PERSISTENCE OF ESCHERICHIA COLI STRAINS IN THE INTESTINAL TRACTS OF DOGS AND STUDIES ON THE MECHANISMS INVOLVED

by

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INTRODUCTION:

The fundamental studies of Escherich in 1885[1] and 1886[2] established the bacillus now designated as Escherichia coli as the main organism of the fecal mass. Originally it was demonstrated by Breslau in 1886[3] and later confirmed by Nothnagel in 1881[4] that the meconium of new born babies was sterile and that micro-organisms appeared with the advent of the first yellow stools. However the presence of Escherichia coli in the meconium at birth and within minutes after birth has been demonstrated by Hall and O'Toole in 1934[5] and 1935[6] and by Snyder in 1936[7]. Since it is possible to isolate Escherichia coli from the intestinal tract of man throughout life it becomes evident that this interesting organism is present with man from birth until death. Because E. coli has been isolated from the intestinal tract of new-born babies within a very short time after birth, it appears that not only babies, but all through life man must come in contact with various strains of E. coli. The major sources of such strains are:

1. The food we eat.
2. The water we drink.
3. The general surroundings in which the organism is deposited by people and animals.

1. The food we eat.

Jensen in 1945 [8], made the following statement:

> "Reduced to its fundamentals, the manufacture and subsequent handling of food products may be considered as a race between man and microbes to see which will be the first to consume such material."

In view of this and some brief work done by the author on meat samples fed to experimental animals it appears that throughout life man is constantly ingesting many strains of E. coli.

Tracy in 1934 [9] makes note of the presence of E. coli strains in olives. They have also been demonstrated in shell fish [9] and in certain kinds of canned foods [9]. Janes, 1935 [10], reports that dairy products are another source of E. coli. This data is tabulated in table 1.

2 and 3. The water we drink and the general surroundings in which the organisms are deposited.

In support of the hypothesis that man acquires his intestinal flora from the surroundings in which he lives and the water he drinks, Levin's work in 1889 [11] is of importance. He studied the intestinal flora of animals in the arctic region such as seals, white bears, penquins, eiderducks and reindeer whose environment is of such a nature that few bacteria live. He found that the intestinal contents of these animals were in most cases sterile. Levin thinks this paucity of micro-organisms is due to the low temperature. He ascribed these results to the relatively few micro-

TABLE 1

INCIDENCE and ISOLATION OF COLIFORM ORGANISMS
from PASTEURIZED DAIRY PRODUCTS
DELIVERED OVER A FIVE-YEAR PERIOD TO
V. A. HOSPITAL, VANCOUVER, WASHINGTON
for Patient Consumption

Year	Pasteurized Milk & Cream		Ice Cream	
	Number of Spec. Tested	% Coliform Positive	Number of Spec. Tested	% Coliform Positive
APRIL 1948	153	14.9%	53	37.7%
1949	361	4.9	129	42.6
1950	299	3.0	127	4.0
1951	228	1.3	53	2.0
1952	387	20.7	82	8.5
APRIL 1953	68	28.0	21	24.0
Totals	1496		465	

Reprinted from Janes[10].

organisms he was able to demonstrate in the air and water of this region.

From the foregoing discussion it appears that man is constantly being subjected to not a few, but to a great number of strains of E. coli coming to him from various sources.

The question now arises as to whether many different strains or only a few strains are present in the intestinal tract at any one time. It is possible to determine this by frequent sampling of the intestinal contents over a period of time. Sears, Brownlee and Uchiyama in 1950 (12), and Sears and Brownlee in 1952 (13), found that at any one time the strain composition of the fecal material of normal individuals consists of two types of E. coli with respect to their tenure. The first type consists of those strains which establish themselves and continue to multiply over extended periods of time and are designated as residents. The second type consists of those strains of E. coli which are found only in a single or several successive specimens and are designated as transients. They found that the tenure of the resident strains was not permanent and that they do disappear from the intestinal tract and are replaced by other strains which then take up tenure for a period of time. They do not believe, however, that one resident necessarily displaces another as there may be a period of time when the individual harbors no resident, but a number of transients.

We have made considerable use of the term "strains of E. coli" with no indication as to how these various strains are identified. It is well to consider this question now. Kauffmann, 1944 (14), established an antigenic schema which makes it possible to classify strains of E. coli according to their "O" or somatic antigens. Since it has been established by Kauffmann that strains do not change their "O" group, and since strains isolated from foods and the environment show a great diversity of "O" groups, we are justified in assuming that strains ingested by a single individual will show a like diversity of antigenic makeup. Thus we may assume that if cultures belonging to the same "O" group are isolated over a period of time from a single individual, this is an indication that these cultures represent the progeny of a single strain which has been multiplying in the intestinal tract, and not the progeny of strains continuously ingested. A change of "O" group would likewise be indicative of a new and different strain.

Sears, Brownlee and Uchiyama (12) have pointed out that the tenure of the resident strains is not permanent. Thus far there is no work reported in the literature that would indicate the reason for the sudden disappearance of the resident strains. However, several possibilities suggest themselves.

1. INCREASED CLEARING OF THE INTESTINAL TRACT DUE TO SPONTANEOUS DIARRHEIC ATTACKS.

Sears, Brownlee and Uchiyama [12] reported some observations supporting this hypothesis, yet some of their studies indicated that considerable disturbance of the normal intestinal function may take place without interfering with the resident E. coli strain or strains. Janes [10], working with two human subjects suffering from chronic irritable bowel, found that even with increased emptying of the intestinal tract, these individuals did not have a greater number of E. coli "O" groups than do persons with a normally functioning intestinal tract. In spite of frequent diarrheic attacks these individuals carried a resident and a few transient strains. The frequent clearing of the bowel did not displace the resident strains from either subject. The results of the study by Janes [10] throws considerable doubt on the hypothesis that diarrhea causes a loss of the resident E. coli strains from the human bowel.

2. DIETARY CHANGES

Little work has been found in the literature correlating the sudden disappearance of resident E. coli strains with changes in the diet. The basis for assuming that dietary changes may have some bearing on the E. coli strain composition of the bowel lies in the fact that the food we eat determines to some extent the pabulum in the intestinal tract upon which the bacteria grow and multiply.

Lembke in 1896 [15], found that in 81 human cases examined, E. coli remained present in spite of dietary changes. He did note that under a restricted diet there was a reduction in the relative number of organisms in the bowel.

Cushing and Livingood [16] considered all the various species of bacteria in the intestinal tract to be only temporary, since it was possible to completely remove all bacteria through a sterile dietary regime followed by a fast long enough to allow the bowel to empty itself.

Sears and Brownlee in 1952 [13] reported that changes in the diet seemed to have no effect upon the resident E. coli strain composition of the intestinal tract of humans.

3. BACTERIOPHAGE

There has been little work done on determining if there is a bacteriophage active against a resident strain of E. coli which has suddenly disappeared from the bowel. Sears, Brownlee and Uchiyama in 1950 [12] were unable in a single attempt to demonstrate such a phage.

Wallick and Stuart in 1943 [17] also failed to demonstrate a bacteriophage active against a resident strain suddenly disappearing from the bowel.

The possibility of lysogenic strains of E. coli playing a role in the tenure and disappearance of other strains of E. coli seems at the present time to be very intriguing, and one which is in need of investigation.

By lysogenic strains of E. coli we mean those strains which carry phage capable of lysing other strains of E. coli. Thus a resident strain of E. coli within an individual might be lysogenic and destroy almost all of the other strains of E. coli which would be deposited in the intestinal tract from the various sources previously mentioned. Such a lysogenic strain could remain in the bowel until another lysogenic strain possessing a phage specific for the resident strain would be deposited in the bowel and thus the resident would be displaced. Two residents could be present in the intestinal tract as they might possess phage capable of destroying the other strains of E. coli with which they come into contact, but not possess phages capable of lysing each other. This would account for the tenure and sudden disappearance of resident E. coli strains of the intestinal tract.

4. ANTAGONISM OF STRAINS OF E. COLI TOWARDS ONE ANOTHER.

It has long been recognized that antagonistic relationships between micro-organisms exist. De Bary in 1879 (18), was the first to emphasize the significance of the antagonistic relationship. Waksman in 1941 (19) presented the broad antagonistic relations between micro-organisms living in association, either in simple mixed cultures or in complex natural populations. He emphasized the fact that pathogenic bacteria would not survive long in water containing sapro-

phytic bacteria. The pathogens were found to survive approximately three times as long in sterilized tap water.

Waksman[19] emphasized the following various types of antagonisms.

1. Antagonism in vivo versus antagonism in vitro.
2. Repressive, bactericidal, and lytic forms of antagonism.
3. Direct, indirect and true antagonsim.
4. Hetero-antagonism between strains of different species.
5. Iso-antagonism between strains of the same species.

It is with reference to iso-antagonism between strains of the same species of E. coli that we are particularly interested. Sears, Brownlee and Uchiyama in 1950[12], following the studies of Gratia and Fredericq in 1947[20], investigated the possibility that antibiotic substances called colicins, which are produced by some strains of E. coli, might be the cause of the sudden loss of resident E. coli strains from the bowel. However, they were unable to find evidence in support of this hypothesis. They found no correlation between the antagonistic activity of E. coli strains and length of tenure in the bowel.

It is well to note that the following differences were recognized between the action of antagonists and that of phage.[19]

1. The filtrate of the antagonist may be active against other bacterial species.
2. Both living and dead bacteria of the antagonized species are dissolved by phage.
3. The action of the antagonist is not as specific as that of phage.
4. Races of E. coli resistant to phage are dissolved by the filtrate of the antagonist.

5. MASS OVERWHELMING OF THE RESIDENT E. COLI STRAIN BY ANOTHER STRAIN OF E. COLI.

It was thought that mass overwhelming of the resident flora might be responsible for the sudden disappearance of this flora. By mass overwhelming of the resident flora we mean that simply by weight of numbers one strain of E. coli might be able to displace another. If, for example, one has a definite number of one strain of E. coli in the bowel, these organisms may be assumed to be multiplying and dying at about an even rate so that the number of organisms in the bowel at any one time remains relatively constant. However, if massive numbers of another strain of E. coli are ingested their growth curve should be similar to that described above. That is, they also will multiply and be destroyed at a constant rate and thus be expected to maintain a constant number. Since their numbers would be much greater than the resident strain they would tend to displace the resident

strain. This they would do because of the competition for the limited material and area within the intestinal tract.

6. INGESTION OF MASSIVE DOSES OF NEW STRAINS OF E. COLI ACCOMPANIED BY A REDUCTION IN THE INTESTINAL FLORA.

This hypothesis is based on the assumption that if there is a marked reduction in the resident flora and a new strain of E. coli is introduced in sufficient number, this new strain will have decreased competition for survival in the intestinal tract and may thus become a resident. The strain which was the previous resident would then be displaced. However, it is possible that the new strain would not displace the resident, but that the two strains could exist together as residents. That two strains occasionally exist as residents of the intestinal tract of humans has been shown by Sears, Brownlee and Uchiyama in 1950[12].

7. CONDITIONS OF SANITATION

Since it has been shown that the main source of the E. coli of the intestinal tract of man are foods and general environment, it would appear that if conditions of sanitation were responsible for the tenure of resident strains in the bowel the composition of the E. coli flora would be extremely complex. An individual living under unsanitary conditions would greatly increase the sources from which he would obtain the E. coli he ingests and thus increase the number of various strains to which he would be exposed. However, if

the composition of the intestinal tract is due to the multiplication of the progeny of a strain deposited in the bowel and not to the progeny of constantly ingested strains the strain composition at any one time would appear to be relatively simple in that one or two residents with a few transients would be expected.

8. PER ANUM INVASION OF THE BOWEL AS A FACTOR INFLUENCING THE TENURE OF RESIDENT STRAINS OF E. COLI.

The possibility of strains of E. coli gaining access to the intestinal tract through the anus cannot be overlooked. Snyder[7] showed that out of thirteen cases of infants studied, four infants showed the same bacterial species in the meconium shortly after birth as was found in the region around the anus. This he interpreted as suggesting either the occurance of per anum invasion of the bowel or direct exterior contamination of the meconium specimen from the skin. The possibility of per anum invasion by the bacterial species should not be overlooked.

EXPERIMENTAL STUDIES

STATEMENT OF PROBLEM

The purpose of this study was to obtain evidence through the experimental approach as to whether certain of the possibilities previously mentioned are the cause or causes for the spontaneous change of the resident E. coli flora of the intestinal tract.

Relative to the previous hypotheses given, the ones to be investigated are:

1. Sanitary Living Conditions.
2. Mass Overwhelming of The Resident E. coli Flora by a Foreign Strain of E. coli.
3. Ingestion of Massive Doses of a New Strain of E. coli Accompanied by a Reduction in the Normal Intestinal Flora.
4. Per Anum Invasion of the Bowel by a Foreign Strain of E. coli.

METHODS

A. Experimental Animals:

Since we had at our disposal two well trained dogs, and since dogs would simulate the condition of human beings who would continuously be ingesting enormous numbers and diverse strains of E. coli, it seemed useful to use these two animals as the subjects for our

experimental work. The two mongrel dogs used had been the subject of studies conducted by the Department of Physiology of the University of Oregon Medical School. These studies in no way altered the normal physiology of the dogs and therefore had no effect on studies reported in this paper, other than to provide us with two mongrel dogs containing intestinal loops. These loops were isolated segments of the intestinal tract open to the outside of the body and containing the same blood and nerve supply as the remaining intact intestine. Dog Rex had a loop formed from the upper jejunem, which was started approximately 6 cm. distal to the ligament of Treitz. The jejunal segment was approximately 36 cm. long ($14\frac{1}{2}$"). Dog Duke had a loop similar to that of dog Rex, except that the jejunal segment was approximately 18" long. The loops are designated by the term "pouches" in the section on experimental work.

The two dogs were kept in two large adjacent cages in the animal room of the University of Oregon Medical School. The animals were fed a combination of horsemeat and dog bisquits once each morning. The cages were hosed out once a day and antiseptic solution used to clean the floors.

B. Obtaining and Preparing Cultures for Antigenic Classification.

Since it is assumed that the E. coli strain composition of rectal swabs would be the same as that of stool specimens, and since rectal swabs were easier to obtain, this method of obtaining specimens was used.

Into small 4" x 5/8" sterile tubes containing cotton swabs, 0.5 cc. of sterile saline was pipetted. The moist swabs were then inserted into the rectum of the experimental animals for about 2 to 3 inches, removed and reinserted into the original tubes containing sterile saline. A small amount of the saline was then poured onto an Eosin Methylene Blue Agar plate (Difco), the plate streaked and incubated at 37° C. for approximately 24 hours. Ten E. coli colonies were then picked at random from the plate. Each colony picked was inoculated to nutrient agar slants in 4" x 5/8" tubes for the production of stock cultures and incubated 24 hours. A loopful of material from each stock culture was inoculated into brain-heart infusion broth (Difco) for antigen production and incubated for 24 hours. The 24-hour broth cultures were then heated in the arnold sterilizer at 100° C. for one hour to destroy the "K" or capsular antigens of the bacillus which cause "O" inagglutinability. Three to four drops of 0.3% formalin solution was then added to each broth culture for the purpose of preserving the antigens.

C. Method of Taking Pouch Specimens.

Two methods were employed.

1. The first method was the same as that used in securing the rectal specimens except that the cotton swabs were inserted into the pouches of the two experimental animals. This method did not prove very satisfactory for obtaining specimens and was discontinued early in the experimental work.

2. Into a 4" x 5/8" sterile tube, containing a small capillary pipette with a rubber bulb attached to its upper end, 0.5 cc. of sterile saline was injected. A small amount of saline was drawn up into the pipette and then injected into and withdrawn from the pouch of the animal. The fluid was then reinjected into the original tube and cultures prepared as in the method for securing rectal specimens.

D. Antigenic Classification of the E. coli Strains.

Classification of the cultures was made on the basis of their "O" antigens. We are fortunate in having in our laboratory anti-sera for each of the 112 Kauffmann "O" groups. Stock solutions of anti-sera to a dilution of 1:160 preserved with merthiolate were kept in a refrigerator. To 0.5 cc. of the antiserum corresponding to the suspected antigen 0.5 cc. of the prepared antigen was added. Thus every antigen was

first tested by means of a single dilution of 1:320. If agglutination occurred in the 1:320 dilution serial dilutions to 1:2560 were performed. Agglutination was usually obtained in dilutions of 1:1280. When the serum titer with the unknown antigen was 1:1280 the culture was considered to belong to the specific "O" group represented by the known anti-sera used. It was by this method that we classified all of the 2453 cultures isolated in this study. This method of using E. coli sera for classifying unknown strains has been used in this laboratory for several years and shown to be entirely reliable.

For reasons mentioned in the introduction we depend upon the classification of cultures into "O" groups for differentiation into strains. A biochemical study of a number of our cultures was made to determine the possible corroborative value of such a study. The details of this study are found in appendix 1. We reached the conclusion from this work that biochemical differences between strains of E. coli are neither sufficiently great nor constant to be used for strain differentiation. This conclusion was also reached by Janes[10], Vahine[21] and Kauffmann[22].

E. Experimental Work.

1. Sanitary Living Conditions.

The purpose of this experiment was to determine whether it would be possible to displace the resident E. coli strain and establish a new resident simply through what to humans would be considered an unsanitary regime. Dogs would simulate this unsanitary regime in their normal daily life. It would be expected that if conditions of sanitation were the main factor in determining the tenure of resident E. coli strains, we would find a most complex E. coli flora in the dogs examined. Thus the problem here was simply to determine whether the E. coli flora of our experimental animals was a simple one with one or two residents and several transients, or whether the composition was extremely complex.

As is shown in Tabe 2, dog Rex was followed over a period of approximately 13 months, during which time nothing was done to the dog other than the taking of rectal specimens, and classifying antigenically the cultures obtained. Out of a total of 494 cultures, 36 were 01, 213 were 04, 204 were 083, 18 were other strains, and 23 were not identifiable. It should be noted that for a period of approximately two months at the beginning of the study, strains 01 and 04 were both present. Following this period, for approximately

TABLE 2

"O" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Rex over a period of 394 days.

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" groups				
			1	4	83	Others	Not Ident.
4-7-52	1	10	5	5			
4-14	7	10		10			
4-21	14	10	2			03,078,083,39	4
4-28	21	10	5			033,088	3
5-5	28	10	8	2			
5-12	35	10	7	3			
5-19	42	10				ten 021	
5-26	49	10	3	6			1
6-2	56	10	2			02,037	6
6-16	70	10		9			1
6-23	77	10		10			
6-30	84	10		10			
7-7	91	10		10			
7-14	98	10		7			3
7-21	105	10		10			
7-29	113	10		10			
8-4	119	10		10			
8-19	134	10		10			
9-12	141	10		10			
9-15	148	10		10			
9-17	150	10		9			1
9-19	152	10		10			
9-22	159	10		8			2
9-24	161	10		9			1
9-26	163	10		10			

TABLE 2 (cont.)

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" groups				
			1	4	83	Others	Not Ident.
9-29	166	10		10			
10-2	169	10	1	9			
10-6	173	10	3	7			
10-21	188	10		6	4		
11-4	202	10		1	9		
11-18	216	10			9		1
12-2	230	10			10		
12-16	244	10			10		
12-30	258	10			10		
1-13-53	271	10			10		
1-19	277	10			10		
2-6	295	10			10		
2-11	300	10			10		
2-24	313	10			10		
3-2	319	10			10		
3-6	323	10			10		
3-23	340	10		2	8		
3-25	342	10			10		
3-27	344	9			9		
4-6	356	7			7		
4-13	363	8			8		
4-20	370	10			10		
5-5	385	10			10		
5-12	392	10			10		
5-19	394	10			10		
Totals		494	36	213	204	18	23

6 months, strain 04 was the sole resident, after which strain 083 established itself as the resident strain. It is seen, therefore, that the E. coli strain composition of this dog was extremely simple, consisting of no more than two residents at any one time and for the most part containing but one resident.

Table 3 shows the results of following dog Duke for a period of 397 days. Out of a total of 410 cultures, 8 were 01, 14 were 04, 14 were other "O" groups and 373 were 083, and one was not identifiable. This dog maintained group 083 as its resident throughout this experiment.

Conditions of sanitation appear to have no effect upon the tenure of E. coli strains in the intestinal tract. In this experiment, it was found that the intestinal E. coli of the two experimental animals was similar to that of normal humans as shown by Sears, Brownlee and Uchiyama in 1950[12] in that there appeared to be but one resident in each dog, with several transients. The complex flora which would be expected if conditions of sanitation were influencing the tenure of resident strains was entirely absent. Since the life of the dog can be taken to simulate that of humans living under the most flagrant violations of san-

TABLE 3

"O" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Duke over a period of 397 days.

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" groups				
			1	4	83	Others	Not Ident.
4-7-52	1	10			9		1
4-14	7	10			8	Two K02	
4-21	14	9	1		5	Three K088	
4-28	21	10			10		
5-5	28	10			10		
5-12	35	10			10		
5-19	42	10			10		
5-26	49	10			10		
6-2	56	10			10		
6-16	70	10			4	Six K088	
6-23	77	10			10		
6-30	84	10			10		
7-14	98	10			10		
7-21	105	10			10		
7-29	113	10			10		
8-4	118	10			10		
8-11	125	10			10		
8-18	132	10			10		
8-25	139	10			10		
9-3	148	10			10		
9-8	153	10			10		
9-15	160	10			10		
9-23	166	10			10		
10-7	181	5			5		
10-21	195	10			10		
11-4	208	10		6	4		
11-18	222	10			9	K088	

TABLE 3 (cont.)

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" groups				
			1	4	83	Others	Not Ident.
12-2	236	1			1		
12-16	250	6			6		
12-30	264	10			10		
1-13-53	278	10			10		
2-6	302	10	1		9		
2-11	307	10		8		Two K021	
2-24	320	10			10		
3-2	326	10			10		
3-6	330	10			10		
3-16	340	10			10		
3-27	351	10	1		9		
4-6	361	10	5		5		
4-13	368	10			10		
4-20	375	9			9		
5-5	390	10			10		
5-12	397	10			10		
Totals		410	8	14	373	14	1

itary conditions it appears to be a safe assumption that conditions of sanitation have no effect upon the tenure of resident E. coli strains in the bowel of the human subject.

2. Mass Overwhelming of the Resident E. coli Flora by Another Strain of E. coli.

This experiment was carried out in two parts.

a. The contents of agar slants of E. coli 028 taken from 6" by 3/4" tubes were suspended in milk and fed over a period of several days to dogs Duke and Rex in their regular drinking pans. Cultures which had been incubated for 24 hours were used for each feeding. As is shown in Tables 4 and 5, these feedings had no apparent effect upon the tenure of the resident E. coli strains. Table 4 shows that dog Rex was fed a total of 13 agar slants of E. coli 028 over a period of 43 days. Out of 106 cultures isolated, all were 083, the original resident. The 028 strain was isolated from a human subject.

From Table 5 it is seen that a total of 14 agar slants of 028 were fed to dog Duke over a period of 57 days. Out of 125 rectal cultures isolated during this time, 122 were 083, the original resident and 3 were unident-

TABLE 4

"0" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Rex over a period of 43 days, during which time the dog received massive feedings of E. coli 028 suspended in milk.
)83 represents the resident strain.

Date Spec. Taken	Day of Spec.	Date of feeding on agar slant 028	Number of Cultures Taken	Number of Cultures in specified "0" groups	
				028	083
		5-25-53			
5-26	1		10		10
		5-26			
5-27	2		10		10
		5-27			
		5-28			
5-29	4		10		10
		5-29			
6-4	10		10		10
		6-4			
		6-8			
6-9	15		7		7
		6-12			
		6-13			
		6-15			
		6-16			
6-17	23		9		9
		6-17			
6-18	24		10		10
		6-19			
6-20	26		10		10
6-25	31		10		10
6-30	36		10		10
7-7	43		10		10
Totals			106		106

TABLE 5

"O" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Duke over a period of 57 days, during which time the dog received massive feedings of E. coli 028 suspended in milk.
083 represents the resident strain.

Date Spec. Taken	Day of Spec.	Date of feeding one agar slant 028	Number of Cultures Taken	Number of Cultures in specified "O" groups		
				028	083	Not. Ident.
		5-18-53				
5-19	1		10		10	
		5-19				
5-20	2		10		10	
5-21	3		10		10	
		5-21				
5-22	4		10		10	
		5-22				
		5-23				
		5-25				
5-26	8		2		2	
6-4	17		10		10	
		6-4				
		6-8				
		6-9				
6-10	23		8		6	2
		6-12				
		6-13				
		6-15				
		6-16				
6-17	30		8		7	1
6-19	32		7		7	
		6-19				
6-20	33		10		10	
6-25	38		10		10	
6-30	43		10		10	
7-7	50		10		10	
7-14	57		10		10	
Totals			125		122	3

ifiable. The ingested strain was not recognized at any time in the feces of the animal in spite of the enormous doses given.

After the conclusion of these two attempts to alter the resident E. coli strain of the intestinal tracts of the experimental animals we decided to change our method of feeding the foreign strain of E. coli to the two dogs. This we did because we realized that the E. coli might not be reaching the intestinal tract where they could multiply, but were perhaps being killed as they passed through the gastric system. The second method is as follows:

b. Dogs Duke and Rex were fed the contents of several agar slants of E. coli 02 placed in enteric treated capsules. These capsules were gelatin and enteric treated with 10% formalin solution and allowed to stand two weeks before use, after the method outlined in Remington's Practice of Pharmacology[23]. The capsules, with their contents, were fed to the dogs in small balls of horsemeat which were rolled into the cages of the dogs. These balls containing the capsules of E. coli were readily swallowed by the dogs without being

chewed. Thus, we felt reasonably certain that the E. coli were getting into the intestinal tract where they could multiply, and were not being destroyed while passing through the stomach.

From Table 6 it can be seen that dog Rex was fed a total of 9 enteric treated capsules of E. coli 02. Over a period of 50 days 129 cultures were isolated, 5 of which were 02, the other 124 being the original resident 083. The fact that 5 cultures of the fed strain were recovered in the feces is indicative that the bacteria were getting in to the intestinal tract where they could multiply. However, the fact that 5 cultures of the fed group were isolated out of a total of 129 cultures appears to indicate that massive feedings of the resident strain by itself is not sufficient to displace the resident E. coli strain.

Table 7 shows that dog Duke received 10 feedings of E. coli 02. Out of 127 cultures isolated over a period of 41 days, all 127 cultures were 083, the resident, and none were 02, the fed group.

It appears from the results of this ex-

TABLE 6

"O" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Rex over a period of 50 days while receiving enteric treated capsules containing E. coli 02.

083 represents the resident strain.

Date Spec. Taken	Day of Spec.	Date of feeding one Agar slant 02	Number of Cultures Taken	Number of Cultures in specified "O" groups.	
				02	083
7-14	1		10		10
		7-22			
7-23	10		10		10
		7-23			
7-27	14		10		10
		7-27			
7-28	15		10		10
		7-28			
		7-29			
7-30	17		10		10
		7-30			
		8-1			
8-3	21		9	5	4
		8-4			
		8-5			
8-6	24		10		10
8-15	33		10		10
8-20	38		10		10
8-25	43		10		10
8-28	46		10		10
8-31	49		10		10
9-1	50		10		10
Totals			129	5	124

TABLE 7

"O" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Duke over a period of 41 days while receiving enteric treated capsules of E. coli 02.

083 represents the resident strain

Date Spec. Taken	Day of Spec.	Date of feeding one agar slant 02	Number of Cultures Taken	Number of Cultures in specified "O" groups	
				02	083
		7-22-53			
7-23	1		10		10
		7-23			
7-27	5		10		10
		7-27			
7-28	6		10		10
		7-28			
		7-29			
		7-30			
		8-1			
8-3	12		9		9
		8-4			
		8-5			
		8-6			
8-7	16		10		10
8-10	19		10		10
8-13	22		10		10
8-15	24		10		10
8-20	29		9		9
8-25	34		10		10
8-28	37		10		10
8-31	40		9		9
9-1	41		10		10
Totals			127		127

periment that massive doses of a foreign strain of E. coli have no effect on the tenure of the resident E. coli strain. The resident strain was in no instance displaced, nor was the fed strain established as a resident.

3. Ingestion of Massive Doses of a Foreign Strain Accompanied by a Reduction in the Intestinal Flora of the Experimental Animal.

This problem was approached in two ways:

a. Massive doses of a foreign strain (02) were administered orally, after the normal flora had been markedly reduced by the administration of sulfa-guanidine.

b. Massive doses of a foreign strain (02) were administered orally, after the normal flora had been markedly reduced by the flushing out of the intestinal tract by means of an enema.

a. From Table 8 it can be seen that dog Rex was given seven feedings of sulfa-guanidine over a period of nine days. For the first two days this animal was given one gram in two equal doses per day and for the last five feedings was given ½ gram in two equal doses per day. As a

TABLE 8

"O" group distribution of Escherichia coli cultures isolated from the intestinal tract of dog Rex over a period of 88 days. The animal was fed enteric treated capsules of E. coli 02 after the E. coli contents of the bowel were first reduced by administration of sulfa-guanidine.

083 represents the resident strain of the bowel.

Date Spec. Taken.	Day of Spec.	Date Sulfa-guanidine given.	Number of Cultures Taken.	Number of Cultures in specified "O" group. 083	Not Ident.
		9-2-53, 1gm.			
		9-3 1gm.			
		9-4 ½gm.			
		9-7 ½gm.			
		9-9 ½gm.			
		9-10 ½gm.			
		9-11 ½gm.			
9-9-53	1		10	10	
9-10	2		10	10	
9-11	3		10	10	
9-12*					
9-14	6		10	10	
9-14*					
9-15	7		10	10	
9-15*					
9-16	9		10	1	9
9-16**					
9-17	10		10		10 cent.Agg.
9-17**					
9-18	11		10	10	
9-24	17		10	1	9
10-1	24		10	2	8
10-6	29		10	10	
10-8	31		10	10	
10-12	35		10	10	
10-15	38		10		10 Cent.Agg.
10-16	39		10	6	4 Cent.Agg.
10-19	42		10	7	3 Cent.Agg.
10-20	43		10		10 Cent.Agg.
10-28	51		10	10	
11-4	58		9	9	
11-8	62		10	10	
11-18	72		10	10	
11-30	84		10	10	
12-1	85		10	10	
12-4	88		10	10	
Totals			239	176	63

* on these dates one capsule of 02 was fed.

** on these dates two capsules of 02 were fed.

result of these feedings the number of E. coli cultures isolated from the intestinal tract was greatly reduced. Before administering the sulfa-guanidine the colonies grown on the E. M. B. plates were so numerous as to be impossible to count. Since care was taken to use approximately the same amount of material for streaking the plates each time, and since after the administration of the sulfa-guanidine the rectal specimens yielded colonies which were greatly reduced in number and very easy to count, we assumed that the intestinal E. coli were correspondingly reduced.

Upon the detection of the reduced number of colonies dog Rex was started on oral feedings of enteric treated capsules of E. coli 02. The resident strain of this dog was still, at this time, 083. Dog Rex was given seven feedings from whole agar slants in 6" x 3/4" tubes. From Table 8 it is seen that out of 239 cultures isolated over a period of 88 days, 176 were the resident 083, 26 were neither 02 nor 083 and proved to be un-

identifiable, and 37 were rough colonies which agglutinated without serum. The fed strain, 02, was not detected in any of the specimens.

b. In the second approach to the problem, dog Rex was given an enema consisting of one quart of warm water with one tablespoonful of soda. The dog had defecated approximately two hours earlier. The enema tube was inserted approximately one-and-one-half feet into the bowel and a considerable amount of water and feces was quickly discharged by the animal. Immediately afterwards, one enteric treated capsule of E. coli 02 was fed to the animal in a meat ball. He received another capsule on each of the two following days.

Table 9 shows that out of 87 cultures isolated from this animal during the 49 days of the experiment, 12 were 02, the fed strain, and 75 were 083, the resident strain. No specimen taken during the experiment failed to yield the resident 083 and 2 days after the last feeding of 02 the 083 group was the only

TABLE 9

"O" group distribution of the Escherichia coli cultures isolated from the intestinal tract of dog Rex over a period of 49 days, after the administration of an enema consisting of one quart of water and one tablespoonful of Soda on 12-4-53.

083 represents the resident strain.

Date Spec. Taken	Day of Spec.	Date of feeding one agar SLANT 02	Number of Cultures Taken	Number of Cultures in specified "O" group	
				02	083
		12-4-53			
12-5	1		7	5	2
		12-5			
12-7	3		10	3	7
		12-7			
12-8	4		10	4	6
12-9	5		10		10
12-11	7		10		10
12-16	12		10		10
1-8-54	35		10		10
1-15	42		10		10
1-22	49		10		10
Totals			87	12	75

group which was isolated and continued to be the only group isolated for the remainder of the experiment.

An enema appears to be no more successful than sulfa-guanidine in preparing the bowel for the multiplication of a new strain of Escherichia coli.

4. Per Anum Invasion of the Intestinal Tract by a Foreign Strain of E. coli.

Six loops of E. coli 02 were suspended in 1.0 cc of sterile saline and injected approximately five inches into the rectum of dog Duke by means of a 1 cc. syringe with a long, rounded needle. From Table 10 it is seen that the 02 strain appeared as a transient on two successive days only. The resident strain, 083, was never completely displaced, and continued as the resident strain during the entire experiment.

TABLE 10

"O" group distribution of the Escherichia coli cultures isolated from the intestinal tract of dog Duke over a period of 24 days after the injection of a saline suspension of the contents of six loops of E. coli 02 directly into the rectum of this animal.

083 represents the resident strain.

Date Spec. Taken	Day of Spec.	Date of Injection of 02	Number of Cultures Taken	Number of Cultures in specified "O" group	
				02	083
11-8-53	1	11-8	10		10
11-9	2		10	7	3
11-10	3		10	2	8
11-11	4		10		10
11-18	11		10		10
11-30	23		10		10
12-1	24		10		10
Totals			70	9	61

STUDIES ON POUCHES OF EXPERIMENTAL ANIMALS

Work was done in which Escherichia coli, other than the resident strain, were injected directly into the pouch of dog Duke. The pouches of both dogs, Duke and Rex, as mentioned previously, were isolated segments of the proximal end of the jejunem and were open to the outside of the body. They were similar to the rest of the intestinal tract in that they had the same blood and nerve supply. Being isolated segments they did not receive the material ingested as food.

Table 11 shows the strain composition of E. coli for the pouch of dog Duke taken over a period of 558 days. Out of 410 cultures isolated 120 were 04, 282 were 01, 5 were 051, and 3 were not identified as to their "O" group. It is interesting to note that the resident strains here are not the same as the resident in the intact small intestine. The loop E. coli contents were similar to the intact intestine, however in that there was only one resident strain at any time and several transients.

Table 12 shows the results of injecting directly into the pouch of dog Duke a saline suspension of the contents of one whole agar slant of E. coli 02. Out of 22 cultures isolated, 17 were the resident 01 strain, and 5 were the injected 02 strain. The resident strain was at no time completely displaced.

The E. coli strain composition of the pouch of dog Rex

TABLE 11

"O" group distribution of Escherichia coli cultures isolated from the pouch of dog Duke over a period of 558 days.

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" group			
			04	01	Others	Not Ident.
5-26-52	1	10		10		
6-2	8	10		10		
6-16	22	10		10		
6-23	29	10		10		
6-30	36	10		10		
7-7	43	10		10		
7-14	50	10		10		
7-21	57	10		10		
7-29	65	10		10		
8-18	85	10		10		
8-25	92	10		10		
9-3	101	7		7		
9-8	106	6		1	five 51	
9-15	113	10	10			
9-23	121	10	10			
10-7	135	10	10			
10-21	149	10	10			
11-4	163	10	10			
11-18	177	10		10		
1-19	239	10	10			
2-6	257	10	10			
2-11	262	10	10			
2-24	275	10	10			
3-2	281	10	10			
3-6	285	10	10			
3-16	295	10	10			
3-27	306	10		10		
4-6	316	10		10		
4-13	323	10		10		
4-20	330	6		6		
5-5	345	10		10		
5-12	352	10		10		
5-20	360	10		10		
5-21	361	10		10		
5-22	362	10		10		
5-26	366	8		5		3
6-4	375	10		10		
6-10	381	10		10		
6-30	401	10		10		
7-7	408	10		10		
7-14	415	10		10		

TABLE 11 (cont.)

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" group			
			04	01	Others	Not Ident.
9-11	474	1		1		
11-4	528	1		1		
11-18	542	3		3		
11-30	554	1		1		
12-1	555	4		4		
12-4	558	3		3		
Totals		410	120	282	5	3

TABLE 12

"O" group distribution of the Escherichia coli cultures isolated from the pouch of dog Duke over a period of 6 days, during which time saline suspensions of E. coli 02 were injected directly into the pouch.

O1 represents the resident strain.

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of cultures in specified "O" group	
			O1	O2
12-4-53*				
12-5	1	5	2	3
12-5*				
12-6	2	4	4	
12-7*				
12-8	4	5	3	2
12-9	5	4	4	
12-11	6	4	4	
Totals		22	17	5

* On these days saline suspension of the contents of whole agar slants of E. coli 02 were injected into the pouch.

is shown in Table 13. Over a period of approximately 309 days, 234 cultures were isolated. Of these, 219 were 04, one was 083, and 14 were not identifiable as to their "O" group. The resident strain here was also different from that of the intact intestine, but the E. coli contents of this pouch were similar to the intact bowel in that one resident with several transients were present.

SUMMARY OF DOG REX

Several interesting facts were brought to light in the study of the 1055 cultures isolated from the rectum of dog Rex. As seen in table 14, by far the greatest number of cultures isolated belonged to group 083, with strain 04 having the next greatest numbers. During the first two months of the study strains 01 and 04 were the dominant strains in the bowel. Following this period, and lasting for a time of five months, strain 04 became the sole resident of the intestinal tract of this dog. At the end of this five-month period, strain 04 yielded to strain 083, which then became the resident and was maintained as resident until the termination of the study, some 14 months later.

Approximately five-and-one-half months after strain 083 became the resident, the dog was given oral feedings of milk suspensions of E. coli 028. This in no way altered the resident 083 strain. This was also the case in the following two month period, during which time the dog was fed enteric

TABLE 13

"O" group distribution of Escherichia coli cultures isolated from the pouch of dog Rex over a period of 309 days.

Date Spec. Taken	Day of Spec.	Number of Cultures Taken	Number of Cultures in specified "O" group.		
			04	083	Not Ident.
6-2-52	1	2	2		
6-23	22	10	10		
6-30	29	10	10		
7-14	43	10	10		
7-21	50	10	10		
9-15	106	10	10		
9-17	108	10	10		
9-19	110	10	10		
9-22	113	10	8		2
9-24	115	10	9		1
9-26	117	10	9		1
9-29	120	10	8		2
10-2	123	10	9		1
10-6	127	10	9		1
11-4	156	1	1		
11-18	170	10	10		
1-13-53	226	10	10		
1-19	232	10	10		
2-6	250	1	1		
2-11	255	10	10		
2-24	278	10	10		
3-2	284	10	10		
3-6	288	10	10		
3-23	305	10	10		
3-25	307	10	10		
3-27	309	10	3	1	6
Totals		234	219	1	14

TABLE 14

Summary of the Escherichia coli "O" group distribution of cultures isolated from the intestinal tract of dog Rex from 4-7-52 to 5-19-53.

Period of Study	Number of cultures Isolated	Number of cultures in specified "O" group					
		083	04	01	02	Others	Not Ident.
4-7-52 to 5-19-53	494	204	213	36		18	23
5-26-53 to 7-7-53 Dog was fed milk suspensions of E. coli 028	106	106					
7-14-53 to 9-1-53 Dog was fed enteric treated capsules of 02	129	124			5		
9-9-53 to 12-4-53 Dog was fed enteric treated capsules of E. coli 02, after reducing E. coli flora with sulfa-guanidine.	239	176					63
12-5-53 to 1-22-54 Dog was fed enteric treated capsules of E. coli 02, after giving dog an enema	87	75			12		
Totals	1055	695	213	36	17	18	86

treated capsules of E. coli 02. For a three-month period following the administration of the first enteric treated capsules the dog was fed more enteric treated capsules after first reducing the E. coli contents of his bowel through the use of sulfa-guanidine. In spite of the reduction in numbers of the resident E. coli strain, and the massive feedings of the foreign, 02, strain, the resident strain of the bowel continued to be strain 083. Reducing the E. coli contents through the administration of an enema followed by massive feedings of enteric treated capsules of E. coli 02, likewise failed to displace the resident 083 strain during the following, and last two months, of the study.

During the entire period of study of approximately 20 months, from 4-7-52 to 1-22-54, 1055 cultures were isolated. Of these, 695 were 083, 213 were 04, 86 were not identifiable, 36 were 01, 18 were other strains, and 17 were 02.

SUMMARY OF DOG DUKE

As seen from Table 15, during the course of the study on dog Duke, a period of approximately 19 months from 4-7-52 to 12-1-53, 732 cultures were isolated from the intestinal tract of this dog. It is interesting to note that E. coli 083 was the resident strain at the beginning of the study and was maintained as resident throughout the entire period of this work. After following the strain composition of the bowel of this dog for a period of 13 months, during which

TABLE 15

Summary of the Escherichia coli "O" group distribution of cultures isolated from the intestinal tract of dog Duke from 4-7-52 to 12-1-53.

Period of Study	Number of Cultures Isolated	Number of cultures in specified "O" group.					
		083	04	01	02	Others	Not Ident.
4-7-52 to 5-12-53	410	373	14	8		14	1
5-19-53 to 7-14-53 Dog fed milk suspensions of E. coli 02.	125	122					3
7-23-53 to 9-1-53 Dog was fed enteric treated capsules of E. coli 02	127	127					
11-8-53 to 12-1-53 Injected directly into the rectum of this dog-saline suspension of E. coli 02.	70	61			9		
Totals	732	683	14	8	9	14	4

time E. coli 083 was definitely determined to be the resident strain, the dog was given oral feedings of milk suspensions of E. coli 028. This lasted for a period of two months and had no effect on altering the resident strain of the bowel. During the succeeding two months the dog was fed enteric treated capsules of E. coli 02. This also did not affect the tenure of the resident E. coli strain. For another two-month period following this, no work was done on this dog. During the last month of the study the dog's intestinal E. coli contents were observed after first injecting saline suspensions of E. coli 02 into the rectum of this dog. This proved no more successful than the other methods employed to bring about an alteration in the resident E. coli strain.

During this entire study, covering a period of approximately 19 months, from 4-7-52 to 12-1-53, 732 cultures were isolated. Of these 683 were 083, 14 were other strains, 8 were 01, nine were 02, and four were not identifiable.

Remarks:

At the present time there are approximately 125 different "O" groups known. We are fortunate in having in our laboratory 112 of these "O" groups. It is possible that the 53 colonies which were not able identifiable belong to one of the remaining 13 "O" groups. The 37 cultures which were agglutinated without serum were most likely rough variants.

DISCUSSION AND CONCLUSIONS

By following, for a period of several months, the E. coli strain composition of two experimental dogs, and comparing the results with those obtained from the study of human subjects, it was determined that the resident E. coli strain composition of dogs is like that of humans. In dogs, as in humans, there is but one resident (occasionally two may be present), with several transient strains. This similarity between human subjects living under normal conditions of sanitation, and dogs living a normal existence, is just the opposite of what one would expect if conditions of sanitation played an important role in determining the tenure of resident E. coli strains of the bowel. As pointed out previously in this study, man comes into contact with various strains of E. coli through the food he eats, the water he drinks and through his general environment. It is logical to assume, then, that a dog, whose conditions of sanitation are low by human comparison, would be exposed to more sources of contact with E. coli than would the normal human. It would also seem that if conditions of sanitation were the main factors influencing the tenure of resident strains of E. coli, the number of various strains within the bowel would increase in direct proportion to the number of sources of contact. Since the dogs' sources of contact with E. coli are more numerous than that of man, his strain composition at any

one time would be expected to be more complex than man. However, since this was not the case, and the strain composition of the intestinal tracts of the two experimental dogs were quite simple, it appears that conditions of sanitation have no great effect on the tenure of resident E. coli strains.

It was thought that ingestion of massive doses of a foreign strain of E. coli might be sufficient to establish the foreign strain as resident and to displace the original resident. Since there is definite competition in the bowel for the limited sub-strate and space available, it was thought that the strain of E. coli present in the largest numbers would be the one to survive. However, since massive feedings of a foreign strain failed to overwhelm the resident strain, which was present in smaller numbers, it appears that massive numbers of a strain are not the important factor in determining the resident strain of the bowel. This also appears to be the case whether the E. coli contents of the bowel are first reduced by oral administration of sulfaguanidine or through the use of an enema. In no instance was the resident strain completely displaced, and at the conclusion of the studies the resident strain was present in numbers comparable to those present at the onset of the experimental proceedure.

From this study it appears that per anum invasion of the intestinal tract by a foreign strain is of no great sig-

nificance in determining the E. coli strain composition of the bowel. Per anum invasion was simulated in this study by per anum injection of a foreign strain of E. coli into one of the experimental dogs. This proceedure did not establish the foreign strain as resident nor displace the resident strain.

From the experimental proceedures carried out in this study, it would seem that a factor, or factors, other than those investigated in this work must be responsible for the sudden loss of a resident strain of E. coli from the intestinal tract.

SUMMARY

A study was made on two mongrel dogs in an attempt to determine the factor or factors responsible for the sudden disappearance of resident strains of Escherichia coli from the bowel.

1. Conditions of sanitation as a factor influencing the tenure of resident E. coli strains in the bowel were investigated and found to play no important part in the tenure of resident strains.
2. Ingestion of massive doses of strains of E. coli other than that acting as resident have no marked effect on the tenure of the resident E. coli strain.
3. The administration of massive doses of a foreign strain of E. coli at a time when the resident strain was in greatly reduced numbers, did not displace the resident strain from the intestinal tract, nor establish the foreign strain as resident.
4. A foreign strain was injected directly into the rectum of dog Duke without causing the displacement of the resident strain, nor establishing itself as resident.
5. Studies were conducted on the pouches of the two experimental animals. It was not possible to

alter the resident pouch strain of dog Duke by direct injection of a foreign strain of E. coli. The pouch strains were not always the same as the residents of the intact intestinal tract, although the pattern of one resident with several transients was present.

6. Biochemical studies show that biochemical differences between strains of E. coli were neither sufficiently great nor constant to be used for strain differentiation.

APPENDIX I

Biochemical Studies

From cultures isolated over a six-month period from the intestinal tracts and pouches of the two experimental dogs, 48 were examined for ability to ferment the following carbohydrates: salicin, dulcitol, sucrose and inositol. The purpose of this study was to determine whether it would be possible to classify strains of Escherichia coli through their biochemical characteristics. To insure uniformity of results, each of the carbohydrate media mentioned above was prepared by the author. This was done as follows: Into 25 cc. of distilled water 1.25 grams of the carbohydrate was added and the solution sterilized at 15 pounds pressure for six minutes in the autoclave. Each carbohydrate was then added aseptically to a final concentration of 0.5% to sterile meat extract containing Andrades indicator. The carbohydrate media were tubed under aseptic conditions and used for fermentation tests. Results of the fermentation tests were read after 24 hours and are shown in Table 16. The Indol, Methyl Red, Vogus Proskauer and Citrate tests were also conducted on these 48 cultures. These results are recorded in Table 16.

From Table 16 it can be seen that the biochemical differences between strains of E. coli are neither sufficiently great nor constant to be used as a basis for strain differ-

entiation.

Table 16 shows that all of the 48 cultures studied here fall into the general IMViC pattern usually obtained for species of the genus Escherichia.

TABLE 16

Results of biochemical studies conducted on 48 cultures isolated over a period of six months from the intestinal tracts and pouches of dogs Duke and Rex.

A equals acid. G equals gas.

Date Spec. Taken	Dog and Site of Isolation	"O" group of Culture	Carbohydrates				I	M	V	C
			Sal.	Dul.	Suc.	Inos.				
1-13-53	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Rex - rectum	83	AG	AG	A	-	+	+	-	-
	Rex - pouch	4	AG	AG	AG	-	+	+	-	-
1-19	Duke - pouch	4	AG	AG	A	-	+	+	-	-
	Rex - pouch	4	AG	AG	A	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
3-2	Duke - pouch	4	AG	AG	A	-	+	+	-	-
	Rex - rectum	83	AG	AG	A	-	+	+	-	-
3-16	Duke - pouch	4	AG	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	AG	A	-	+	+	-	-
3-27	Rex - pouch	4	-	AG	AG	-	+	+	-	-
	Rex - pouch	Not ident.	AG	AG	A	-	+	+	-	-
	Rex - pouch	Not ident.	A	AG	A	-	+	+	-	-
	Rex - rectum	4	A	AG	A	-	+	+	-	-
	Rex - pouch	83	AG	AG	A	-	+	+	-	-
	Duke - pouch	1	A	AG	A	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Duke - rectum	1	A	AG	-	-	+	+	-	-
4-6	Rex - rectum	83	A	AG	AG	-	+	+	-	-
	Duke - rectum	1	A	AG	AG	-	+	+	-	-
	Duke - rectum	83	-	AG	A	-	+	+	-	-
	Duke - pouch	1	A	AG	-	-	+	+	-	-
	Rex - pouch	83	AG	AG	AG	-	+	+	-	-
4-13	Rex - rectum	Not. ident.	A	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Duke - pouch	1	A	AG	AG	-	+	+	-	-

TABLE 16 (cont.)

Date Spec. Taken	Dog and Site of Isolation	"O" group of Culture	Carbohydrates				I	M	V	C
			Sal.	Dul.	Suc.	Inos.				
4-20	Rex - rectum	83	A	AG	AG	-	+	+	-	-
	Duke - pouch	1	A	AG	A	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
5-5	Rex - rectum	83	AG	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Duke - pouch	1	A	AG	A	-	+	+	-	-
5-12	Rex - rectum	83	A	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	A	AG	-	+	+	-	-
	Duke - pouch	1	A	AG	A	-	+	+	-	-
5-19	Rex - rectum	83	A	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
5-26	Rex - pouch	Rough	A	AG	AG	-	+	+	-	-
	Duke - pouch	1	A	AG	A	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
6-10	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Rex - rectum	83	A	AG	AG	-	+	+	-	-
6-17	Duke - pouch	Not ident.	-	AG	AG	-	+	+	-	-
	Duke - rectum	83	AG	AG	AG	-	+	+	-	-
	Rex - rectum	83	A	AG	AG	-	+	+	-	-
6-19	Duke - rectum	83	A	AG	AG	-	+	+	-	-
	Rex - rectum	83	A	-	-	-	+	+	-	-

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