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EXPERIMENTAL STUDIES OF THE INTESTINAL FLORA *

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INTRODUCTION

The following experiments were carried out with the purpose of ascertaining the effect of various foods commonly used in infant feeding on the bacterial flora of the intestinal tract.

It was necessary to ascertain whether the number and variety of organisms varied in different parts of the intestinal tract. It is well recognized that but a small percentage of the bacteria occurring in the feces are viable. Bacteriologic studies of the feces, therefore, have justly been criticized on the ground that the findings give inaccurate data concerning the flora at other levels. Many workers in the field of the intestinal bacterial flora of infants have claimed that definite characteristic flora occur at certain parts of the intestinal tract. Attention has been called especially to the peculiarities of the duodenal flora.

Attention has been directed by many writers to the effect certain foods containing relative high percentages of carbohydrates or proteins have on the intestinal flora. Certain popular conceptions of the different types of intestinal disturbances in infants are based on the bacterial changes these foods are supposed to produce. If a diarrhea, for instance, occurs after a diet with a high carbohydrate content, this is called a "fermentative" condition. Under these circumstances the intestinal flora is considered to consist of organisms that act primarily on the carbohydrates. The products of this action are thought to produce the diarrhea. Similarly a disturbance associated with the feeding of a diet containing a high percentage of protein is considered to be due to "putrefaction."

It is a matter of dispute whether these intestinal disturbances are caused by primary chemical changes, or whether they are due to initial bacterial activity. As food containing a large amount of sugar is frequently associated with diarrhea in infants, studies were made to see

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if the condition was accompanied by changes in the bacterial flora. Attention was paid not only to whether a change took place, but also at what level the change occurred.

Studies were made to determine the effect of starvation on the bacterial flora at different levels, both after a normal diet and after a diet which caused diarrhea.

EXPERIMENTAL STUDIES. SERIES 1

Technic.—The experiments were carried out on puppies a few weeks of age. The animals were cared for in a private house where ample space could be given for normal activities, as it was found that the animals after being weaned did very poorly under ordinary laboratory conditions. The attempt was made to eliminate from the series all animals with intestinal parasites. The technic employed for the study of the intestinal flora was as follows: The puppies were etherized, without previous administration of a sedative. A laparotomy was then performed under antiseptic precautions. After placing presection sutures and clamping the intestinal wall at a distance of about 3 to 5 cm. on either side of the suture, the gut was punctured with a medium sized record syringe needle and 3 c.c. of sterile saline introduced. This was drawn back into the syringe and the procedure repeated three or four times in order to insure a thoroughly mixed specimen of the fecal contents. Material was removed from the duodenum at the level of the tail of the pancreas, from the ileum about 8 cm. proximal to the ileocecal valve, and from the cecum about 8 cm. distal to the ileocecal valve. A specimen of the contents of the colon was also recovered in each experiment by means of a rectal tube. The animals made excellent operative recoveries.

The bacteriologic studies were carried out as follows: Two or more smears, stained by Gram's method, were made from the specimens recovered. Examination of these preparations gave helpful information as regards the actual presence of organisms, a general impression of the relative number present and a picture of the various types from a morphologic point of view. Four plates were made from each specimen on the following mediums: plain agar, dextrose litmus agar, 2 per cent. acid dextrose agar, and whey agar. Dilutions were not usually made from the duodenal and ileal specimens. In a few instances in which smears showed no organisms, or very few, cultures were made, after incubation of the specimen. Special studies for acidophilic organisms were carried out by making three transfers to 3 per cent. acid broth, after intervals of two days, and finally plating on 2 per cent. acid whey agar. As spore-bearing forms are easily inhibited by *Bacillus coli*, one specimen of the intestinal content, in each instance, was heated for fifteen minutes at 80 C., and then plated on the usual mediums.

GROUP 1. *Controls.*—Observations were first made on the intestinal contents of five puppies, which were used as controls. The diet consisted exclusively of whole milk (pasteurized), fed over a period of seven to ten days. The following experiments show the characteristic results.

These experiments show that the bacterial flora of young puppies fed on cow's milk is essentially the same as that of infants on a similar diet. The acidophilic bacilli found in small numbers in stools of infants fed on cow's milk were not noted in these studies. All the various organisms observed in the colon (the findings of the colon were identical with those of the cecum and have not been recorded) were found at the other levels. Fewer organisms, especially after

ingestion of small amounts of food followed by periods of starvation, were found in the duodenum than elsewhere. There seems to be a gradual increase in the number of organisms from the duodenum to the cecum. Although *Bacillus coli* is the predominating type, the variety of organisms present is so great and their relative number so variable that one cannot speak of a characteristic flora at any level.

TABLE 1.—EXPERIMENT 6. NORMAL

Age	Diet	Fasting	Duodenum	Ileum	Cecum
1 mo.	Whole milk; 2 ounces at last feeding	4 hrs.	Smear: Organisms, +++ G— bacilli, ++ G+ cocci, + Large G+ bacilli and yeasts Culture: B. coli and B. lac- tis-aerogenes, ++ Strep. lact., + Spore bearing forms, rare Acidophilic ba- cilli, 0	Smear: Organisms, +++ G— bacilli, ++ G+ bacilli, + G+ cocci, — Spirillae, + Culture: B. coli, ++ Acidophilic ba- cilli, 0	Smear: Organisms, +++ G— bacilli, ++ G+ bacilli, ++ G+ cocci, rare Spirillae, + Culture: B. coli, ++ B. lactis- aerogenes, + Spore bearing forms, + Acidophilic ba- cilli, 0

* In this and the following tables +++ = very numerous, ++ = many, + = few, G = gram.

TABLE 2.—EXPERIMENT 9. NORMAL

Age	Diet	Fasting	Duodenum	Ileum	Cecum
1½ mos.	Whole milk; 1 ounce at last feeding	5 hrs.	Smear: Organisms, +++ G— bacilli, ++ G+ bacilli, ++ G+ cocci, rare Culture: B. coli, + Strep. lact., rare Acidophilic ba- cilli, 0	Smear: Organisms, +++ G+ bacilli, ++ G+ cocci, + Culture: B. coli, ++ Spore bearing forms Acidophilic ba- cilli, 0	Smear: Organisms, +++ G— bacilli, ++ G+ bacilli, + Spirillae, + G+ cocci, + Culture: B. coli, ++ Spore bearing forms, + Acidophilic ba- cilli, 0

TABLE 3.—EXPERIMENT 15. NORMAL

Age	Diet	Fasting	Duodenum	Ileum	Cecum
2½ mos.	Cow's milk for past 11 days; 2 ounces at last feeding	8 hrs.	Smear: Organisms, + G— bacilli, rare Culture: B. coli, rare Strep. lact. No acidophilic bacilli	Smear: Organisms, ++ G— bacilli, + G+ bacilli, rare G+ cocci, rare Culture: B. coli, ++ Yeasts, rare Strep. lact., rare No acidophilic bacilli	Smear: Organisms, +++ G+ bacilli, rare G— bacilli, rare G+ cocci in clus- ters, + Culture: B. coli, ++ Strep. lact., rare Yeasts, rare Spore bearing forms No acidophilic bacilli

GROUP 2. *Experiments with High Sugar Diets.*—Eleven puppies were fed pasteurized cow's milk, with additional lactose, four with additional sucrose. With rare exceptions the animals took this modification well. Typical experiments of this group are given.

TABLE 4.—EXPERIMENT 11. LACTOSE FEEDING

Age	Diet	Fasting	Duodenum	Ileum	Cecum
2 mos.	Milk +, 10% extra lactose for 9 days; amount last feeding, 2 ounces	2 hrs.	Smear: Organisms, + G— bacilli, + G+ cocci, rare G+ bacilli, rare Culture: B. coli, + B. lact.-aerog., + Spore bearing forms, + Strep. lact., + Acidophilic bacilli, 0	Smear: Organisms, +++ G+ bacilli, + G— bacilli, + G+ cocci, + Culture: B. coli, ++ Strep. lact., rare Spore bearing forms, rare Acidophilic bacilli, 0	Smear: Organisms, +++ (poor definition) G— bacilli with + granular appearance G+ bacilli, + G+ cocci, rare Culture: B. coli, ++ Strep. lact., + Spore bearing forms, + No acidophilic bacilli

TABLE 5.—EXPERIMENT 22. LACTOSE. FASTING AFTER DIARRHEA. STARVATION PERIOD 18 HOURS

Age	Diet	Period	Duodenum	Ileum	Cecum
1 mo.	Whole milk, 10% extra lactose; diarrhea, ++	4 days; 18 hrs. fasting	Smear: G+ bacilli, rare Organisms, rare (most fields no organisms) Culture: Strep. lact., rare Spore bearing forms All isolated colonies 1-10 on a plate Acidophilic B., 0	Smear: G— bacilli, rare G+ bacilli, rare Most fields without organisms (rarely 1 to 5) Culture: Spore bearing forms B. coli, + B. lact. aerogenes (isolated colonies of above, 10-20 on a plate) Acidophilic B., 0	Smear: Organisms, +++ G— bacilli, + G+ bacilli, + G+ cocci, + Culture: B. coli, ++ Spore bearing forms, ++ Strep. lact., ++ Acidophilic B., 0

TABLE 6.—EXPERIMENT 19. LACTOSE FEEDING (STARVATION)

Age	Diet	Starvation	Duodenum	Ileum	Cecum
2 mos.	Whole milk + 10% lactose; fed for past 10 days without diarrhea; amount last feeding, 2 ounces	20 hrs.	Smear: Organisms, rare* G— bacilli, rare Cultures: Strep. lact. B. coli Spore bearing forms (isolated colonies of each) Acidophilic bacilli, 0	Smear: Organisms, rare, few† G— bacilli B+ bacilli G+ diplococcus Cultures: B. coli Strep. lact. Yeasts (few colonies of each) Acidophilic bacilli, 0	Smear: Organisms, +++ G— bacilli, ++ G+ bacilli (few) Spirillae G+ cocci Cultures: B. coli and L. aerogenes, + Strep. lact. (many colonies of each) Acidophilic B., 0

* Rare; many fields without any organisms.

† Few, 2 to 5 in a field.

TABLE 7.—EXPERIMENT 25. LACTOSE WITH DIARRHEA (STARVATION)

Age	Diet	Fasting	Duodenum	Ileum	Cecum
7 wks.	Whole milk + 10% extra lactose for past 10 days; diarrhea 86 hrs. before operation (not at time of operation); 6 ounces at last feeding	20 hrs.	Smear: Organisms, + G— bacilli G+ cocci Culture: B. coli, + Strep. lact., + (scattered colonies of each) No acidophilic bacilli Presence of spore bearing forms Mesentericus; large spreading colonies	Smear: Organisms, ++ G— bacilli, + G+ bacilli, + Culture: B. coli, + Strep. lact., + Spore bearers, + No acidophilic bacilli Presence of spore bearing forms Profuse growth; mesentericus and cereus	Smear: Organisms, +++ G— bacilli, + G+ bacilli, + G+ cocci, + Culture: Strep. lact., + B. coli, ++ No acidophilic bacilli Presence of spore bearing forms Colonies of mesentericus subtilis (pseudotetanus)

TABLE 8.—EXPERIMENT 14. CANE SUGAR

Age	Diet	Starvation Period	Duodenum	Ileum	Cecum
2½ mos.	Whole milk with cane sugar to 18%	7 hrs.	Smear: Organisms, + G. bacilli G+ bacilli G+ cocci, all rare Culture: B. coli Acidophilic organisms, 0 Spore bearing forms, +	Smear: Organisms, + G— bacilli, + G+ bacilli, + Culture: B. coli, rare Acidophilic, 0 (desquamated epithelial cells)	Smear: Organisms, +++ G— bacilli, ++ G+ cocci, rare Culture: B. coli, ++ Strep. lact. Strep. (fecalis) Acidophilic bacilli, 0

The studies of the bacterial flora of puppies fed diets containing from 10 to 15 per cent. of lactose or sucrose show that the same organisms are present as under normal dietary conditions. Even with diets with a very high carbohydrate content, no acidophilic organisms are present at any level. As in the previous group of experiments, there is a close agreement between the microscopic and cultural findings. Examination of the intestinal contents of puppies with a profuse diarrhea after a high carbohydrate diet show no essential variation from the findings previously noted. The same diminution in the number of organisms after starvation is noted in this group of experiments as in the foregoing.

In addition to the effect of dietary changes on the intestinal bacterial flora, the results of the foregoing experiments show that after periods of starvation of from eight to twenty hours, the number of organisms in the small intestine is greatly reduced. This diminution is more marked in the duodenum, but is evident as far down as the cecum. The reduction in the number of organisms seems to be in pro-

portion to the length of the fasting period and the amount of food taken before the starvation, the longer periods of fasting and the previous ingestion of little food giving the greatest reduction in organisms. Although many of the plates and smears from specimens under those conditions showed no organisms, in every experiment bacteria were demonstrated at each level. No changes in types of organisms were shown to exist after periods of fasting.

GROUP 3. *Protein Milk Diet*.—Some difficulty was experienced in feeding the puppies protein milk. In but one instance did the animal take this well.

TABLE 9.—EXPERIMENT 10 (PROTEIN MILK)

Age	Diet	Fasting	Duodenum	Ileum	Cecum
2 mos.	Protein milk; 2 ounces last feeding partially vomited	2 hrs.	Smear: Organisms, ++ G- bacilli, + G+ bacilli G+ cocci Culture: B. coli and B. lact. aerogenes, ++ Special test for spore bearing forms, + Acidophilic, 0	Smear: Organisms, +++ G- bacilli, ++ G+ cocci, + G+ bacilli Culture: B. coli, ++ Spore bearing forms, +	Smear: Organisms, +++ G- bacilli and spirillae, ++ G+ bacilli and cocci, ++ Culture: B. coli, ++ Spore bearing forms, +

TABLE 10.—EXPERIMENT 24. PRESENCE OF SPORE BEARING FORMS

Age	Diet	Fasting	Duodenum	Ileum	Cecum
2 mos.	15% lactose without diar- rhea; period feeding, 1 week; 4 ounces last feeding	20 hrs.	No growth	Many colonies of spore bearing forms mesentericus cereus subtilis yeasts	Few colonies of spore bearing forms mesentericus

The bacterial intestinal flora of a dog fed on protein milk is essentially the same as was found under normal dietary conditions. Although the spore-bearing forms are more numerous, they do not become the predominating type. It is seen that spore-bearing organisms occur at all levels in relatively large numbers, in animals fed on a diet high either in carbohydrates or in proteins.

EXPERIMENTAL STUDIES. SERIES 2

A second series of studies was made on infants. A duodenal catheter was passed, as has been described by Hess,¹ and duodenal con-

1. Hess, A. F.: The Use of a Simple Duodenal Catheter in the Diagnosis and Treatment of Certain Cases of Vomiting in Infants, AM. JOUR. DIS. CHILD., 1912, 3, 133.

tents were then aspirated. The specimens were studied as in the foregoing series of experiments. Typical reports of this series are given below. Ten cases were studied.

TABLE 11.—STUDY 2

Subject	Diet	Hours after Feeding	Results
Baby B., aged 5 mos.; normal	Whole milk	2	<ol style="list-style-type: none"> 1.5 c.c. of yellow turbid material with soft whitish curds; recovered Smear: Organisms, +++ (not unlike fecal specimen); gram ++ cocci; gram — bacilli Cultures: Staphylococci, +; Streptococcus lacticus, +; B. coli and Lactis aerogenes, ++; yeasts and spore bearing forms, rare

TABLE 12.—STUDY 4

Subject	Diet	Hours after Feeding	Results
Normal, 4 months' infant	Whole milk modification	2	<ol style="list-style-type: none"> ¾ c.c. rather viscid, yellow, turbid material Smear: Organisms, ++; gram— bacilli, +; gram+ cocci, + Culture: B. coli and B. lactis-aerogenes, +

TABLE 13.—STUDY 5, BY MODIFICATION OF FORMER TECHNIC *

Subject	Diet	Hours after Feeding	Results
Normal, 5 months' infant	Breast milk, with supplementary feedings of cow's milk	5	<ol style="list-style-type: none"> Small amount (0.5 c.c.) slightly turbid fluid Smear: No organisms noted Cultures: B. coli, rare; B. lactis-aerogenes, +; Streptococcus lacticus, +

* In this instance, 10 c.c. of normal saline was introduced into the duodenum before aspiration.

The observations made on infants by means of a duodenal catheter show that not only a large number of organisms may be present in the duodenum, but that these may be very similar to the organisms occurring in the feces. Cocci of various types are more numerous in the duodenum than are commonly found in the fecal specimens. The predominating organisms at this level when large numbers of forms are present is a gram-negative bacillus; these are *Bacillus coli* and *Bacillus lactis-aerogenes*. It will be noted that the coccus forms referred to above are similar to those present normally in the mouth.

DISCUSSION

Localization of Flora.—From the experiments recorded it is seen that the flora of the intestinal tract at various levels is essentially the same, as determined by our present bacteriologic methods. Although the organisms recovered from the feces showed poorer definition and staining properties than those recovered from other levels, the same types of organisms were noted microscopically, and culturally they were found to be nearly the same in all regions. It seems, therefore, that the study of fecal specimens gives quite a true, adequate picture of the flora that may occur at any of the different levels of the entire intestinal tract. It is of special interest to find that so many forms may be present in the duodenum of the normal infant, and that these may be very similar to forms found at lower levels. It has not infrequently been stated that such a condition was in all probability associated with gastro-intestinal disorders. Tobler² asserts that the digestive processes of the duodenum are unaccompanied by the activities of bacteria. The method of recovering the specimens with the duodenal catheter is not entirely satisfactory, as it is impossible to avoid completely contaminations from the mouth and upper alimentary tract. In regard to the relative number of organisms present at the various levels, the experiments show that during the early periods of digestion there is no recognizable variation. Where so many organisms are present, we feel that we have no satisfactory method of determining with sufficient accuracy an estimate of the absolute number.

Effect of Diet on the Flora.—Moro³ and others have called attention to changes in the bacterial flora of infant stools after various diets. An acidophilic, gram-positive group of bacilli was considered characteristic of infants fed on breast milk. The feces of children fed on cow's milk was found to contain few of these organisms and numerous *B. coli*, cocci and proteolytic forms. Wollstein⁴ investigated stools of infants fed on high protein and carbohydrate diets. On carbohydrate high diet, "cocci, colon bacilli, acidophilus and bifidus bacilli were most numerous." "During the periods of high protein content in the food, *B. mesentericus* gained the ascendancy, *B. aerogenes-capsulatus* sometimes persisted, and the colon bacilli were increased in numbers. But the acidophilus and bifidus did not entirely disappear." From the writings of these authors it is to be assumed that in their belief certain quantitative changes result from varying the food. Herter and

2. Tobler, L., and Bessau, G.: Krankheiten durch abnormen Ablauf der Ernährungsvorgänge und des Stoffwechsels, 1914.

3. Moro, E.: Jahrb. f. Kinderh., 1900, 52, 38.

4. Holt, L. E.; Brown, A.; Wollstein, M., and Courtney, A. M.: The Influence of High Protein Feeding on the General Metabolism, on the Intestinal Flora and on the Body Temperature of Infants, AM. JOUR. DIS. CHILD., 1912, 4, 265.

Kendall⁵ fed monkeys and cats high protein diet and assert that a proteolytic flora was obtained.

Few experimental studies of the intestinal bacteriology of animals, after changes in diets, have been made. Rettger and Horton⁶ showed that when rats were fed on a diet containing a single protein the intestinal flora became simpler. The normal flora was replaced by the acidophilic group of organisms. The authors consider that the change was "independent of the pure proteins, but bore a definite relation to the protein-free milk which formed a part of the pure protein diet." From a more recent series of experiments, Rettger⁷ concludes that the intestinal flora of white rats and the domestic fowl undergoes "profound alterations" when fed on a milk diet. These changes consist in the establishment of a flora consisting of *Bacillus acidophilus* or *Bacillus bifidus*, or both of these organisms may predominate. From a further series of experiments carried out on rats fed various carbohydrates, Rettger⁸ concludes that lactose in itself will cause the changes above mentioned.

Of special interest were the findings in the experiments as presented by me after feeding cow's milk containing relative high percentages of additional sugars. The flora was not only unchanged in the colon, but all the levels examined showed essentially the same organisms as after diets of unmodified cow's milk. An acidophilic flora, as noted by Rettger, in the sense of a predominating gram-positive bacillus, showing cultural characteristics of *Bacillus bifidus* and *Bacillus acidophilus*, or both of these organisms, was never obtained, either after lactose or sucrose feedings. It is possible that the difference in the species of the animals utilized for experimentation may in part explain these contradictory results. The cultural methods employed to isolate the acidophilic organisms may, indeed, have failed to show the presence of rare bacilli of this type. That they never were in predominating numbers is shown conclusively by the microscopic findings, as a smear made up chiefly of gram-positive bacilli was never encountered.

It is very difficult to say whether the diarrhea elicited by the high sugar feedings in these experiments is similar to that occurring in infants under similar conditions. The animals, in many instances, became inactive, would not play and took their feedings poorly. The

5. Herter, C. A., and Kendall, A. I.: Jour. Biol. Chem., 1908-1909, **5**, 293.

6. Rettger, L. F., and Horton, G. D.: Centralbl. f. Bakteriöl., Part 1, Orig., 1914, **73**, 362.

7. Rettger, L. F.: Jour. Exper. Med., 1915, **21**, 365.

8. Rettger, L. F.: Footnotes 6 and 7.

diarrhea was easily controlled by withdrawing the sugar from the diet. The younger puppies developed diarrhea much more quickly than older ones.

The experiments with protein milk are too few to allow definite conclusions. Nevertheless, it seems significant that essentially the same types of organisms may be present in the intestinal tract on this diet as on unmodified cow's milk. It is possible that the organisms normally present under dietary conditions may change their function and act on proteins instead of carbohydrates. The reason for the beneficial effect of protein milk must still be considered as a matter of conjecture.

From those studies it seems probable that protein milk induces no marked alteration in the bacterial flora, and that, therefore, the changes produced in digestion are primarily chemical rather than bacteriologic. The fact that spore-bearing forms were found at all levels examined deserves mention. It has formerly been thought that these organisms sporulated primarily in the colon and there produced toxic compounds which caused certain digestive disturbances. In puppies, it is clear that the spore-bearing forms may occur in large numbers at upper levels of the intestinal tract without causing any unfavorable symptoms. The same statement is undoubtedly true for infants, as foods such as protein milk are known to contain large numbers of these forms, and it has been shown by Ford and Knox⁹ that the ingestion of these organisms may cause no clinical symptoms.

In relation to the experiments on the effects of starvation presented in this paper, reference should also be made to the work of Cushing and Livingood.¹⁰ These authors made observations on the number of intestinal organisms in dogs after a twenty-four-hour fast. A laparotomy was performed on an adult dog previously fed on a milk diet and starved for twenty-four hours. Specimens were then taken from ten different levels, after incision of the intestine. The specimens consisted of single platinum loopfuls of intestinal contents. Smears and cultures were made. No mention is made either of the mediums used or the method employed for counting the organisms. It should be stated that a single loopful of intestinal contents may vary so much in consistency and in number of bacteria present that estimations of the number and kind of organisms by this method are of very relative value. From their experiments the authors conclude that a relative amicrobism of the small intestine results after a period of twenty-four hours' starvation. Study of the distal loops of two patients with permanent fistula of the jejunum showed a sterile condition. These and further

9. Knox, J. H. M., Jr., and Ford, W. W.: *Bull. Johns Hopkins Hosp.*, 1915, **26**, 27.

10. Cushing and Livingood, L. E.: *Contributions to the Science of Medicine*, by Pupils of Welch, 1900, p. 543.

observations on a series of twenty-seven dogs and eight rabbits confirmed the conclusion given above. These last experiments were undertaken from a surgical rather than from a strict bacteriologic point of view. Careful dietary precautions were not taken. These authors also conclude from their experimental studies that one is not justified in speaking of a local flora at particular levels.

Effects of Starvation.—The examinations of the intestinal contents after periods of starvation show definite changes in the number of organisms. In many instances smears showed rare forms, and many times the plates showed but isolated colonies, in contrast to the profuse growth found normally. Changes in the kinds of organisms persisting after periods of starvation were not noted. The greatest diminution was observed in the duodenum. In some instances, however, only rare organisms were present in the contents of the ileum. The contrast between the paucity of organisms at this level and the large number present in the cecum was striking. Although the number of organisms is, to a great extent, dependent on the food residue present, absolutely sterile specimens, even after the food residue was undoubtedly absent, were never obtained. In other words, the condition produced by starvation is one of relative amicrobism.

CONCLUSIONS

From the experimental studies on the intestinal flora of puppies, the following conclusions can be drawn:

1. In puppies the types of organisms occurring at the duodenum, ileum, cecum and rectum are in all instances similar. One cannot speak of a characteristic local flora occurring in these regions.
2. Feedings to puppies of cow's milk mixtures with high percentage of sucrose or lactose does not cause characteristic changes in the intestinal flora at any level, even when a diarrhea is produced.
3. Starvation of twenty-four hours renders a relative amicrobism of the small intestine.