A STUDY OF THE BACTERIAL FLORA OF ISOLATED INTESTINAL SEGMENTS

by

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The studies reported in this paper were made upon animals (dogs) operated upon and used in a much larger study of isolated intestinal segments being carried on in the Department of Physiology of this institution. We are much indebted to Drs. G. E. Burget and Karl Martzloff for permission to make the study and for their kindness in taking the many samples used.

All the dogs used were in normal health and had had no previous surgical work done on them. Food was withheld from them on the day before and the day of operation, but water was permitted at any time. Thirty minutes before the operation the animal was given hypodermically ½ grain of morphine sulphate and 1/100 grain atropine sulphate.

In order to obtain isolated segments for study the technic of Whipple was used. A section of the small intestine was taken out and the ends inverted and closed. This segment, averaging fifteen centimeters in length, was sutured to the peritoneum in the midline of the anterior abdominal wall. A stitch was taken through the skin above the position of the segment to mark the spot for future punctures. The continuity of the intestine was reestablished by bringing the two free ends together in an end to end anastomosis. Thus there was no obstruction to the flow of intestinal content. The blood and nerve supply to the segment were left intact, thus making available a closed section of the small intestine separated from the rest, brought near enough to the surface so that it could be reached easily by a needle puncture.

The sections used for isolated segments were taken from various levels of the small intestine. Two duodenal, eighteen jejunal and twenty ileal loops were studied.

Among the experimenters who have used isolated intestinal segments Dragstedt and his co-workers have studied the bacterial flora to the greatest extent. Dragstedt, Cannon, and Dragstedt (1922) report isolated intestinal segments made with a technic similar to ours. Their method differed in that they replaced the segment in the abdominal cavity instead of suturing it to the anterior abdominal wall. On smears taken from the jejunal content at operation they found a preponderance of Gram positive organisms. The fecal flora was strongly aciduric. In every case at death the bacteria in the closed loops were almost entirely Gram negative. In a series of isolated loops washed with ether and sterile water there appeared in four or five days an accumulation of Gram negative organisms. Neither toxemia nor distention occurred in loops which were washed with tannic acid before closure. The dogs in this series survived indefinitely. After several months there were large numbers of Gram negative organisms and the Gram positive organisms had disappeared. The authors believed the disappearance of the Gram positive aciduric organisms was due to the absence of utilizable carbohydrate and to alkaline reaction of the medium.

Meleney, Jobling, and Berg (1927) experimented with chronic duodenal obstruction in dogs. Their obstruction was maintained by bands of transversalis muscle and fascia wrapped loosely around the intestine about 15 centimeters from the pylorus. They found that after partial obstruction the number of organisms increased enormously, varying with the degree of obstruction and the extent of dilatation. The bacteria they identified were 70% B. welchii, 50% varieties of non-hemolytic streptococci, and 40% B. coli. The greatest increase occurred in B. coli, but no striking differences were noted. This increase was maintained for a considerable period but in some cases there was a decrease in numbers even though the obstruction persisted. They also found that the flora of any one animal was not constant, but first one type predominated, then another. They found fewer organisms below than above the obstruction.

Our animals were allowed water the third day after operation, but food was withheld until the fifth day. If the operation was successful the dog recovered quickly, acted normally, ate well, and had no rise of temperature. Pressure in the segment was kept down as well as possible. When an animal showed loss of appetite, rise in temperature, weakness and vomiting, pressure in the loop was indicated and a puncture was made to remove fluid.

Peritonitis was the most frequent cause of premature death. If the anastomosis gave way and bacteria gained access to the peritoneal cavity peritonitis followed. Some of the segments leaked due to the loosening of the sutures at the ends, while in some cases pressure in the segments caused rupture and peritonitis. It can easily be seen that many factors were involved in the post-operative condition of the dogs.

In table I the length of life of each dog is listed. The dogs which died within three days after operation never fully recovered from the procedure. In No. 46 peritonitis was caused by the leakage of the anastomosis. No. 69 probably had his abdomen contaminated at the operation for a loop of ileum herniated beneath the skin. In No. 82 the segment leaked at the upper end, and in 85 the rupture was due to insufficient blood supply from high distention. The above cases give typical illustrations of the causes of death as determined by autopsy.

Several of the dogs were killed with chloroform or ether after varying periods of time. Dogs 15 and 33 seemed to be in perfect health but were worthless for further experimentation. In these cases the segments were filled with a putty-like material which was impossible to remove by needle puncture. Dog 32 is still being used for experimentation 411 days after her operation. She seems to be in good condition.

In obtaining samples of loop content for our study all precautions of aseptic technic were observed. The dogs were stretched out on their backs. The hind legs were tied down to the table and the front legs were held by an assistant. The abdomen was then cleaned with alcohol. A sterile needle was inserted over the marked position of the loop, and the fluid withdrawn into a sterile syringe. For the bacteriological study some of the fluid was put immediately in a sterile test tube and placed in the ice box until examination.

For the first few days after operation the fluid was a very dark red which gradually changed to a light buff. The fluid was thin and watery or thick and pasty probably depending on the amount of secretion and filtration into the loop. The very thick contents were made up largely of debris from the intestinal mucosa rather than bacterial bodies.

If we had attempted to isolate and identify every organism present in the segments, the problem would have become much larger than we could manage in the time available. We felt that the results obtained would not warrant a long and complicated procedure. For our purpose the following routine examination seemed sufficient.

1. A direct smear of the material was made on a slide and stained by Gram's method. The Gram stain was used to give an indication of the types of organisms present and to show the relative numbers present in any one series of specimens. In certain cases organisms were seen in the stains which could never to recovered from the cultures. For instance, dog 57 had a spirillum present in large numbers, but which we failed to isolate.

2. A loopful of the material was streaked on a blood agar plate. (Horse or rabbit blood was used). We felt that in this enriched medium we could grow most of the common types of bacteria.

The chief difficulty encountered was that if Bact. coli were present in large numbers they grew so rapidly that they inhibited the more delicate, slower growing types, such as Streptococcus viridans and non-hemolytic streptococci. Recently we have overcome this difficulty by using a second method. A loopful of the content is placed in two cubic centimeters of a 1% sodium carbonate solution and incubated for two hours. A streak plate on blood agar is then made from the sodium carbonate. The high alkalinity of the sodium carbonate solution destroys the Bact. coli to a greater extent than the streptococci. If streptococci are present they are often found on the plate.

3. One cubic centimeter of intestinal content was placed in a deep milk tube. The tube was heated to 80 degrees Centigrade for twenty minutes to kill all the bacteria but the spore formers. In this way we were able to detect Cl. welchii. If it was present it formed a clot torn by gas bubbles, the so called "stormy fermentation". Its presence was confirmed by a Gram stain from the milk. We stressed the search for Cl. welchii because of the quantity of work that has been reported on its importance in intestinal obstruction, Williams (1927), and because we thought it might have some significance in our study.

4. One cubic centimeter was inoculated into a beef heart mash medium, made by placing about one inch of ground beef heart in a test tube, covering with infusion broth for a depth of two inches and sterilizing at fifteen pounds for one hour. The pH was adjusted to 7.4. This meat made a good medium for the growth of both aerobes and anaerobes, as well as microaerophilic types. By inoculating a large amount of mat-

erial in it and incubating for forty-eight hours we could get growth of types present in very small numbers. For instance, Cl. welchii could be recovered in a few cases where it was not found in the milk. This was probably due to the lack of spores in the material. If only vegetative forms of Cl. welchii were present in the loop content at the time of examination they would be killed when the milk tube was heated. By inoculating a deep milk tube with some of the chopped meat medium that had incubated for several days we could sometimes recover Cl. welchii, for the incubation in the meat tube gave a chance for sporulation. Then by heating the milk tube Cl. welchii could be obtained in pure culture. The meat tube helped in the isolation of other organisms also. In dog 15 we were able to obtain hemolytic streptococci from the meat tube when the blood plate failed because of over-growth by Bact. coli.

During the early part of the work we attempted to make a quantitative estimate of the organisms found. Dilutions of the specimens with sterile water were made for 1/100 to1/10,000,000. One cc. of each dilution was used in an agar pour plate and one cc. inoculated in lactose fermentation tubes. In many cases the loops contained such large quantities of bacteria that it was impossible to count the pour plate made with the 1/10,000,000 dilution. If Bact. coli were present at all, they usually produced fermentation in lactose broth in the 1/10,000,000 tube. The number of bacteria varied widely from day to day, and seemed to give no indication of the health of the dog. For instance on one day the count might be one hundred million, the following day too many to count on the ten million plate, then fall to ten million at the next examination. We discontinued quantitative methods early in our study for the results obtained showed little value in correlation with the rest of the study and the amount of time and materials consumed was great.

Table 1 shows the distribution of the types of organisms in the dogs studied. Chart 1 shows this in graphic form. The specimens were taken at irregular intervals depending on the health of the dog and other research being done upnn it. It will be noted that from some dogs only two or three specimens were obtained, while from others as many as forty were received. This is accounted for by the fact that some of the loops ruptured and death followed peritonitis a short time after operation.

The bacteria we found fell into a few large groups. The coli group, streptococci, staphylococci, and Cl. welchii of the anaerobes were by far the most prominent. It will be seen that Cl. welchii was found in a large proportion, in fact, in every case in which the first specimen was examined within a week after operation. It seems not unlikely that this organism would have been found in every case in specimens taken immediately after operation. This organism persisted for from a few days to several months, then disappeared. Table 2 shows the length of time after operation that we first failed to find Cl. welchii in our deep milk tube and Gram stain of loop content. This means that Cl. welchii was absent, or if present, there was less than one spore per cc. of loop material.

There seemed to be no evidence of a toxemia produced by Cl. welchii in the dogs in which the organism persisted for some time.

TABLE 1.

General Information about Dogs Studied

Dog No.	Location of Loop	No. of Specimens	No. days after operation first specimen examined	Bact. coli	Cl. welchii	Hemolytic streptococci	Strep. ignavus (Bergey)	Non-hemolytic streptococci	Staph. albus	Staph. aureus	Alkaligenes fecalis	Proteus vulgaris	Diphtheroid	No. days dog lived	
12	j	7	106	6										181	
15	j	7	117	7.		3			1					178	killed
20	j	9	101			4								100	killed
26	j	14	70	6		11								96	
32	j	42	17	8		5	22	8	6	1	2			411	still living
33	j	3	165		3								3	398	kill d
45	j	5	1	2	2		1		1					16	
46	j	2	1	2	2									5	
48	j	7	1	6	6						3			2	
49	j	32	1	32	7			4	2					10	
50	j	5	1	5	5			1						6	
51	j	4	1	4	4	2		2						5	
52	j	5	1	5	4	3		1						5	
53	j	7	1		2	7		1						17	
54	i	10	1	10	3	10								14	
56	i	6	1	3	4	3								16	
57	i	8	1	8	5									12	
58	i	3	2	8	5									13	killed
59	i	6	2		3				1			6		13	
60	i	3	7		2		2		3					24	
61	i	6	4		3	1			5					179	still living
62	i	7	2	7	3									111	killed
65	i	3	2	3	3									25	
67	i	16	9	16	3									154	still living
68	i	9	2	1	6			3						139	killed
69	i	1	2	2	2									3	
70	i	10	2	10	9			2	1					91	killed
73	i	3	3	3	2	3		2						11	
75	i	9	3	9	6			4						47	killed
76	i	4	6	2	1	1	1	2						28	
77	j	6	3	6	6									27	
78	j	4	2	3	4			1	2					15	
79	j	10	2	4	8			2	5	1				38	
81	i	6	1	6	6			2	1					11	
82	d	2	1	2	1									3	
83	i	7	1	4	3			2	2					24	killed
84	i	5	1	5	4					1				65	still living
85	d	3	1	3	3	2		2	1					4	
91	i	5	1	5	3			4						21	still living
93	j	3	1		3				1					14	still living

CHART I

Percentage of Dogs Showing Different Species of

Bacteria in Isolated Intestinal Segments

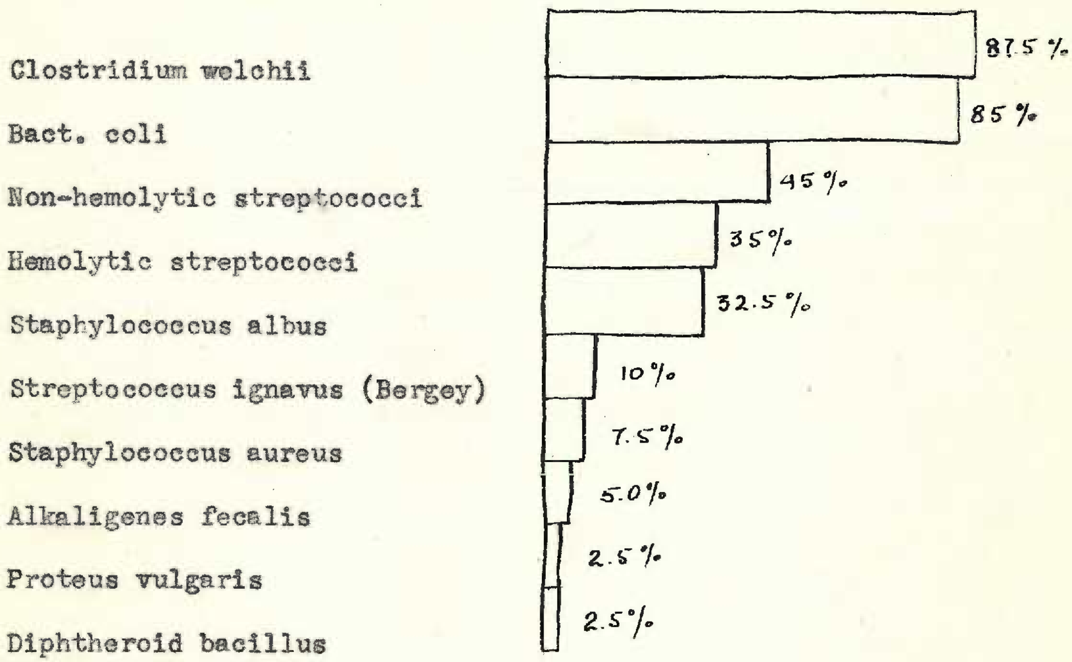

TABLE 2.

Time of Disappearance of Cl. welchii

From Isolated Intestinal Segments

Dog No.	No. of days after operation when cultures for Cl. welchii were first negative.
49	27
53	10
54	5
62	29
67	13
70	60
75	40
76	25
79	31
81	5
83	54
84	22
91	8

Average time 23 days.

In dog 68, Cl. welchii remained for a period over two months. During this time the clinical history recorded from day to day states that the dog was "fine".

One striking fact brought out in this work is that the loops do not tend to become sterile. In two cases the loops appeared to be sterile for a few days only to become filled with organisms later. In dog No. 20 we were unable to find any organisms between August 27, and September 5, 1929, although three trials were made. Subsequently hemolytic streptococci became very abundant. The only other case in which our technic failed to detect bacteria was in dog 26. We were unable to find any organisms between July 9 and 15, 1929. In this dog hemolytic streptococci soon became very prominent and a few weeks later Bact. coli made its appearance. We depended on the Gram stain and the beef heart mash medium for demonstration of the organisms. Since we inoculated one cubic centimeter of loop content into this medium there was less than one viable organism per cubic centimeter or growth would have been found in the tube.

We had only two dogs in which duodenal loops were made. From dog 82 Bact. coli was isolated twice and Cl. welchii once. From dog 86 Streptococcus hemolyticus, non-hemolytic streptococci, and Staphylococcus albus were found as well as Bact. coli and Cl. welchii. These two duodenal loops did not differ in flora from the jejunal or ileal.

The pH of the loops varied from pH 5.80 in dog 32 to pH 8.89 in dogs 65 and 92. The highest change in any one dog was noted in cases 32 and 45 in which the pH varied from 5.80 to 7.11 and 5.84 to 7.45, a change of 1.31 and 1.61 respectively. With this great difference in pH the flora did not alter in type. Knowing that each type of organism has its own optimum pH we expected to find a different flora in acid and alkaline loops. However, this was not the case. We found Staphylococcus albus, Streptococcus viridans, and Cl. welchii in dog 45 when the pH tested 5.84. These same organisms were the only ones isolated in dog 60 when the pH was 8.63. We found Bact. coli predominating in dog 65 when the pH was 8.89 and in dog 45 when the pH was 6.35, or over a range of 2.54 of the pH scale. We wish to thank Charlotte Schwichtenberg and James Newsom for the use of the pH readings taken in the department of Physiology.

SUMMARY

Isolated segments from the small intestines of dogs were studied to determine the types of organisms present. Three hundred seven specimens from forty dogs were examined. Of the segments used twenty were ileal, eighteen jejunal and two duodenal. No significant difference in the flora of the loops was observed. Clostridium welchii was present in 87.5% of the dogs and Bact. coli was present in 85%. Non-hemolytic streptococci were found in 45%, hemolytic streptococci in 35%, Staphylococcus albus in 32.5%, green producing streptococci in 10%, Staphylococcus aureus in 7.5%, Alkaligenes fecalis in 5%, Proteus vulgaris in 2.5%, and an unidentified diphtheroid bacillus in 2.5%.

During the early part of the work quantitative methods were used but were discontinued because they were found to be of little value in

comparison with the extra work required. The total number varied from zero to above ten billion per cubic centimeter of loop material. The Bact. coli content frequently reached the number of 10,000,000 per cubic centimeter.

CONCLUSIONS

1. The organisms most commonly found in isolated loops of the small intestine belong to the Bact. coli, streptococcus, staphylococcus, and Cl. welchii groups.

2. Cl. welchii is present in the loop in a large percentage of dogs soon after operation but tends to disappear from the isolated segment after a few weeks.

3. There is no apparent toxemia produced although Cl. welchii may be present for some time in the loop.

4. Isolated intestinal loops do not tend to become sterile.

5. Contents of segments from different levels do not differ more than individual differences from the same level.

6. The same types of organisms are present over a long range of the pH scale.

7. The number of organisms per cubic centimeter of loop material varies greatly from day to day.

TABLE 3.

Detailed Information about Dog No. 32

Spec. No.	Date	pH	Organisms isolated
32.1	6/14/29		non-hemolytic streptococcus
32.2	6/16/29		non-hemolytic streptococcus
32.3	7/17/29	6.43	non-hemolytic streptococcus
32.4	7/24/29	5.80	Staph. albus, non-hemolytic streptococcus
32.5	7/25/29		Staph. albus, hemolytic streptococcus, Alkaligenes fecalis
32.6	7/29/29	5.84	hemolytic streptococcus
32.7	7/31/29	6.35	Staph. albus, non-hemolytic streptococcus
32.8	8/3/29	6.48	non-hemolytic streptococcus
32.9	8/4/29	7.03	hemolytic streptococcus, Staph. albus, Alkaligenes fecalis
32.10	8/12/29	6.86	hemolytic streptococcus
32.11	8/18/29	7.20	hemolytic streptococcus, Staph. albus
32.12	8/24/29	7.11	Streptococcus ignavus (Bergey)
32.13	9/10/29		Streptococcus ignavus
32.14	9/15/29		Streptococcus ignavus
32.15	9/19/29		Streptococcus ignavus
32.16	9/21/29		Streptococcus ignavus
32.17	10/4/29		Streptococcus ignavus
32.18	10/10/29		Streptococcus ignavus
32.19	10/7/29		Streptococcus ignavus
32.20	10/9/29		Streptococcus ignavus
32.21	10/15/29		Streptococcus ignavus
32.22	10/16/29		Streptococcus ignavus
32.23	10/21/29		Streptococcus ignavus
32.24	11/13/29		Streptococcus ignavus
32.25	11/14/29		Streptococcus ignavus
32.26	11/20/29		Streptococcus ignavus
32.27	11/23/29		Streptococcus ignavus
32.28	12/16/29		Streptococcus ignavus
32.29	12/18/29		Streptococcus ignavus
32.30	1/15/30		Streptococcus ignavus
32.31	2/3/30	7.20	Streptococcus ignavus, Staph. albus
32.32	3/12/30		Streptococcus ignavus, non-hemolytic streptococcus
32.33	3/24/30		Streptococcus ignavus, non-hemolytic streptococcus
32.34	4/15/30		Streptococcus ignavus, non-hemolytic streptococcus
32.35	4/19/30		Bact. coli (few)
32.36	4/24/30		Bact. coli
32.37	4/29/30		Bact. coli
32.38	5/20/30		Bact. coli
32.39	5/22/30		Bact. coli, non-hemolytic streptococcus
32.40	6/27/30		Bact. coli, Streptococcus ignavus
32.41	6/28/30		Bact. coli, hemolytic streptococcus
32.42	7/6/30		Bact. coli, hemolytic streptococcus

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