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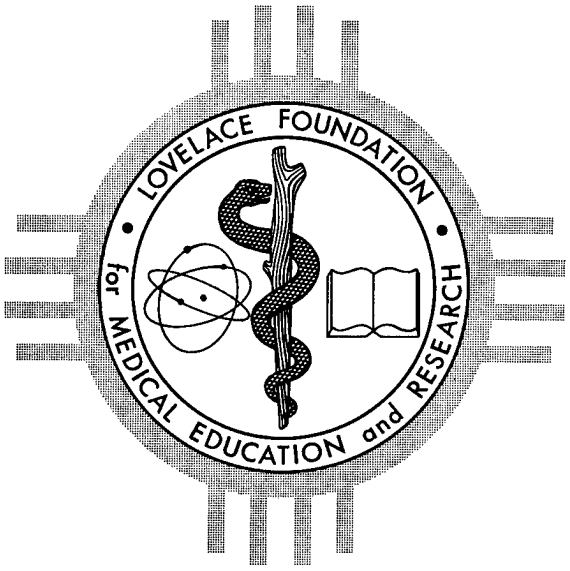

Albuquerque, New Mexico

FLORA OF HEALTHY DOGS
III. INCIDENCE AND DISTRIBUTION OF SPIROCHETES IN THE DIGESTIVE TRACT OF DOGS

by

F. F. PINDAK,
W. E. CLAPPER
AND J. H. SHERROD

November 1964

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ABSTRACT

The spirochetes found in the feces of dogs were divided into three categories based on morphology. These were: borrelias, treponemes, and fairly large double-contoured organisms. Some variation was observed within each group. Classification or further subdivision was not attempted because definitive characteristics necessary for such a purpose were not available. The live organisms could be readily demonstrated when a wet preparation was viewed in phase contrast illumination under 400 X magnification.

Of 54 dogs examined, nearly 80% had more than one type of spirochete in their feces. In addition, 16.7% carried a single type. Only 3.7% of dogs were found to be free of spirochetes. All three types of organisms were encountered in almost the same percentage of animals, although in some animals the borrelias were present in greater numbers than the remaining two types. It appears that the mere presence of these organisms in the feces cannot be interpreted as a sign of illness. However, when found in large numbers, they may be related to disease.

As demonstrated by repeated examinations of 12 dogs, the number of spirochetes in the feces can fluctuate from day to day. Therefore, in order to establish whether a dog carries any of these organisms, multiple samplings are necessary.

The examination of the entire digestive tracts of three dogs revealed that some spirochetes can be found in the oral cavity and in the stomach, but by far the greatest accumulation of all three types was confined to cecum, colon, and rectum. It is not known whether their localization in the lower part of the digestive tract can be accounted for by the physiology of the gut, the dependency of these organisms on the intestinal flora, or other factors.

Orally administered Furadantin, sulfaguanidine, or penicillin, in the dosages used, had no effect on the spirochetal population of the rectum. Terramycin caused a temporary elimination of all three types of organisms, but their recurrence was seen four to five days after termination of treatment. Dogs given a combination of the four chemotherapeutic agents were freed of all spirochetes for at least 41 days after the treatment, but became reinfected during two to thirteen days of association with spirochete-carrying dogs. Penicillin, sulfaguanidine, or Furadantin might therefore be administered orally to suppress other bacterial flora and thus facilitate the isolation and cultivation of the spirochetes.

None of the organisms studied was an obligatory anaerobe, as evidenced by their maintenance in viable state in small amounts of saline for a considerable length of time. They were maintained at 4°C for at least four days. At room temperature and at 37°C, they retained viability for several days, provided that the pH of the medium was maintained in the vicinity of neutrality. Successful cultivation of these spirochetes may depend on a metabiotic or other relationship to some microorganisms of the lower intestine.

ACKNOWLEDGMENTS

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III. INCIDENCE AND DISTRIBUTION OF SPIROCHETES IN THE DIGESTIVE TRACT OF DOGS

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INTRODUCTION

In the preparation for microbiological studies on beagles exposed to inhalation of radioactive fission products, their normal bacterial, fungal, and viral flora have been determined and reported (4, 5). The intestinal spirochetes were not studied because routine methods for culture, isolation, and identification were not readily available in the literature. There were some reports of their pathogenicity, although past studies of this nature were sometimes conflicting as will be noted subsequently. Since these organisms might represent a health hazard for dogs, it was clear that further investigation of their occurrence among the experimental animals was needed. Our attention was first directed to the problem when an examination of fecal material from a dog with hemorrhagic diarrhea revealed innumerable spirochetes. Accordingly, a pilot study was undertaken to determine the occurrence and persistence of the spirochetal flora in the digestive tract of beagles considered to be healthy.

MATERIALS AND METHODS

1. Dogs Studied

Sixty-nine dogs were included in this study. All were apparently normal male and female beagles, whose ages ranged from six weeks to over two years. Most of them were housed in small groups outdoors in pens consisting of a crushed rock floor and wire fence walls. Others were kept indoors in individual isolation cages. A number of dogs were

brought in from outside sources and some were raised at our own installation. They all were fed commercial dried food supplemented by fresh meat and vitamins. Their drinking water came from a chlorinated supply used routinely for human consumption.

2. Collection and Handling of Specimens

Sterile dry cotton swabs were used for collection of fecal material from the rectum of the dog. These were immediately submerged in 2 ml of sterile saline in plastic test tubes measuring approximately 15 mm in diameter. The specimens reached the laboratory within one hour after collection, at which time they were examined for the presence of spirochetes.

3. Examination of Specimens and Recording of Results

The fecal material was dispersed in the saline and one drop of the fluid was deposited on a slide. If necessary, it was further diluted with saline solution to a density which permitted better visualization of individual microorganisms. The preparations were examined in phase contrast illumination, using a 40X objective. A minimum of ten fields were examined. The numbers of each type of spirochete per field were averaged and recorded as follows:

More than 50 spirochetes per field	4+
11 to 50 spirochetes per field	3+
2 to 10 spirochetes per field	2+
1 per field but not less than 1 per 10 fields	+
None in 10 fields	0

4. Oral Chemotherapy

The antibiotics were administered orally on three successive days in the following dosages:

Dogs No. 58 and No. 59	900,000 units of procaine penicillin G (Bio Ramo Drug Co., Baltimore, Md.)
	500 mg cosa-terramycin tablets (Chas. Pfizer & Co., New York, N.Y.)
	1,500 mg sulfaguanidine tablets (Lederle Lab., New York, N. Y.)

	100 mg Furadantin (Eaton Lab., Norwich, N. Y.)
Dogs No. 60 and No. 61	900, 000 units of penicillin
Dogs No. 62 and No. 63	250 mg Cosa-terramycin
Dogs No. 64 and No. 65	2, 000 mg sulfaguanidine
Dogs No. 66 and No. 67	50 mg Furadantin
Dogs No. 69 and No. 70	No treatment (controls)

5. Media Used for Maintenance and Culture of Spirochetes

Attempts were made to culture and to maintain the spirochetes in the following media: 1) 0.85% sodium chloride in distilled water, 2) tryptose phosphate broth (TPB) (Difco), 3) thioglycollate broth (Th B), 4) brain heart infusion broth (BHIB), 5) tissue culture medium 199. All were tried both with and without 10% heat inactivated (56° C for 30 minutes) calf serum. The incubation temperatures were 37°C, 20°C (room temperature), and 4°C.

RESULTS

1. Description of Organisms Found

The spirochetes encountered in the feces were divided into three main groups. These were 1) a highly flexible organism with loosely wound spirals (Borrelia), 2) a rigid tightly coiled organism (treponeme), and 3) a thick, loosely coiled, double-contoured organism. In addition, there was a small borrelia-like organism and a slender treponeme, distinctly different from the larger ones of the more commonly observed varieties. Most of them can be seen in Figure 1, which includes photomicrographs of a fecal suspension, dried and stained by the Giemsa method. The division of organisms into these categories was arbitrary and not intended to be a classification into genera bearing the names Borrelia and Treponema. Indeed, none of the three groups were entirely homogeneous in respect to morphology. Most of the borrelia-like organisms were 10-15 μ long and less than 1 μ thick. All had a common method of propulsion, i.e., characteristic skip-rope-like undulation, apparent rotation about the longer axis and intermittent formation of 1-5 coils followed from time to time by relaxation and extension into a flaccid filament.

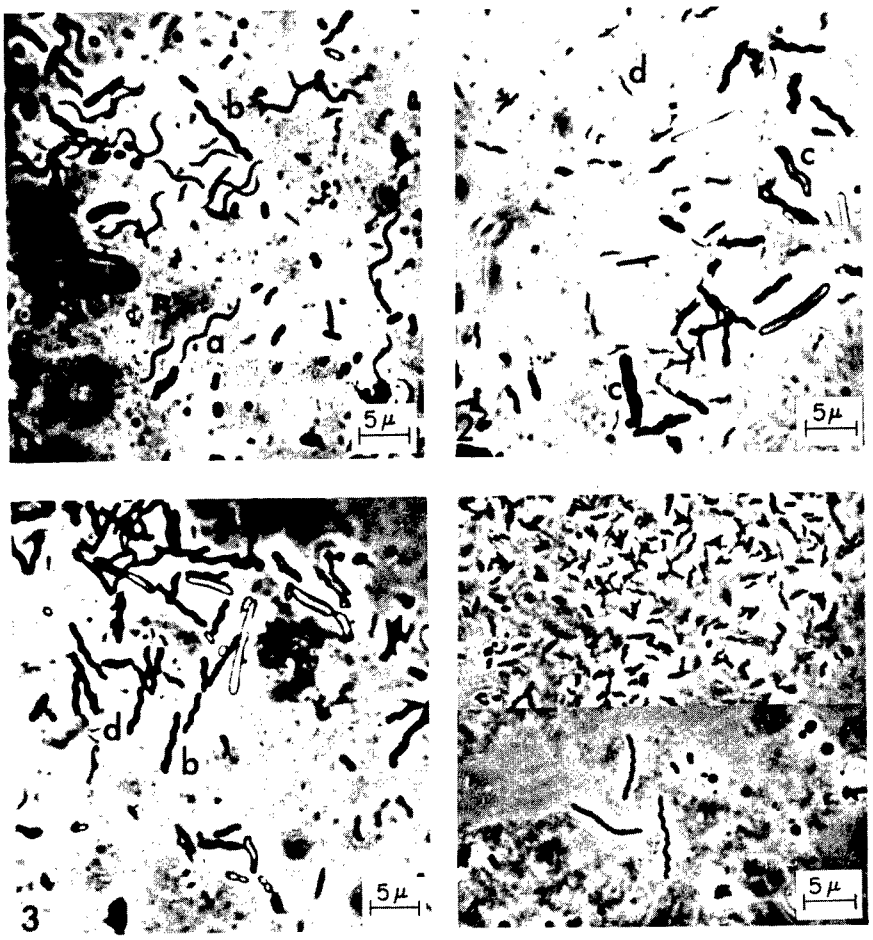

Fig. 1 Intestinal Spirochetes of Dogs

1, 2, 3 --- Direct smears of fecal samples, stained by the Giemsa method:

a. Borrelia
b. Treponeme
c. Double-contoured spirochete
d. Small borrelia

4 --- Culture of treponemes:

Upper half, early culture of short organisms;
Lower half, long spirochetes in old culture.

The treponeme-like organisms were about 1μ thick and 3-10μ long. They were single-contoured spirals with blunt ends, rather rigid, with only occasional slight bending and no definite flexion. They exhibited corkscrew-like motility, spinning on the long axis. The motion was reversible.

The double-contoured organisms were by far the largest. Their thickness was roughly twice that of the treponeme-like spirochetes. They had one to three loose coils, though an occasional organism had as many as five. Under proper illumination, the double contour was quite distinct. The motility consisted of rather slow rotation on the long axis producing a forward and backward motion. At times, the shorter ones were tumbling or gyrating on one end. When the organism remained stationary, a whirling of the small fecal particles could be observed in the vicinity of either end, suggesting the presence of terminal flagella, although none were distinctly seen.

2. Single Samplings of 54 Dogs

As noted in the introduction, examination of fecal material from a dog with hemorrhagic diarrhea revealed innumerable borrelia-type spirochetes. A very few double-contoured organisms were also seen along with many red blood cells (Figure 2). That the spirochetes occur in the absence of diarrhea was demonstrated by examining rectal swabs taken from a total of 54 apparently normal beagles. The spirochetes noted were recorded according to their morphology and numbers. Table 1 shows that the borrelias were found in 34 dogs, the treponemes in 39, and the double-contoured organisms in 38. The small borrelia and the thin treponeme were seen in one dog each. Only two dogs were found free of any spirochetes.

Inasmuch as the mere presence of the spirochete in the dog feces was quite common, attention was focused on the relative numbers of them. It can be seen from Table 2 that only one dog had more than 50 borrelias (4+) per field. Eight dogs had 11 to 50 per field (3+).

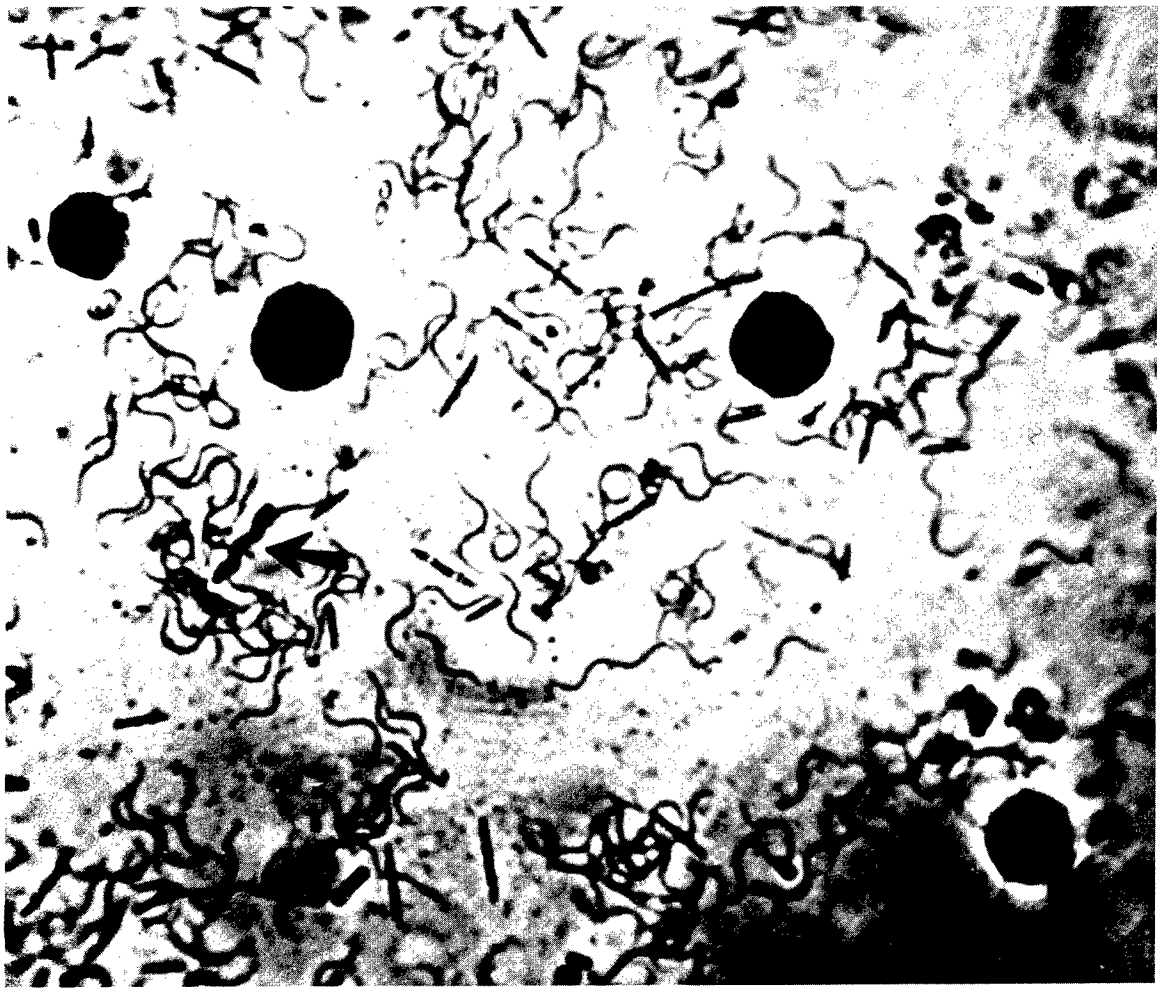

Fig. 2 Direct Smear of Fecal Sample from Dog with Hemorrhagic Diarrhea

Note the abundance of borrelias, four red blood cells, and one double-contoured spirochete (see arrow).

TABLE 1

DISTRIBUTION OF INTESTINAL SPIROCHETES
IN 54 HEALTHY BEAGLES

Spirochete	Number of dogs	Percent of total
Borrelia	34	62.96
Treponeme	39	72.22
Double-contoured	38	70.37
Small borrelia	1	1.85
Thin treponeme	1	1.85
None	2	3.70

TABLE 2

DISTRIBUTION OF INTESTINAL SPIROCHETES ACCORDING TO RELATIVE NUMBERS - 54 HEALTHY BEAGLES

Spirochete	Number of dogs	Percent of total
Borrelia:		
* 4+	1	1.85
3+	8	14.81
2+	11	20.37
+	14	25.93
0	20	37.04
Treponeme:		
4+	0	0
3+	2	3.70
2+	15	27.78
+	22	40.74
0	15	27.78
Double-contoured:		
4+	0	0
3+	1	1.85
2+	5	9.26
+	32	59.26
0	16	29.63

* See page 2 for exact evaluation of number of spirochetes.

The treponemes, although found in more dogs, were in smaller numbers, than the borrelias. The greatest concentration of treponemes (11 to 50 per field) was found in only two dogs. The double-contoured spirochetes, distributed essentially in the same percentage of the dogs as the treponemes, were in still lower numbers. The greatest concentration (11-50 per field) was found in only one dog.

It was observed from the beginning that most dogs harbored more than one type of spirochete. Table 3 shows that the distribution of the spirochetes either singly or in various combinations was more or less random in normal dogs.

3. Repeated Samplings of Dogs

Since 96% of normal dogs carried at least one type of spirochetes, it was of interest to determine whether their presence in any given dog was constant or varied from day to day. To determine this, 12 dogs housed singly in metabolism cages were sampled nine to ten times in the span of 14 days. Table 4 summarizes the result of this experiment. It can be seen that, in this group of dogs, the borrelia-type organism was found every time in seven, more than 75% of the time in three, and at least 50% of the time in one. Of 115 rectal swabs taken from these dogs, this organism was found in 102, or 88.7%. The treponeme was present in 80 swabs (69.6%). It was found in one-half or more of the swabs taken from nine dogs. The double-contoured spirochete showed a greater fluctuation, but it also was present in nine dogs 50% or more of the time.

The relative number of these organisms, as found in day-to-day examinations, can be seen in Table 5. It is quite evident that a dog carrying significant numbers of spirochetes one day may have only a few or none demonstrable the following day, and vice versa. Such a situation was encountered many times, particularly in the case of borrelias. The same is true with the other two organisms, though to a lesser degree.

TABLE 3

COMBINATIONS OF TYPES OF INTESTINAL SPIROCHETES IN 54 DOGS

Spirochete	Number of dogs	Percent of total
Borrelia, treponeme, double-contoured	16	29.63
Borrelia and treponeme	6	11.11
Borrelia and double-contoured	7	12.96
Treponeme and double-contoured	14	25.93
Borrelia only	5	9.26
Treponeme only	3	5.56
Double-contoured only	1	1.85
None	2	3.70

TABLE 4

THE INCIDENCE OF INTESTINAL SPIROCHETES
IN 12 NORMAL DOGS OVER A 14-DAY PERIOD

Dog	Borrelia		Treponeme		Double-contoured		Samplings		Percent positive		
Number	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	totals		Borrelia	Treponeme	Double-contoured
58	10	0	4	6	2	8	10		100.0	40.0	20.0
59	10	0	4	6	7	3	10		100.0	40.0	70.0
60	8	1	6	3	6	3	9		88.9	66.7	66.7
61	9	0	7	2	4	5	9		100.0	77.8	44.4
62	7	2	9	0	8	1	9		77.8	100.0	88.9
63	10	0	9	1	8	2	10		100.0	90.0	80.0
64	10	0	5	5	4	6	10		100.0	50.0	40.0
65	9	0	4	5	5	4	9		100.0	44.4	55.6
66	9	1	9	1	7	3	10		90.0	90.0	70.0
67	10	0	8	2	6	4	10		100.0	80.0	60.0
69	4	5	7	2	5	4	9		44.4	77.8	55.6
70	6	4	8	2	7	3	10		60.0	80.0	70.0
Total of all dogs	102	13	80	35	69	46	115	More than 50% incidence in:			
Percent of all samplings	88.7	11.3	69.6	30.4	60	40			11 dogs (91.7%)	8 dogs (66.7%)	9 dogs (75%)

TABLE 5

THE INCIDENCE OF INTESTINAL SPIROCHETES IN NORMAL DOGS. REPEATED SAMPLINGS

Dog Number	58			59			60			61			62			63		
Spirochete	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc
Sampling date																		
5-4	2+	0	0	+	0	0	+	0	+		ND		2+	2+	0	2+	0	2+
5-5	3+	0	0	+	+	0	3+	0	0	+	0	0	+	2+	+	3+	+	+
5-6	2+	0	+	2+	0	0	+	+	+	2+	+	0	2+	+	+	2+	+	+
5-7	3+	0	0	3+	0	+	+	+	0	+	+	+	+	2+	+	+	2+	+
5-8	2+	+	+	2+	0	+	0	2+	+	+	+	+	2+	2+	+	+	+	+
5-11	3+	0	0	2+	0	+	+	2+	+	+	+	+	2+	3+	2+	4+	+	0
5-12	2+	0	0	3+	+	+	+	2+	+	+	+	0	+	2+	+	+	2+	+
5-13	2+	+	0	+	0	2+		ND		2+	0	0		ND		4+	2+	2+
5-15	3+	+	0	+	+	+	2+	2+	0	4+	+	0	0	3+	+	3+	2+	0
5-18	2+	2+	0	3+	+	+	+	0	2+	+	+	+	0	+	+	3+	2+	+
Dog Number	64			65			66			67			69			70		
5-4	2+	0	0	2+	0	+	3+	0	0	+	+	0		ND		0	2+	+
5-5	+	+	+	+	0	+	2+	+	0	4+	+	0	0	+	0	+	+	0
5-6	3+	+	0	+	0	+	4+	2+	+	3+	+	+	2+	+	0	3+	+	0
5-7	3+	0	0	2+	0	2+	4+	+	0	3+	+	0	0	2+	+	0	+	+
5-8	+	0	2+	+	0	+	2+	2+	2+	2+	0	+	0	2+	0	0	0	+
5-11	3+	0	0	2+	2+	0	2+	2+	+	+	2+	2+	+	+	+	+	2+	+
5-12	+	+	+	3+	2+	0	2+	2+	+	3+	2+	+	+	+	0	0	2+	2+
5-13	+	0	0		ND		2+	3+	+	3+	0	0	0	0	+	2+	0	+
5-15	2+	3+	+	2+	2+	0	+	2+	+	2+	+	+	+	2+	+	+	2+	+
5-18	+	2+	0	+	+	0	0	2+	+	2+	+	+	0	0	2+	+	2+	0

ND = not done; Bo = borrelia; Tr = treponeme; Dc = double-contoured spirochete

4. Distribution of Spirochetes in the Disgestive Tract of Dogs

No specific reference has been found in the literature to the incidence of spirochetes in the anatomical subdivisions of the entire digestive tract of dogs. Because some spirochetes were present in almost all of the rectal swabs, it was of interest to determine what other segments of the digestive tract might harbor them.

Three dogs were used for this purpose. One dog (A), a male beagle six months old, was allowed water ad lib., but not food for 24 hours before the examination. Dog B, an eight weeks old female beagle, and dog C, a male mongrel of unknown age and background, took meals one to two hours before they were euthanized. The entire digestive tracts were then surgically removed and their portions examined immediately. The results are given in Table 6. In the oral cavity, swabs were taken from tongue, alveolar crypts, and from the anterior aspect of the tonsils. An organism resembling the borrelia-type spirochete was recovered only from the alveolar crypts of the mongrel. Rigid treponemes, some slightly longer and thicker than those found in the rectum, were found in dog A and in the mongrel (C). In addition, the alveolar crypts of all three dogs contained fine, long, flexible treponemes. Some were 20-30μ long. They exhibited corkscrew boring and lashing motility. The double-contoured organisms were not found.

Three samples were taken from the mucosa of the esophagus of each dog: one about two inches from the proximal end, one from the middle, and one about two inches from the distal end. The only organisms found were a few treponemes in dogs A and C.

The stomach mucosa of all three dogs contained only treponemes. Some were slightly thicker and longer than those usually seen in fecal smears.

Dog A had a few treponemes in the upper segment of the duodenum.

TABLE 6

DISTRIBUTION OF SPIROCHETES IN THE DIGESTIVE TRACT OF DOGS

Spirochete	Borrelia			Treponeme			Double-contoured			Other
Dog	A	B	C	A	B	C	A	B	C	
Tongue	0	0	0	0	0	+	0	0	0	
Alveolar crypts	0	0	+	2+	0	+	0	0	0	Fine, long, flexible treponemes (A, B, C)
Tonsils	0	0	0	2+	0	+	0	0	0	
Esophagus, upper	0	0	0	+	0	0	0	0	0	Fine, long, flexible treponemes (C)
Esophagus, middle	0	0	0	0	0	0	0	0	0	
Esophagus, lower	0	0	0	0	0	+	0	0	0	
Stomach, upper	0	0	0	+	0	+	0	0	0	
Stomach, center of greater curvature	0	0	0	+	+	+	0	0	0	
Pylorus	0	0	0	+	+	2+	0	0	0	
Duodenum, 2 inches below pylorus	0	0	0	+	0	0	0	0	0	
Duodenum, middle	0	0	0	0	0	0	0	0	0	
Duodenum, lower	0	0	0	0	0	0	0	0	0	
Jejunum, 4-inch segments	0	0	0	0	0	0	0	0	0	
Ileum, 4-inch segments	0	0	0	0	0	0	0	0	0	
Ileum, just above cecum	0	0	0	0	0	0	0	0	0	
Cecum, upper tip	0	0	0	2+	3+	2+	+	+	2+	
Cecum, middle	+	0	0	+	2+	3+	+	2+	+	
Cecum, lower	0	0	0	2+	3+	2+	+	2+	2+	
Colon, just below cecum	2+	0	2+	2+	3+	2+	2+	2+	2+	
Colon, middle	3+	0	2+	2+	3+	2+	+	+	2+	
Colon, lower	2+	0	3+	3+	2+	2+	+	2+	+	
Rectum	2+	+	3+	2+	2+	2+	2+	+	+	

Dogs: A = male beagle, 6 months old, fasting (24 hours).
B = female beagle, 8 weeks old, full stomach.
C = male mongrel of unknown age and background, full stomach.

Otherwise, no spirochetes were encountered in any of the dogs in the intestinal segments between the stomach and the cecum, despite the fact that both jejunum and ileum were sampled at four-inch distances.

Beginning with the cecum and continuing to the anus, the spirochetes were found in moderate to great numbers. A few borrelias were seen in the cecum of one dog. Their greatest prevalence was in the colon and in the rectum of two dogs, (A) and (C). The treponemes and double-contoured spirochetes were fairly uniformly distributed from cecum to anus in all three dogs.

5. Effect of Oral Chemotherapy on the Spirochetal Population in the Intestine

Anticipating attempts to grow intestinal spirochetes in vitro, it was desirable to obtain some knowledge about what chemotherapeutic agents could be used to suppress the growth of the usual coliform flora and hopefully to facilitate the isolation of spirochetes in pure culture.

Twelve dogs, kept singly in metabolism cages and studied previously in some detail for the presence of spirochetes in their fecal material, were chosen for this purpose.

The results of such treatment are shown in Table 7. The spirochetal population in dogs treated only with penicillin, sulfaguanadine, or Furadantin, respectively, remained essentially unaltered. Twenty-four hours after the treatment was initiated, one of the dogs treated with terramycin was negative; but it was not certain that this finding was significant, since on the preceding day the organisms were present only in very small numbers.

On the second day of treatment an effective suppression of the spirochetes was observed in dogs treated with the combination of all four chemotherapeutic agents (dogs No. 58 and No. 59) and with terramycin alone (dogs No. 62 and No. 63). On the third day of treatment, and for the first three days thereafter, the dogs given terramycin remained negative. However, on the following day, the spirochetes reappeared.

TABLE 7

THE INCIDENCE OF INTESTINAL SPIROCHETES IN DOGS

BEFORE, DURING, AND AFTER TREATMENT WITH SELECTED ORAL CHEMOTHERAPEUTIC AGENTS

Therapy	Pen., Terra., Sulfa, Furad.						Penicillin						Terramycin					
Dog Number	58			59			60			61			62			63		
Spirochete	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc
Sampling date																		
5-4	2+	0	0	+	0	0	+	0	+		ND		2+	2+	0	2+	0	2+
5-5	3+	0	0	+	+	0	3+	0	0	+	0	0	+	2+	+	3+	+	+
5-6	2+	0	+	2+	0	0	+	+	+	2+	+	0	2+	+	+	2+	+	+
5-7	3+	0	0	3+	0	+	+	+	0	+	+	+	+	2+	+	+	2+	+
5-8	2+	+	+	2+	0	+	0	2+	+	+	+	+	2+	2+	+	+	+	+
5-11	3+	0	0	2+	0	+	+	2+	+	+	+	+	2+	3+	2+	4+	+	0
5-12	2+	0	0	3+	+	+	+	2+	+	+	+	0	+	2+	+	+	2+	+
5-13	2+	+	0	+	0	2+		ND		2+	0	0		ND		4+	2+	2+
5-15	3+	+	0	+	+	+	2+	2+	0	4+	+	0	0	3+	+	3+	2+	0
5-18*	2+	2+	0	3+	+	+	+	0	2+	+	+	+	0	+	+	3+	2+	+
5-19*	+	2+	+	+	0	+	2+	2+	+	+	0	+	0	0	0	3+	0	+
5-20*	+	0	0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	0
5-21	0	0	0	0	0	0	2+	2+	0	4+	4+	0	0	0	0	0	0	0
5-22	0	0	0	0	0	0	+	0	+	0	2+	2+	0	0	0	0	0	0
5-23	0	0	0	0	0	0	2+	0	+	0	2+	2+	0	0	0	0	0	0
5-25	0	0	0	0	0	0	2+	3+	+	0	+	+	0	0	0	0	0	0
5-26	0	0	0	0	0	0	0	+	+	+	+	+	0	+	0	0	0	+
5-27	0	0	0	0	0	0	2+	2+	2+	3+	0	0	0	2+	0	0	3+	0
5-28	0	0	0	0	0	0	0	+	+	+	+	+	2+	2+	0	0	3+	2+
6-4	0	0	0	0	0	0	2+	2+	2+	2+	+	+	+	+	0	+	2+	+
6-16	0	0	0	0	0	0	3+	+	+	2+	+	+	+	0	0	0	2+	0
7-1	0	0	0	0	0	0	3+	+	0	2+	0	+	+	0	0	+	2+	+

*Indicates days when therapy was administered

ND = not done; Bo = borrelia; Tr = treponeme
Dc = double-contoured spirochete.

TABLE 7 (CONTINUED)

THE INCIDENCE OF INTESTINAL SPIROCHETES IN DOGS

BEFORE, DURING, AND AFTER TREATMENT WITH SELECTED ORAL CHEMOTHERAPEUTIC AGENTS

Therapy	Sulfaguanidine						Furadantin						None					
Dog Number	64			65			66			67			69			70		
Spirochete	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc	Bo	Tr	Dc
Sampling date																		
5-4	2+	0	0	2+	0	+	3+	0	0	+	+	0		ND		0	2+	+
5-5	+	+	+	+	0	+	2+	+	0	4+	+	0	0	+	0	+	+	0
5-6	3+	+	0	+	0	+	4+	2+	+	3+	+	+	2+	+	0	3+	+	0
5-7	3+	0	0	2+	0	2+	4+	+	0	3+	+	0	0	2+	+	0	+	+
5-8	+	0	2+	+	0	+	2+	2+	2+	2+	0	+	0	2+	0	0	0	+
5-11	3+	0	0	2+	2+	0	2+	2+	+	+	2+	2+	+	+	+	+	2+	+
5-12	+	+	+	3+	2+	0	2+	2+	+	3+	2+	+	+	+	0	0	2+	2+
5-13	+	0	0		ND		2+	3+	+	3+	0	0	0	0	+	2+	0	+
5-15	2+	3+	+	2+	2+	0	+	2+	+	2+	+	+	+	2+	+	+	2+	+
5-18*	+	2+	0	+	+	0	0	2+	+	2+	+	+	0	0	2+	+	2+	0
5-19*	0	2+	+	2+	+	+	0	2+	2+	2+	2+	+	2+	+	2+	2+	+	+
5-20*	+	+	+	+	0	0	+	+	+	2+	2+	+		ND		+	+	+
5-21	+	+	0	0	+	+	+	2+	2+	2+	2+	+	+	+	+	+	+	+
5-22	2+	0	0	0	2+	+	+	0	0	2+	+	+	0	2+	0	0	2+	2+
5-23	2+	0	0	0	2+	+	2+	+	+	2+	2+	+	+	2+	+	+	+	+
5-25	+	2+	0	0	0	0	+	2+	+	+	2+	+	+	+	2+	2+	+	+
5-26	2+	+	0		ND		4+	3+	0	3+	2+	+	0	2+	0	0	2+	2+
5-27	3+	2+	2+	+	+	+	3+	2+	0	3+	+	2+	0	2+	+	0	2+	2+
5-28	+	+	0	0	+	0	2+	+	2+	4+	2+	+	+	0	2+	0	2+	+
6-4	+	0	+	+	0	0	3+	+	+	0	0	+	+	0	+	0	2+	0
6-16	2+	+	0	2+	0	0	3+	+	+		ND		0	2+	+	+	2+	0
7-1	3+	+	+	2+	0	0	3+	2+	+	+	+	0	0	+	+	+	2+	+

*Indicates days when therapy was administered.

ND = not done; Bo = borrelia; Tr = treponeme; Dc = double-contoured spirochete.

Those treated with the combination of agents were negative on the last (third) day of treatment. Nine subsequent examinations made during the next 41 days were also negative. At this time, they were housed together with dogs treated previously with sulfaguanidine, but still carrying all three types of spirochetes. Two days later, the "cured" dogs again had the treponemes and the double-contoured spirochetes in their feces. The borrelias reappeared 11 days later.

6. Maintenance and Culture of the Spirochetes

Initially, the rectal swabs were submerged in approximately 2 ml of Th B and the microscopic examination was done within one hour after collection. Later, it became apparent that 0.85% NaCl solution was just as suitable for short-term preservation of the spirochetes. Moreover, whether the specimens were kept in saline or in the media listed under Materials and Methods, they could remain at room temperature for up to four hours without any appreciable loss of motility of the organisms. When stored at 4°C, the borrelias remained motile for at least one day. The other two types of organisms retained their viability for up to four days.

At 37°C, the number of motile borrelias in all these media began to diminish after four hours and only very few could still be seen after overnight incubation. At this time, a decrease of the treponemes and the double-contoured spirochetes was also evident. In subsequent attempts, neither the amount of the media in test tubes nor their overlaying with a 3-cm column of sterile mineral oil improved the maintenance of these organisms. It was noted, however, that during the overnight incubation, there was a great proliferation of other bacteria accompanied by accumulation of acid in the medium.

This lowering of the pH of the media at 37°C was considered a possible cause of destruction of the spirochetes. Consequently, the pH of all media was adjusted to 7.4. Sets of tubes were inoculated with equal amounts of pooled fecal suspensions and incubated at 37°C. No viable spirochetes were seen in these tubes after 24 hours. In a similar

experiment, the pH of the media was periodically adjusted to 7.4 during working hours. To reduce acid production by the growing organisms, the cultures were stored at 4°C and incubated again at 37°C the following day. This resulted in an improved maintenance of both treponemes and the double-contoured spirochetes, but not of the borrelias. There was no increase in numbers of spirochetes in any of the cultures.

Since it could be possible that 37°C was not optimum for growth of the spirochetes, cultures in all media were incubated at room temperature and at 4°C. The pH of these cultures reached 6.3 and 7.1, respectively, as compared to 4.7 for those at 37°C. No diminution in numbers of any of the spirochetes was observed at 4°C, and about half of those at room temperature remained motile. These results suggest that the acid produced by the other organisms was a deleterious factor and the higher temperature allowed more acid production. Maintenance of pH at around 7 is apparently more favorable to survival of these organisms.

Inasmuch as the methods just described offered no help in isolating and culturing of the spirochetes, attempts were made to take advantage of their high motility for this purpose. Specimens were inoculated into thioglycollate medium with calf serum, contained in approximately two-foot lengths of glass tubing bent in the middle at a 90 degree angle. The rectal swab was submerged in one arm. The motile organisms were allowed to migrate into the other arm. After several hours of incubation at 37°C, they could be seen as a moving front of haze ascending in the uninoculated part of the tube. When this was about two inches away from the swab, a long capillary pipette was used to withdraw some of the organisms through the sterile arm of the V-shaped tube. This fluid was transferred to the bottom of a similar tube of sterile medium. At one time, after four such transfers, an apparently pure culture of treponemes was obtained, as illustrated in Figure 1. The organisms were then subcultured five times in 15 x 150 mm tubes of thioglycollate broth before they were lost. The older cultures had rather long spirochetes and some irregularly shaped granules, believed to be involution forms. Unidentified

bacteria were found in the cultures after the fifth transfer. Repeated attempts to isolate spirochetes in pure culture by this method were unsuccessful.

DISCUSSION

Occurrence of spirochetes in man and animals. It seems appropriate in a discussion of the spirochetes of the digestive tract of dogs to consider the occurrence of these microoorganisms in other animals, including man. They were, apparently, first described by Rappin (25) in 1881, who noted their presence in the stomachs of dogs. During the following 50 years, they were found in various anatomical parts of the digestive tract of many other animals. Some authors believed that they produced disease, while others considered them as a harmless part of the microbial flora of the digestive system. Not much progress was made in regard to their characterization, classification, and pathogenicity and little attention was given to them in the last two decades.

Too many names were assigned to various organisms by the early investigators whose chief criteria of classification were the morphology of the spirochetes and the species of animals from which they were recovered. The main difficulty, even today, is that there apparently are no satisfactory methods for their isolation and cultivation.

The original discovery of spirochetes in the alimentary canal of dogs by Rappin (25) was confirmed and further investigated by others. For example, Bizzozero (1) saw them in the intracellular canaliculi and in the cytoplasmic vacuoles of the stomach mucosa. Similar findings were presented by Salomon (28), Regaud (26), and Kasai and Kobayashi (17), and recently by Weber, Hasa, and Sautter (32). They were also seen in the lower intestine of dogs by Lucet (19), Macfie (21), Jungherr (16), and by Craige (6-10).

It soon became evident that such organisms can be found also in cats (13, 17, 18, 26, 28, 30, 31), monkeys (11, 12, 17, 20, 21), rats, (21, 24, 28), mice and guinea pigs (24), cattle (2, 3, 21), and in sheep, goats, and pigs (21). Fantham (14) reported that Kowalski, in 1893, noted the presence

of spirochetes in stools of cholera patients, and that Escherich, in 1894, observed them in normal human feces. Doenges (11, 12) found them in 43% of 242 stomachs examined during autopsies. However, Palmer (23) did not find any spirochetes in aspirated samples of stomach mucosa of 1,000 living adults. This led him to believe that infestation of the human stomach with spirochetes takes place during an agonal or post-mortem process and that the source is the oral cavity. Various types of spirochetes were also described in the feces of live humans (14, 21, 22, 24, 27, 29, 33).

Classification of spirochetes of the digestive tract. It would no doubt be useful if these organisms could be properly divided into genera and species. However, it appears unlikely that each of the many proposed species names represents a different organism. With the knowledge at hand, it is impossible to determine their mutual relationship, or lack of it. For example, 50 years ago, Macfie (21) quoted as many as ten different "species" of spirochetes to be found in the oral cavity of man. Werner (33) described two spirochetes in human stools and called them Spirochaeta eurygyrata and Spirochaeta stenogyrata. The principal distinguishing characteristic between them was their thickness. Fantham (14) believed that both these organisms were identical and should be called Spirochaeta eurygyrata. Jungherr (16) observed a very similar spiral organism in the stomach of dogs and named it Treponema eurygyrata. The description of spirochetes which invaded epithelial cells of the fundic glands of the cat's stomach could fit the organism(s) named above, but they were given the name of Spirella regaudi by Edkins (13). Macfie (21) saw a spirochete in the feces of dogs which he called Spirochaeta canis. It was "lashing" and "undulatory", 2-11μ long and 0.25μ thick. Its description seems to fit the Borrelia eurgyrata of Craige (7), or the one which Reinhold and Wagner (27) and Shera (29) found in the rectum of humans.

A recent attempt to classify some of the spirochetes of the digestive tract was made by Craige (10), who recognized four species of these

organisms found in dogs. These were: 1) Spirillum eurygyrata (formerly Treponema eurygyrata and Borrelia eurygyrata), a rigid spiral with 1-4 loose coils, approximately 10μ long, having terminal flagella; 2) Borrelia canis (formerly Spironema), a long, loosely coiled organism with an undulating action like that of a snake; 3) Spirillum minutum (formerly Spirillum minus), a "small borrelia" with 1-4 loosely coiled spirals; and 4) Spirillum rappini (Spirilla canis by other authors).

Some of the spirochetes described in the present study could fall into Craige's classification. However, it is felt that until more pertinent information is available it would be best to avoid the use of either generic or species names. Our arbitrary division of these organisms into three groups was made for convenience and should not be given more than a purely descriptive and tentative meaning.

Pathogenicity of the spirochetes. The question of pathogenicity of the intestinal spirochetes was also subject to controversy. Lim (18) concluded that the clusters of trepneme-like organisms which he saw within the lumina of ducts and glands of the cat's stomach were non-pathogenic parasites. He was certain that they were not found below the pyloric sphincter and that they were not passed in feces. This could indicate that he was dealing with a true "stomach spirochete". Similar results were presented by Edkins (13) who found spirochetes in the stomachs of 80% of cats and thought that they were "as characteristic of the cat's gastric mucous membrane as fleas are of its fur." When Doenges (12) attempted to correlate the incidence of spirochetes in human stomachs examined at autopsy to the illness or death of the individuals who harbored them, he found no relationship.

Other investigators believed that these organisms may possess varying degrees of pathogenicity. Lucet (19) reported hemorrhagic enteritis in dogs which was associated with intestinal spirochetes. Macfie (21) concluded from a rather extensive study of animals that "the intestinal spirochaetes are sometimes present in such enormous numbers that one cannot but suspect that they are not entirely harmless." He further

observed that "in man they are often most abundant in cases of diarrhea."

Jungherr (16) observed that, in dogs, these organisms could cause mucodesquamative enteritis accompanied by profuse diarrhea, anorexia, and fever. Craige (7, 10) found that large numbers of spirochetes were present in the feces of dogs with an otherwise unexplainable diarrhea. He also noted that spirochetes can be detected in vomitus of dogs with signs of gastritis. Weber, Hasa, and Sautter (32) and Weber (30) believed that the spirochetes found in the stomachs of cats and dogs were mildly pathogenic. Doenges (11), studying spirochetes in the stomach of Macacus rhesus, noted some destruction in the parietal cells.

Reinhold and Wagner (27) described a case of a 12-year-old child complaining of fatigue and having bloody stools, which at times consisted only of mucus and blood. Borrelias were the only unusual organism found in such specimens. These authors stated that borrelias are often associated with such involvements as ulcerative colitis, tuberculous intestinal ulcers, disintegrating tumors, and catarrhal and inflamatory processes.

Only two years ago, Shera (29) stated that borrelia-like organisms found in the stools of his patients were the cause of "strawberry lesion" in the colon. He recognized primary and secondary types of the disease, both associated with vitamin D deficiency.

Our findings indicate that several types of spirochetes can be found in the feces of most apparently normal dogs. When present in small to moderate numbers, they should be considered as part of the normal coliform flora. However, if, for unknown reasons, the balance which they seem to maintain with other organisms is disrupted and the spirochetes appear in great quantities, they might assume pathological importance. We have seen one such dog in which excessive numbers of borrelias were associated with intestinal bleeding.

Treatment with chemotherapeutic agents. Several investigators who believed that the intestinal spirochetes caused disease attempted therapy. Nishiama (22) used arsenicals with apparently good results. Craige (6-8)

found that dogs responded favorably to sulfa drugs if the course of the disease was mild. In more severe cases of intestinal spirochetosis, he recommended the use of a combination of sulfa-compounds, streptomycin, and penicillin. In humans, Reinhold and Wagner (27) claimed cure after two days' administration of penicillin. Shera's (29) patients were considered cured after treatment with Stovarsol for ten days.

Our purpose in administering chemotherapeutic agents orally to dogs with intestinal spirochetes was to determine whether their bacterial flora could be suppressed without affecting the spirochetes. Sulfaguanidine, penicillin, and Furadantin were found to accomplish this. Such treatment combined with in vitro use of chemotherapeutic agents which do not interfere with the viability of the spirochetes, will be used in future attempts to establish them in pure cultures. The combination of terramycin, penicillin, sulfaguanidine, and Furadantin did not meet this objective. However, since it produced an apparently permanent elimination of all types of spirochetes from the lower intestine, it could be used when treatment is desirable.

Natural source of the spirochetes. There is no ready answer to the question of the source of intestinal spirochetes. It is generally assumed that they come from the oral cavity and have little, if any, pathogenic significance, but Doenges (12) believed that the organisms he found in the human stomach were not those of Vincent's angina. Based on our study of the distribution of spirochetes in the digestive tract, we believe that, in dogs, the organisms pass through the mouth and stomach before they establish themselves in the lower intestine. However, we cannot say that they are the oral spirochetes. Of the three types described, only treponemes were definitely found in the oral cavity. Even these may not be the same ones which were found consistently in the fecal specimens. Morphological similarity alone cannot be accepted as evidence of species identity. An interesting means of isolation of mouth spirochetes was reported by Wichelhausen and Wichelhausen (34) who used filters of such

porosities that the spirochetes could pass through while most bacteria would be retained.

Transmission studies. The fecal samples of dogs treated in this study with a combination of chemotherapeutic agents were freed of any spirochetes for a considerable time. However, after only a few days of association with spirochete-carrying dogs, they became re-infected with all three types. The literature shows clearly that spirochetes are found in the alimentary tract of other animals as well. Whether these are the same organisms passed from one animal species to another cannot be determined by observing morphology alone.

Salomon (28) was able to transmit spirochetes from dogs to mice, but not to birds or frogs. In 1919, Kasai and Kobayashi (17) transmitted spirochetes from a dog's stomach into mice and rats with relative ease, but guinea pigs could be infected only if they were first "infected by scarlet fever or measles." Similarly, they could produce a spirochetal infection in rabbits accompanied by punctate hemorrhages of the stomach mucosa, but only if an inoculation with rabies virus was given first. This relationship between rabies virus and intestinal spirochetes could be of importance in dogs, since they are susceptible to rabies and other viruses. Association of intestinal spirochetosis with other pathogens was also noted by Macfie (20) and Reinhold and Wagner (27).

Distribution of the spirochetes throughout the digestive tract. The present study shows that, whereas the intestinal segment from cecum to anus contains moderate to great numbers of all three types of spirochetes, only treponemes exist in the stomach and in the oral cavity and, even then, in considerably smaller numbers. In the mouth, and in one instance in the upper esophagus, there was also a long flexible treponeme not encountered elsewhere. Otherwise, no spirochetes were found in the esophagus and in the intestinal portion between the stomach and the upper end of the cecum.

It is therefore very likely that all three types of spirochetes actively multiply in the lower intestine which is their normal habitat. It is possible that their propagation in some other part of the intestine could result in a pathological process. Their appearance in the mouth or some other part of the digestive tract can be considered as a result of anus-to-mouth re-infection incidental to the dog's natural habit of self-licking. However, since the organisms are quite motile, it should not be overlooked that, in a group of dogs, the anus-to-anus route of dissemination is also possible.

Maintenance and culture of the spirochetes. Most reported efforts to isolate these organisms in pure culture were unsuccessful. Lim (18) attempted to grow spirochetes from the stomach of cats in deep agar, cooked meat, gastric digests with and without pepsin, with hydrochloric acid, and with serum, all without success. Jungherr (16) claimed that intestinal spriochetes from dogs "could be maintained and subcultured for several passages," but did not mention how and in what medium this was achieved. Two years ago Weber and Schmittdiel (31) were unable to obtain growth of the spirochetes from stomachs of cats and dogs. Negative results were obtained recently by Reinhold and Wagner (27) who attempted to culture the borrelia-like organisms from human stools. In 1962, Shera (29) claimed their maintenance for up to four weeks, but could not subculture them. Bryant (2) was able to grow a strictly anaerobic spirochete from bovine rumen.

The limited experience in this laboratory with maintenance and growth of these organisms suggested the possiblity of some inter-relationship between the spirochetes and the remaining coliform organisms. At one time a culture of treponeme-like organisms was established. The subsequent negative attempts of the same nature might be explained in several ways. It is possible that the fecal samples then used either contained bacteria which produced a substance toxic to the spirochetes, or lacked certain microorganisms producing metabolites necessary for their growth.

Hardy, Lee, and Nell (15) have recently reported that filtrates of cultures of certain bacteria enabled them to isolate spirochetes from the human oral cavity.

The importance of multiple examinations for detection of the spirochetes. The inadequacy of examinations of single fecal specimens was brought out by our repeated samplings of a number of dogs. It was shown that both the numbers and the types of spirochetes in an animal can change from day to day. Therefore, in order to determine whether a dog is infected, its fecal samples must be screened over a period of several successive days. This assumes particular importance when the effectiveness of chemotherapy is studied.

Conclusions. The relative scarcity of reports on intestinal spirochetes during the last two decades indicates a diminished interest in this field. Moreover, with methods commonly used in the routine clinical microbiology laboratories, it is very likely that the presence of spirochetes in feces may go undetected. The organisms cannot be isolated on the usual media. Examinations of direct stained smears of feces, as a rule, are not done. If they are, the spirochetes would have to be present in rather large numbers before they could be observed. Ordinarily, they would be obscured by the fecal debris or could be mistaken for artifacts. However, wet preparations observed under phase contrast illumination can easily reveal them, even if only a few are present, but this is not a routine procedure for handling stool specimens for bacteriological examination. It can, therefore, be expected that, should the interest in these organisms be renewed and the proper techniques be adapted, more reports on these spirochetes will be forthcoming. With improved methods of isolation, the organisms, in all probability, will be grown in pure culture. This can lead to their characterization, classification, and to determination of their relation to disease in dogs and other hosts.

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