BACTERIOLOGY OF THE INTESTINAL TRACT IN CERTAIN DISEASES

II. The Possible Inhibition of Colon Bacilli by Pathogenic Streptococci and Staphylococci

GEORGE H. CHAPMAN*
and
CLARENCE W. LIEB, M.A., M.D.*
New York, N. Y.

Reprinted from
THE REVIEW OF GASTROENTEROLOGY
Vol. 5, No. 3; pages 234 to 240, September, 1938

BACTERIOLOGY OF THE INTESTINAL TRACT IN CERTAIN DISEASES

II. The Possible Inhibition of Colon Bacilli by Pathogenic Streptococci and Staphylococci

GEORGE H. CHAPMAN*
and
CLARENCE W. LIEB, M.A., M.D.*
New York, N. Y.

In the previous paper[1] it was shown that there was insufficient evidence to incriminate the presence of sporulating anaerobes, Friedlander bacilli, *Aerobacter aerogenes, Escherichia coli* (*B. coli*), degraded colon bacilli (paracoli), and the absence of aciduric organisms in the etiology of "intestinal intoxication", colitis or irritable colon.

The role of *B. coli* toxins was thought to be a minor one because there was considerable reduction in the number of colon bacilli in many patients with intestinal complaints. In this paper we shall try to show that reduction in the number of *B. coli,* suggestive of inhibition, is related to the presence of pathogenic streptococci in the feces and pathogenic staphylococci in the nasal and oral cavities.

As in the previous paper, the term "colon bacilli" will be used for the group as a whole, including Escherichia, Aerobacter and non-lactose fermenters. The term *"B. coli"* will be used to designate the genus Escherichia, in harmony with the trend of recent bacteriologic literature to eliminate "species" based on fermentation reactions and to consider members of the genus Escherichia as a single unit.

Inhibition of B. Coli in Feces

Felsen[2] reported a case in which *B. coli* was absent in daily stool cultures over a period of 2 months, and mentioned a similar observation by Herter and Kendall. In our laboratory a large number of instances have been noted in which the number of *B. coli* was low constantly. A few examples are given in table 1.

*Clinical Research Laboratory, 604 5th Ave., New York, N. Y. Aided by grants from the Ophthalmological Foundation, Inc.

TABLE I

Illustrating the relative constancy of *B. coli* in feces.

Patient	Date	*B. coli* (millions per 100 gms. dry feces)	
R.H.K.	July 9, 1929	30,000,000	
	Jan. 7, 1930	4,000,000	
	Mar. 25,	5,300,000	
	Dec. 17,	8,000,000	
	April 7, 1931	12,500,000	
	July 6,	6,100,000	
	Nov. 5,	460,000	
	July 30, 1932	1,200,000	
	Nov. 7,	1,800,000	
	Sept. 10, 1934	500,000	
	May 8, 1936	70,000	
	April 15, 1937	700,000	
	June 23,	2,000,000	
Mrs. W. Harold H.	April 22, 1929	11,000	
	May 6,	20	
	June 11,	41	
	July 22,	200	
	Sept. 9,	1,100	
	Dec. 2,	1	
	Jan. 27, 1930	70	
	April 25,	370	
	Oct. 8,	23	
	March 2, 1931	1,200	
	April 2,	230	
	May 7,	16	
	Oct. 5,	190	
	Dec. 1,	9	
	Nov. 1, 1932	1	
Mrs. Wm. H. H.	Nov. 7, 1930	1,000	
	May 7, 1931	230	
	July 21,	350	
	Dec. 16,	0	
C.A.	Dec. 29. 1930	60	
	Jan. 21, 1931	1,000	
	Feb. 3,	30,000	
	Feb. 17,	77,000	
	Mar. 2,	5	
	April 21,	20	
	June 24,	2,400	
	Nov. 23,	0	
	April 29, 1932	3,000	
	Oct. 14,	9,000	
E.F.J.	Nov. 3, 1926	100	
	Feb. 3, 1927	2,000	
	Sept. 28,	180	
	Jan. 30, 1928	0	
	Oct. 9.	0	
	March 28, 1929	5	
	Sept. 11.	0	
	May 5, 1930	0	Daily "implantations" of *B. coli* for 3 months
	July 6,	0	
	March 9, 1931	0	
	Sept. 25.	30	
	Oct. 20.	400	
	Nov. 13.	70	
	Dec. 8.	0	
	Dec. 12	0	
	April 18, 1932	0	
	Aug. 21.	800	

It is difficult to establish the normal number of *B. coli* because of the difficulty of establishing that "normal" persons are free from bacterial infection or intoxication, and because factors affecting variations in the number of colon bacilli are but imperfectly understood. Therefore, it is impossible to make comparative studies using "normals" as controls.

Stabler and Pemberton[3] observed a greater number of "coliforms" in the stools of 17 patients with arthritis than in 9 "normal" persons. However, there are several reasons for rejecting their findings. Lactose broth, which they used for initial isolation, is unacceptable from the quantitative standpoint. The number of determinations was too small for statistical analysis, particularly in view of the extreme variations in different patients and in the "controls".

Stools which showed gross fermentation, considered evidence of small intestinal hypermotility by Owles[4], contained the smallest number of colon bacilli (table 2). The number was larger in semisolid stools and largest in formed specimens. This suggests that, the longer the specimen is in the intestinal tract, the larger the number of colon bacilli.

TABLE II

Relationship between the consistency of feces, the number of total colon bacilli, and the presence of pathogenic streptococci.

Consistency	Number of specimens examined	Average number of total colon bacilli	% of specimens containing pathogenic streptococci
Formed	35	422,012	29
Semisolid	159	296,640	33
Liquid	13	155,304	50
Frothy	4	138,325	25

Clumps of fecal material may produce marked irregularities in the distribution of colon bacilli in different parts of the sample. The technic used to obtain uniform distribution was suggested by Mr. H. G. Dunham of Difco Laboratories, viz. a wad of absorbent cotton was placed in the mouth of a test tube containing the specimen and pushed to the bottom of the tube by means of a sterile pipette. The suspension filtered through the cotton and was free from clumps.

The distribution of specimens with high, medium and low numbers of total colon bacilli was not materially different in Stabler and Pemberton's[3] arthritis series, one of our earlier series, and a recent series (table 3), which suggests that the number is independent of any particular disease.

TABLE III

Frequency of *B. coli* in feces in different series of tests.

Number (millions per 100 gms. dry feces)	Stabler and Pemberton Arthritis	Present authors	
		1926	1937-8
	%	%	%
100.000 and over	41	31	32
10,000 to 100.000	18	15	24
less than 10.000	41	53	42

Bile did not appear to be a factor in controlling the number of colon bacilli in feces because staphylococci and the colon group grew quite well in undiluted ox bile, while streptococci were inhibited, even when it was diluted 1:1,000.

The number of *B. coli* seemed to be inversely proportional to the severity of the intestinal disease, although this would be difficult to prove because of lack of suitable clinical methods. If true, it would suggest that factors responsible for inhibition of *B. coli* might be related to the toxi-infectious agent.

It had been shown by Chapman[5] that the electrophoretic migration velocity (P. D.) remained constant and could be used to identify a strain. Dozois[6] apparently construed this to mean that *B. coli* could be differentiated from *A. aerogenes* by this method. However, it was intended to mean that a strain with such characteristic velocity as our # 87, which was used for "implantations", could be recognized in cultures, even though it might be mixed with other strains of *B. coli.* Conversely, strains with dissimilar velocities are not likely to be related. By this method, and from counts of the number of *B. coli,* it was possible to show on numerous occasions that the strain used for "implantation" did not remain in the intestinal tract. However, when steps were taken to overcome factors considered responsible for inhibition of *B. coli* (see paper III of this series), the number of *B. coli* returned to normal, even without "implantation".

Since this organism could not be implanted by rectal instillation, the relief from symptoms attributed to bacterial infection suggested an antigenic effect, rather than to the ability of *B. coli* to dominate the intestinal flora. This was supported by the observation that best results were obtained after the culture had passed the logarithmic, or most vigorous, phase of growth.

Relationship Between Pathogenic Streptococci and Staphylococci and the Number of Colon Bacilli

It was noted that the administration of streptococcal vaccines often was followed by considerable increase in the number of *B. coli.* This suggested that streptococci might be implicated in some way in the inhibition of *B. coli* in the feces. The experiments to be described offer evidence in favor of this hypothesis. Since there was a possibility that staphylococci, which are known to affect the gastrointestinal tract, also might affect the number of colon bacilli, search was made for them also.

Cultures were obtained from patients with different types of chronic disease, most of whom had clinical evidence of focal infection. Since they are rarely found in levels below the duodenum probable pathogenic staphylococci were isolated from nose and throat cultures, using the methods of Chapman, Lieb, Berens and Curcio[7], and Chapman, Lieb and Curcio[8], and tested for pathogenicity by the methods of Chapman, Berens, Nilson and Curcio[9]. Cultures also were made of the feces of these patients, counting the number of *B. coli, A. aerogenes* and non-lactose fermenters (paracoli) by plating serial dilutions on Bacto Levine's eosine methylene blue medium. Streptococci were isolated from blood agar plates, differentiated from enterococci[10], and tested for probable pathogenicity by the method of Chapman and Curcio[11].

When the results were arranged according to the presence of pathogenic streptococci in the feces and pathogenic staphylococci in the nose and throat (table 4), there was an inverse relationship between the average number of total colon bacilli, the presence of pathogenic streptococci and, to a certain extent, pathogenic staphylococci. The lowest count of total colon bacilli was associated with the presence of pathogenic streptococci

in the feces and pathogenic staphylococci in the nose and throat. In the second group, which was similar to the first except that pathogenic staphylococci were not found, there were more than twice as many *B. coli* as in the first group. The number of *B. coli* was highest in the third and fourth groups, with no pathogenic streptococci in the feces.

TABLE IV

Relationship of streptococci and staphylococci to the number of colon bacilli in feces.

Number of specimens examined	Pathogenic streptococci in feces	Pathogenic staphylococci in respiratory tract	Average (millions per 100 gms. dry feces)			
			Total colon group	*B. coli*	*A. aerogenes* & intermediates	Paracoil (non-lactose fermenters)
22	+	+	110,256	40,883	7,827	61,543
12	+	0	177,018	108,600	10,084	58,334
56	0	+	285,920	219,196	61,298	5,443
29	0	0	290,000	216,117	73,132	756

The degradation of Aerobacter to paracoli was discussed in the previous paper[1], and was attributed to the harmful influence of pathogenic streptococci and, to a slight extent, pathogenic staphylococci. The failure of staphylococci to reach lower levels of the gastrointestinal tract may account for the minor influence of this organism. The smallest number of paracoli and, consequently, the least degeneration of colon bacilli, occurred in the group from which no pathogenic streptococci were isolated from the feces and no pathogenic staphylococci from the nose and throat. The highest number of paracoli, representing the greatest degradation, was found in the group from which pathogenic streptococci were found in the feces and pathogenic staphylococci in the nose and throat (table 4).

Out of 34 specimens with pathogenic streptococci in the feces, 83.3 per cent had less than 100,000 million total colon bacilli. In the absence of pathogenic streptococci only 53.6 per cent of 85 specimens had less than 100,000 million total colon bacilli per 100 grams dry feces.

These results suggest that the average number of total colon bacilli bears an inverse relationship, and the average number of paracoli a direct relationship, to the presence of pathogenic streptococci in feces, and to a lesser extent to the presence of pathogenic staphylococci in the nose and throat. However, the correlation did not hold in individual specimens or small series, as shown in table 5. This may be explained by errors due to random sampling, differences in consistency of the specimens, difficulties in isolating pathogenic streptococci, differences in oxidation-reduction potential of the feces, etc.

TABLE V

Comparison of frequency of pathogenic streptococci in feces with different proportions of colon bacilli.

Number (millions per 100 gms. dry feces)	*B. coli*		*A. aerogenes*		Non-lact. ferm.		Total colon group	
	Number	% showing strep.	Number	% showing strep.	Number	% showing strep.	Number	% showing strep.
1,000,000 and over	14	7	4	0	1	100	18	16
100,000 and over	36	19	10	40	6	33	37	19
10,000 and over	41	41	17	35	6	0	42	43
1,000 and over	31	26	12	17	12	42	28	25
100 and over	10	60	8	27	3	0	11	64
10 and over	8	27	4	50	2	100	8	27
less than 10	14	57	99	34	124	33	10	60
						Total	155	30.4

Excessive Numbers of Colon Bacilli

In some instances, a stool from a patient with intestinal symptoms showed a considerable increase in the number of *B. coli*. It could not be demonstrated that the strains were capable of growing more luxuriantly than other strains in streptococcal filtrates. Therefore, the excess numbers in these specimens were probably not due to greater resistance of the strains to streptococcal inhibiting substance, and must have been due to other factors.

In searching for possible explanations it was recalled that one of the characteristics of the intestinal flora of patients with pernicious anemia, where the number of fecal bacteria is characteristically high, is the ascendency of the colonic flora into the duodenum, possibly connected with the gastric anacidity which is a constant finding in this disease. Thus, an abnormally high count of colon bacilli in the feces could be due to proliferation of this organism in the duodenum. This would necessitate postulating that the inhibiting agent was inactivated or rendered ineffective in the duodenum. When insufficient acid is produced in the stomach, it is likely that the alkalinity of the duodenal fluid will be only slightly reduced. Such an excessively alkaline fluid might inactivate streptococci, which tolerate a narrower range of pH than does *B. coli*.

In certain patients, there was an excessive number of *B. coli*, although the patients were ill for several years and had streptococcal foci of infection. When the illness became more severe, and the foci showed evidence of increased activity, the number of *B. coli* in the feces was materially reduced. This suggests that originally the foci were either closed or mild in character.

Thus, an abnormally large number of colon bacilli in the feces could be attributed to: (1) Absence or diminution of pathogenic streptococci, or a metabolite produced by them, in the gastrointestinal tract; or (2) ascendency of the colonic flora into the duodenum, as a possible result of gastric anacidity. The subject is being studied further.

SUMMARY

The relationships between streptococci, staphylococci and colon bacilli can be summed up as follows. Except in certain conditions dependent upon little understood changes in the upper respiratory or gastrointestinal tracts, pathogenic streptococci in the gastrointestinal tract, and to a certain extent pathogenic staphylococci in the upper respiratory tract, produce marked inhibition of colon bacilli. Along with this, colon bacilli, particularly Aerobacter, are degraded to paracoli.

The excessive number of colon bacilli in certain stools was attributed to phenomena connected with the upper gastrointestinal tract.

These findings suggest that pathogenic streptococci and staphylococci may be responsible for changes in the number and biochemical properties of commensal intestinal organisms (colon bacilli). It would be logical to expect fundamental changes in gastrointestinal physiology as a result of these influences. Thus, pathogenic streptococci and staphylococci may play a significant role in gastrointestinal symptomatology. This possibility will be considered in the following paper.

REFERENCES

1. Lieb, C. W., Chapman, G. H., Rawls. W. B. and Stiles, M. H.: Bacteriology of the intestinal tract in certain chronic diseases. I Sporulating anaerobes, aciduric organisms and the colon group. Rev. Gastroent. 5: 142.
2. Felsen, J.: Intestinal oxygenation in idiopathic ulcerative colitis; preliminary report. Arch. Int. Med. 48: 786-792, Nov. 1931.
3. Stabler, S. H. and Pemberton, R.: A note on the intestinal bacteria in chronic arthritis. Am. J. Med. Sci. 193: 233-241, Feb. 1937.
4. Owles, W. H.: The etiology of intestinal carbohydrate dyspepsia. Guy's Hosp. Rep. 86: 411-9, Oct. 1936.
5. Chapman, G. H.: Electrophoretic potential as an aid in identifying strains of the *B. coli* group. J. Bact. 18: 339-342, Nov. 1929.
6. Dozois, K. P.: Variations in electrophoretic mobilities of Escherichia, Aerobacter and "intermediate" strains. J. Bact. 31: 211-5, Feb. 1936.
7. Chapman, G. H., Lieb, C. W., Berens, C. and Curcio, L.: The isolation of probable pathogenic staphylococci. J. Bact. 33: 533-543, May 1937.
8. Chapman, G. H., Lieb, C. W. and Curcio, L. G.: The use of bromthymol blue agar and phenol red mannitol agar for the isolation of pathogenic types of staphylococci. Am. J. Clin. Path., Tech. Suppl. 2: 3-11, Jan. 1938.
9. Chapman, G. H., Berens, C., Nilson, E. L. and Curcio, L. G.: The differentiation of pathogenic staphylococci from non-pathogenic types. J. Bact. 35: 311-334, March, 1938.
10. Chapman, G. H.: Studies of streptococci. IV Resistance of enterococci. J. Bact. 32: 41-6, July 1936.
11. Chapman, G. H. and Curcio, L.: Studies of streptococci. II Quantitative differences in resistance to sodium bicarbonate and hexylresorcinol. J. Bact. 31: 333-7, April 1936.