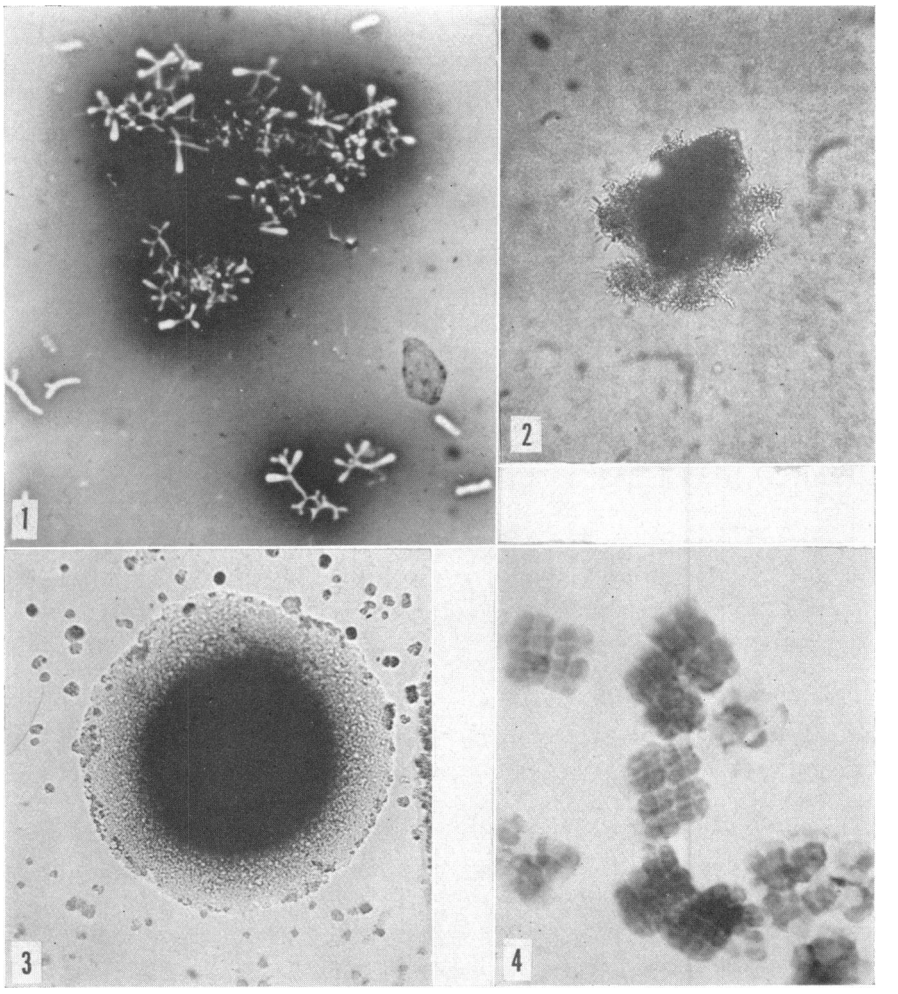
This organism was isolated from the feces and intestinal contents of guinea pigs by plating on tomato agar under either anaerobic or microaerophilic conditions. After repeated transfers in semi-solid tomato cystein agar, cultures could be maintained indefinitely in this medium, and successful cultivation could be brought about in plain tomato broth.

On solid tomato agar the cell morphology was usually that of a small rod 0.5–0.8 by 2.5–8.0 micra in size. The cells occurred single or in pairs, rarely in chains. Gram-stained preparations showed more or less granulation. Varying degrees of pleomorphism were exhibited ranging from single cell to multibranched forms (see fig. 1). Capsules and motility were not observed.

Recently isolated strains produced deep pin-point colonies in tomato agar within 24–36 hours which under the low-power lens appeared circular to elliptical. Colonies which reached the surface of the agar revealed a more or less dense structure from which hair-like filaments extended into the medium (see fig. 2). The colonies increased in size under favorable conditions, but remained delicate and never attained a diameter of more than 300 micra.

Established strains produced slight turbidity in tomato broth within 48 hours; this was followed soon by sedimentation. In tomato broth medium containing 10 per cent gelatin and 1.5 per cent cystein hydrochloride, and inoculated liberally with young cells, a uniform turbidity developed within 24 hours at 37°C.;

by the end of 72 hours incubation there was abundant growth which settled out and occupied one third of the shake tube column. The gelatin was not liquefied by the organism.



FIGS. 1 TO 4

Upper left. Showing cell morphology of the intestinal lactobacillus in tomato broth culture incubated at 37°C. for 72 hours. ×600. Nigrosin stain.

Upper right. Typical deep colony of the lactobacillus after reaching surface of medium; tomato agar incubated in closed chamber with added CO_2 at 37°C. for 72 hours. ×100.

Lower left. Surface colony of anaerobic sarcina on tomato agar after 18 hours incubation at 37°C. ×20. Note broken agar caused by escaping gas.

Lower right. Showing cell morphology of the anaerobic sarcina. Tomato broth culture incubated 6 hours at 37°C. ×1000. Nigrosin stain.

No visible growth took place at 18, 22 and 50°C. At 43° only five of the strains appeared to develop. Good growth occurred at 37° within 48 hours.

Hydrogen ion concentration tolerance was determined in tomato broth ad-

justed to various pH values with the aid of the Beckman glass electrode. The lower limits of growth lay between 4.5 and 5.0. The optimum growth range was pH 6.6–6.8. The upper limits of visible growth were between pH 7.5 and 8.0. The final H-ion concentration attained in 72 hours in soft cystein tomato agar was 4.5–5.0, except for a few strains which brought the pH down to 4.2.

Growth is favored strongly by anaerobic or micro-aerophilic conditions. Total colony counts are very much higher in the presence of added CO_2 or under anaerobic conditions than in plates that are incubated aerobically.

None of the strains exhibited hemolytic properties. Litmus milk was strongly acidified and clotted by only 3 strains. The same strains also reduced the litmus.

All of the guinea pig strains fermented glucose, levulose, maltose and sucrose, without gas production; and all but two attacked lactose and raffinose. However, classification on the basis of fermentation of carbohydrates was not satisfactory, because there was considerable variation in the powers of some of the strains to attack carbohydrates consistently. The total titratable acidity in tomato broth varied between 4.90 and 10.00 ml. N/10 acidity per 10 ml. of the medium. Uncompleted experiments indicated that the amounts of fixed and volatile acids did not differ materially from those produced by known *L. acidophilus* of human origin.

Attempts to relate the guinea pig lactobacillus to other lactobacilli by means of agglutination and precipitin reactions proved unsatisfactory.

Catalase production could not be demonstrated in any of the strains. Tests made at the same time with known strains of *Propionibacter* gave positive results.

THE ANAEROBIC SARCINA ISOLATED FROM THE FECES OF GUINEA PIGS

This organism is given some prominence here, first, because it often occurs in large numbers in the intestine of guinea pigs, and, second, because it is an unusual type of *Sarcina*, with which many bacteriologists do not appear to be familiar. Indeed, Bergey's Manual (1939) lists Goodsir's sarcina as an aerobic coccus, the cells measuring 2.5 micra in diameter. The real nature of Goodsir's *Sarcina* was not fully revealed until Beijerinck (1911) isolated it or a closely related packet form by employing acidified beer wort in flasks which were filled to the mouth and stoppered, to create anaerobic conditions. He succeeded in this manner in separating it from aerobic sarcina forms with which it is so commonly associated, particularly in the stomach.

Smit (1933) claimed that he had isolated and described the true *Sarcina ventriculi* of Goodsir (1842), while engaged in a comprehensive study of anaerobic sarcinae. He reported it to be a very large anaerobic packet form; also a powerful gas producer possessing high acid tolerance. Smit's claims were recognized by Weinberg and his associates (1937). Smit also isolated and described an anaerobic sarcina which was on the whole quite similar to *Sarcina ventriculi*. The two differed, however, in one important respect. The new organism, to which he applied the name, *Sarcina maxima*, was a strong producer of butyric acid, whereas this substance did not constitute one of the fermentation products of *S. ventriculi*.

The organism found by the present authors in the intestine and feces of guinea pigs bore a striking resemblance to Smit's *S. maxima*, and is regarded by them as being identical with it or a close variant of it. It is referred to in this paper, therefore, as *Sarcina maxima*.

The organism is a large gram-positive non-sporulating anaerobic coccus which develops in characteristic packet form. It was first observed on tomato agar plates which had been inoculated with guinea pig feces. Attempts to isolate it failed until an acidified tomato broth and strictly anaerobic conditions were employed. By adjusting the reaction to pH 4.0 the small colony-forming lactobacillus was inhibited.

Deep colonies of a pure culture usually raised the agar from the bottom of the Petri dish. A striking characteristic of the deep colonies was their angular appearance. The larger colonies attained a size of about two millimeters in diameter during twelve hours' incubation at 37°C.

Surface colonies produced under the same conditions were 2–5 millimeters in diameter; they were round and had a raised surface and an internal microscopic structure resembling that of a yeast colony (see fig. 3). When magnified 100 times the large packets were seen to be made up of units of 8 cells. There was very little or no chromogenesis on tomato agar plates. A definite odor of butyric acid was emitted from the anerobic jars at the time they were opened.

Tomato broth growth was obtained readily when large amounts of inoculum from broth or agar cultures were employed. Growth was vigorous by the end of 6 hours incubation at 37°C. It was typified by marked gas evolution throughout the tube, the bubbles being released apparently from the heavy sediment in the bottom of the tube. At the peak of growth the sediment was churned and carried up through the fluid by the gas bubbles. A "head" of foam developed on the surface of the tomato broth after six and up to 12 hours of incubation.

The cell morphology and orientation presented striking microscopic pictures. The cells were 3.3–4.0 micra in diameter, and when alone appeared spherical. In packets of 8 cells or more the adjacent cell surfaces were flattened and seemingly bound together firmly (see fig. 4). The most commonly observed packets were made up of 32 cells. Capsules could not be demonstrated. During the first 6 hours of incubation the cells were strongly gram-positive. After 12 hours practically all of the cells were negative by the gram stain. Spore formation was at no time apparent.

Growth took place at 20, 30 and 37°C., but not at 50. It was most vigorous at 37°C. The lower limit of pH which permitted growth was 4.0, and the upper 8.5. Reactions on the acid side of neutral were more favorable than on the alkaline.

The organism required added carbon dioxide, and almost complete anaerobiosis. Bacto-peptone and "savita" yeast extract were adequate sources of nitrogen. A fermentable carbohydrate was necessary for growth. Slight growth took place when 0.1 per cent glucose was present. For luxuriant development 2.0 per cent was necessary. The sarcina utilized glucose, levulose, galactose, maltose and lactose, but not mannose, xylose and raffinose.

A comparison of this anaerobic coccus with Smit's *Sarcina maxima* reveals a close similarity, except in the following respects: *S. maxima* has a pH tolerance as low as 0.8, while that of the guinea pig organism is 4.0. The former was found in soil and grains and, according to Smit, could not be isolated from the feces of laboratory animals, whereas the latter was not only present in various guinea pigs examined, but could implant itself for varying periods of time in the intestine of guinea pigs.

From the numerous characters which the two Sarcina forms have in common, in spite of the few differences cited here, we may assume the guinea pig coccus to be a variant of *Sarcina maxima*. It is proposed here to apply to it the name, *Sarcina maxima* Smit (variant).

SUMMARY AND CONCLUSIONS

This investigation was undertaken to determine the normal flora of guinea pigs subsisting on a stock diet of lettuce and oats, and to study the effect of changes of ration upon the intestinal flora. Five different diets are reported here.

The normal flora of the guinea pig intestine was found to be relatively simple, consisting of a small number of bacterial types and of yeast-like microorganisms. The predominating organism was a small gram-positive, non-sporulating rod form of the *Lactobacillus* genus. It constituted approximately 80% of the total cultivable flora. The other 20% consisted essentially of what appeared to be yeasts, soil and air bacteria. Throughout the course of the investigation, with one exception, other commonly occurring intestinal bacteria, particularly *Escherichia coli* and the enterococci, were absent, or were present in very small numbers only.

The predominant organism bears a close resemblance to *Lactobacillus acidophilus* of human origin, particularly in its preference for microaerobic and anaerobic conditions, cell and colony form, and nutritional and temperature requirements. It produces smaller and more delicate colonies on appropriate media, and in general appears to be a less hardy lactobacillus than *L. acidophilus* of rat origin. There is every reason to regard it as *L. acidophilus*; for the sake of convenience and host reference the name, *L. acidophilus*, variety *caviae* might well be applied to it.

A second organism of particular interest in the present study was a large anaerobic sarcina form which was highly aerogenic. It occurred in some of the guinea pigs in large numbers and was apparently capable of implanting itself in the intestine of guinea pigs. This organism resembles Smit's *Sarcina maxima* to a very large extent, and should perhaps be designated as such, or a variant thereof.

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