

# THE RELATIVE PERSISTENCE OF BACT. COLI AND BACT. AEROGENES IN NATURE

## I. IN DECAYED STUMPS

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Received for publication, June 17, 1931

A growing question for practical consideration in water control is the relative sanitary significance of *Bact. coli* and *Bact. aerogenes* as criteria of fecal pollution. It is generally agreed that *Bact. coli* is strictly of fecal origin, and its presence in water therefore is accepted as evidence of dangerous pollution. *Bact. aerogenes*, however, occupies a doubtful position as an organism of fecal origin. Some observers regard it as of the same fecal significance as *Bact. coli*, while others consider it of little, if any, value as indicating contamination from fecal sources. There appears to be growing evidence in support of the latter view (Chen and Rettger (1920); Koser (1927); Hinman (1925); Tonney and Noble (1930)). Certainly, the great predominance of *Bact. aerogenes* over *Bact. coli* at large in nature needs some explanation other than the assumption that both are of direct fecal origin, since in feces itself the reverse relationship is true. In previous studies we have found the ratio of *Bact. coli* to *Bact. aerogenes*, by the differential plate count, to be about 100 to 1 in feces of both human and animal origin, and on the other hand about 1 to 20 in soils and vegetation (Tonney and Noble (1930)).

This wide divergence in the numerical relation of the two organisms is against the assumption that they are both of direct fecal origin and hence equally significant of fecal pollution. Possible explanations of the phenomenon, however, are:

1. That *Bact. aerogenes* survives longer and in greater numbers than *Bact. coli* outside of the animal body.

2. That *Bact. aerogenes* at large in nature multiplies to a greater extent than *Bact. coli* in the same environment.

3. That *Bact. aerogenes* is primarily of non-fecal origin and finds its way into the animal body with the food, hence appearing irregularly in the feces as a transient rather than a characteristic organism.

In an effort to throw light on this problem, the experiments here reported were made on the relative persistence of *Bact. coli* and *Bact. aerogenes* under one set of natural conditions. The procedure consisted of planting several series of decayed stumps, some with fecal material and some with washed suspensions of the two organisms, and making differential plate counts of their relative numbers at intervals until they disappeared. By this method we were able to study the relative persistence of the two organisms exposed to the weather under identical conditions, and at all seasons of the year. Observations were made of (1) their relative rate of reduction, (2) the relative time of their survival, (3) their relative rates of growth when the conditions were such as to favor it.

Beginning December 27, 1929, and at suitable intervals as the season advanced, seven series of stumps were planted with sterile distilled water suspensions of human feces and of cultures of *Bact. coli* and *Bact. aerogenes*. The cultures were isolated, respectively, from feces and Lake Michigan water.

#### TECHNIC

Suspensions of the feces were made by emulsifying normal and diarrheal stools in quart bottles of sterile distilled water. Similar suspensions were prepared from culture material, washed from a suitable number of agar slants on which the organisms had been grown. Prior to the planting, sufficient material from the stump was collected for test of its colon-aerogenes content. Immediately thereafter the plant was made by carefully pouring the contents of a bottle into the hollow portions and crevices of the stump. Care was taken to assure complete absorption of the liquid by enough of the stump substance to yield sufficient sampling material over a considerable period of time. After

about thirty minutes a sample of the infected stump was collected in a sterile wide-mouthed bottle.

At suitable intervals thereafter, depending upon the season of the year and the duration of the series, further samples were taken until two successive negative specimens for each organism had been obtained. When the samples could not be examined on the same day, they were held in the refrigerator over night. The procedure of testing was as follows:

Twenty grams of the sample were weighed into a sterile container to which were added 200 cc. of sterile distilled water. The contents were shaken and allowed to settle for one-half to two hours, depending upon the amount of coarse material remaining in suspension. Five 10.0 cc. portions of the supernatant liquid were planted in ferrocyanide-citrate agar in accordance with the modified technic previously reported by us (Tonney and Noble (1931)).

The differential plate counts of *Bact. coli* and *Bact. aerogenes* thus obtained were studied for trends and relationships between the two types of organisms under the natural conditions of the experiment.

#### RESULTS

Since the primary purpose of the study was to show the relative persistence of *Bact. coli* and *Bact. aerogenes* exposed under identical conditions in decaying wood, no attempt was made to evaluate the probable influencing factors. Among these factors are: (1) temperature, (2) rainfall, (3) the kind of wood, its hardness, moisture and chemical content, (4) the presence of molds and fungi, (5) the presence of other bacteria, (6) subsequent secondary infection by birds and animals, and (7) the influence of the distilled water used for suspending the samples. It was merely assured by the conditions of the experiment that these factors occurring under natural conditions were equally operative on both organisms.

For convenience, the data are separated into two groups, those obtained from feces plantings and those from the pure cultures. The feces group is subdivided into three series while the culture group is subdivided into four series.

*The feces group*

In series I of the feces group, five stumps were planted with fresh human feces and two with fresh bovine feces<sup>1</sup> on December 27, 1929. The weather at the time was very cold and a blizzard was in progress. There was a sharp decline in the number of *Bact. coli* from the initial average of 532,000 per gram to 0 per gram on the 18th day, with small numbers recurring up to the 61st day. Unfortunately no tests were made of this group after the initial planting, until the 18th day. The initial absence of *Bact. aerogenes* in the quantities tested is noted. This organism, however, appeared on the 18th and 21st days.

Two stumps constituted series II. They were planted with feces on April 4, in the spring, and the organisms persisted through the warm season until the 153rd day (September 4), when the last recovery was made. The initial *Bact. coli* content was 11,800 per gram, with *Bact. aerogenes* absent in the quantity tested. Recovery of the latter organism, however, occurred more often than in series I and as late as the 153rd day. A difference between series I and series II is the relatively greater number of the *Bact. aerogenes*. This may be due, in part, to the warm weather.

In series III, representing ten stumps, planted with feces, there was a uniformity of ratio between the two types of organisms, with *Bact. coli* predominating, as at the outset. The magnitude of the numbers was somewhat greater in this series, which extended over the warmer season from June 7 to November 16, a total of 172 days. The larger numbers surviving may be the effect of the season, or may be due to a larger initial content or both.

In figure 1, trend curves of these data, indicate a decline of both organisms with little change in relative numbers of one type over the other until the low magnitudes of 10 or less per gram were reached.

Table 1, showing the combined ratios of *Bact. coli* to *Bact. aerogenes* in all of the stumps planted with feces, shows a sustained

<sup>1</sup> The only planting made with animal feces.

characteristic fecal ratio of 100 or more *Bact. coli* to 1 *Bact. aerogenes*, irrespective of season, duration of the series, and magnitudes. Of the 22 items representing samples taken as many as 153 days after infection, only 4 show a reversal of the typical fecal relationship.

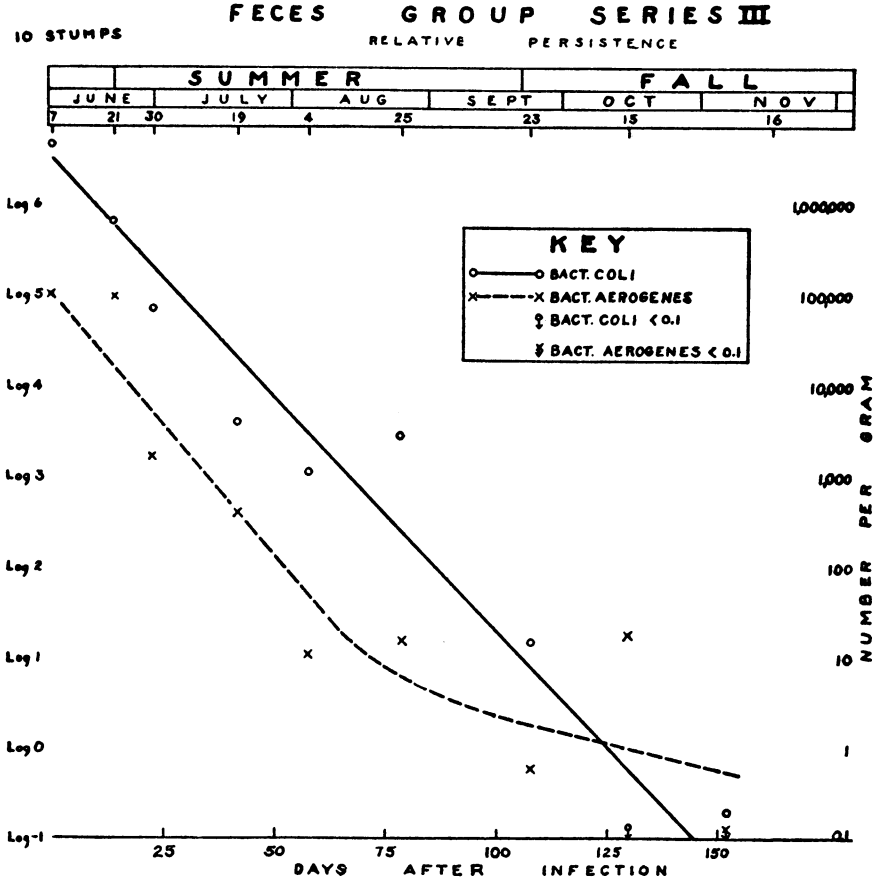


FIG. 1

*The pure culture group*

In series I of the pure culture group, ten stumps were planted December 27, 1929. The initial *Bact. coli* and *Bact. aerogenes* averages per gram were 323,000 and 708,000 respectively.

On the 6th day (December 2) two stumps showed an average of only 2.4 to 10.4 *Bact. aerogenes* per gram respectively. On the 19th day (December 15) these two and five of the others were negative. The remaining three in the series were first tested on the 22nd day (December 18). One showed an average count of 0.2 *Bact. coli* and 0.2 *Bact. aerogenes* per gram of material and

TABLE 1  
*Feces group. Consolidated data of series I, II and III—17 stumps*

DATE	DAYS AFTER PLANTING	PERCENTAGE		RATIO OF BACT. COLI TO BACT. AEROGENES	
		<i>Bact. coli</i>	<i>Bact. aerogenes</i>		
1880					
April 17.....	13	56.6	43.4	1.3:	1.0
June 21.....	14	87.2	12.8	67.6:	1.0
January 14.....	18	0	100.0	0 :	0.2
January 17.....	21	95.4	4.6	20.5:	1.0
June 30.....	23	97.8	2.2	43.1:	1.0
January 21.....	25	100.0	0	0.9:	0
April 30.....	26	100.0	0	5.4:	0
January 28.....	22	100.0	0	0.2:	0
May 14.....	40	0.08	99.92	1.0:1,250.0	
July 19.....	42	88.4	11.6	76.4:	1.0
August 4.....	58	98.6	1.4	63.1:	1.0
February 26.....	61	100.0	0	0.6:	0
June 4.....	61	62.7	37.3	15.0:	1.0
June 18.....	75	100.0	0	0.7:	0
August 25.....	79	98.9	1.1	89.1:	1.0
June 30.....	87	58.4	41.6	1.4:	1.0
September 23.....	108	92.7	7.2	12.6:	0
July 22.....	109	100.0	0	57.0:	0
July 29.....	116	0	100.0	0 :	112.0
October 15.....	130	100.0	0	0 :	12.5
November 6.....	152	100.0	0	0.2:	0
September 4.....	153	100.0	0	0.1:	0

the rest were negative. Samples taken on the 26th, 32nd, 41st, 63rd, 76th, 98th and 111th days were negative except one, which showed an average of 4.2 *Bact. aerogenes* per gram on the 76th day. Results were negative again on the 98th and 111th days.

The significant feature of this series is the failure of the infection to last as long as the first series of the feces group planted at

the same time, during the season of severe cold. Moreover, only *Bact. aerogenes* was found in the few instances that were positive after the sixth day. Their numbers, however, were very small.

Series II, comprising three stumps, is essentially the same as series I. Plantings were made January 28 with an initial average content of 307,000 *Bact. coli* and 456,000 *Bact. aerogenes* per gram. Recoveries on the 9th, but not on the 29th day, showed 2.0 *Bact. coli* and 2.0 *Bact. aerogenes*, as an average per gram respectively. Samples on the 36th, 49th, 62nd and 77th days were negative.

Both series represent winter conditions.

Series III, of 8 stumps, was planted in the early spring (April 4), and the last recovery was made November 19 or the 229th day. More data were available in this series.

In figure 2 there is seen a falling off of numbers up to a time between the 41st and 61st days (May 15 and June 4). From this time on, however, there appeared a gradual increase in the *Bact. aerogenes* content, reaching a peak on the 153rd day (September 4), after which there was a quick decline. *Bact. coli* underwent a slight and irregular increase but in much smaller degree than *Bact. aerogenes*. Thirteen of the 17 items in this series showed an excess of *Bact. aerogenes* over *Bact. coli*. It should be recalled that in the corresponding series planted with feces, during this period *Bact. coli* maintained its typical fecal ratio to *Bact. aerogenes* throughout the experiment (over 100 to 1).

In series IV nine stumps, there is a similar picture (fig. 3). Plantings were made in the late spring (May 27) and the last recoveries of both *Bact. coli* and *Bact. aerogenes* were made on January 10, the 228th day. There was a reduction in numbers of both organisms until the 75th day (August 11) when an increase of both began. This reached a peak between the 148th and 170th days (October 22 and November 13) and declined again between the 183rd and 197th days (November 26 and December 10). Throughout the period the initial excess of *Bact. aerogenes* over *Bact. coli* was consistently maintained and was somewhat increased during the late summer and fall. Only four of seventeen items showed a reversal of this relationship.

Table 2, representing the consolidated data of all the culture

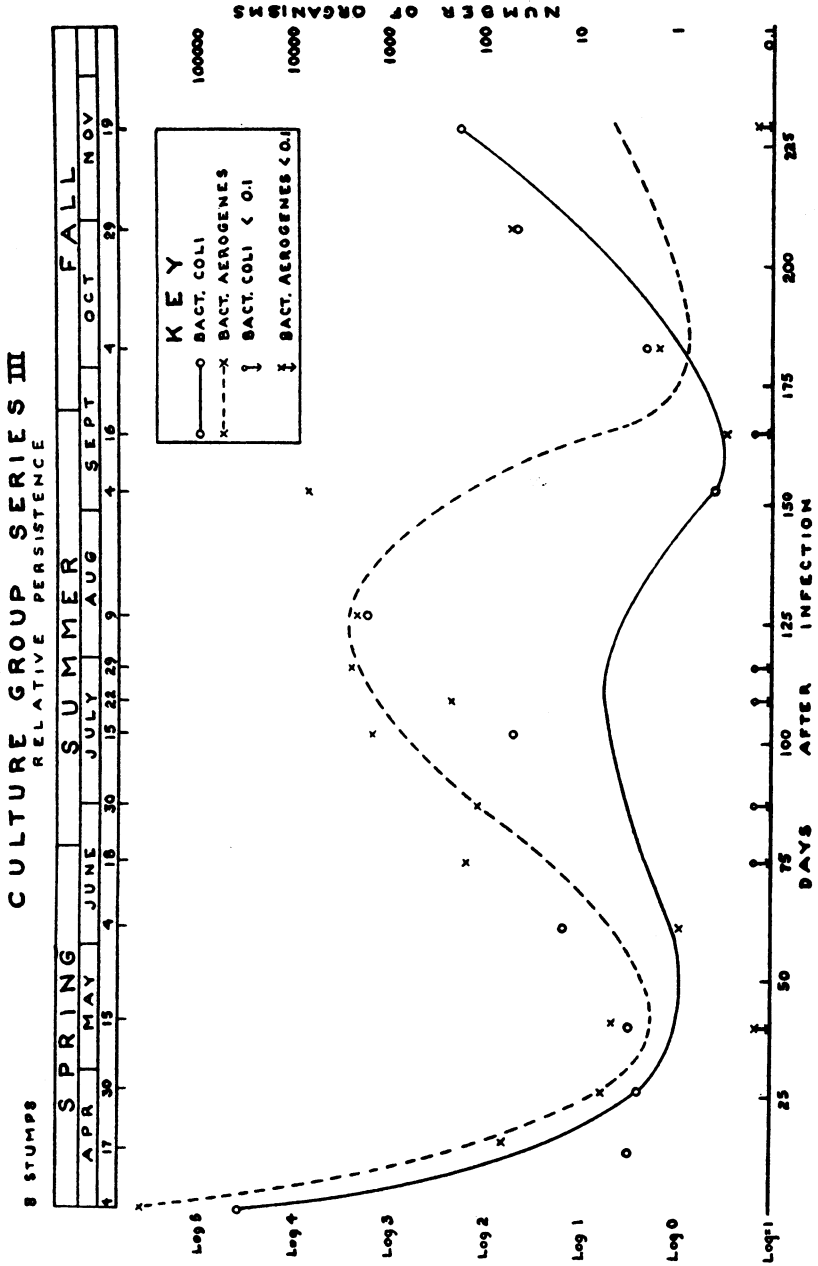


FIG. 2

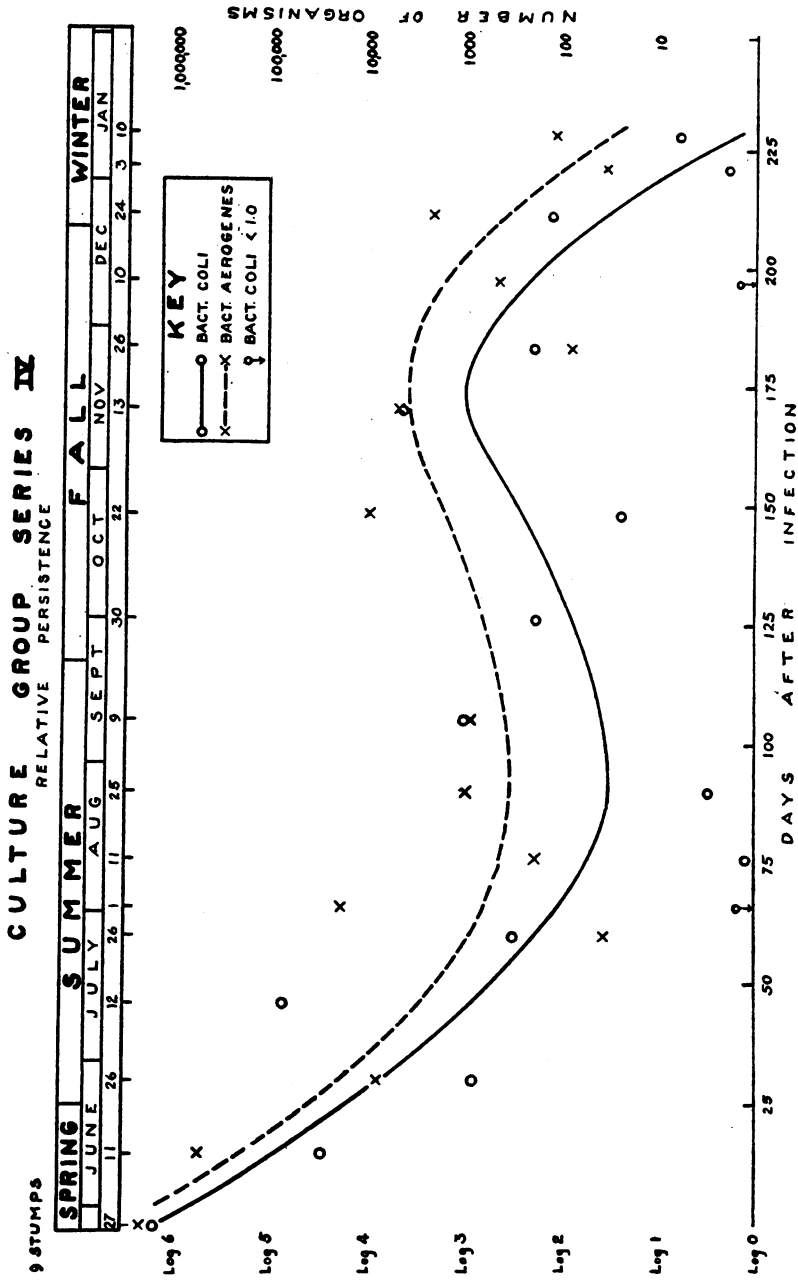


FIG. 3

plantings, shows a sustained ratio of *Bact. coli* and *Bact. aerogenes*, which is the converse of the typical fecal ratio, viz.: 1 or less

TABLE 2  
Culture Group. Consolidated data of series III and IV—17 stumps

DATE	DAYS AFTER PLANTING	PERCENTAGE		RATIO OF BACT. COLI TO BACT. AEROGENES	
		<i>Bact. coli</i>	<i>Bact. aerogenes</i>		
1930					
April 17.....	13	4.5	95.5	1.0:	20.6
June 11.....	15	50.7	49.3	1.0:	1.9
April 30.....	26	29.8	71.2	1.0:	2.4
June 26.....	30	9.2	90.8	1.0:	9.8
May 14.....	40	100.0	0	3.0:	0
May 15.....	41	0	100.0	0 :	4.6
July 12.....	46	88.8	11.2	29.8:	1.0
July 26.....	60	89.7	10.3	8.6:	1.0
June 14.....	61	94.4	5.6	16.9:	1.0
August 1.....	66	0	100.0	0 :	18,300.0
June 18.....	75	0	100.0	0 :	158.0
August 11.....	76	0.6	99.4	1.0:	148.0
June 30.....	87	0	100.0	0 :	120.0
August 25.....	90	0.3	99.7	1.0:	322.0
July 15.....	102	3.3	96.7	1.0:	29.6
September 9.....	105	54.6	45.4	1.2:	1.0
July 22.....	109	0	100.0	0 :	225.0
July 29.....	116	0	100.0	0 :	2,580.0
September 30.....	126	52.7	47.3	1.1:	1.0
August 9.....	127	43.6	56.4	1.0:	1.3
October 22.....	148	0.2	99.8	1.0:	409.0
September 4.....	153	0.005	99.995	1.0:	18,100.0
September 16.....	165	0	100.0	0 :	0.3
November 13.....	170	49.1	50.9	1.0:	1.0
October 4.....	183	58.3	41.7	1.4:	1.0
November 26.....	183	68.1	31.9	2.5:	1.0
December 10.....	197	0	100.0	0 :	472.0
October 29.....	208	45.8	54.2	1.0:	1.2
December 24.....	211	5.5	94.5	1.0:	17.1
1931					
January 3.....	221	14.5	85.5	1.0:	5.9
January 10.....	228	4.5	95.5	1.0:	21.0
November 19.....	229	100.0	0	180.0:	0

*Bact. coli* to 20 or more *Bact. aerogenes*, irrespective of season, duration of series, and magnitudes. Of the 32 items representing

samples taken as many as 229 days after infection, only 7 showed a reversal of the relationship.

## DISCUSSION

*Relative rate of reduction*

The sharpest percentage reduction of *Bact. coli* and *Bact. aerogenes* occurred in the winter plantings of both feces and laboratory cultures. As the season advanced, however, the rate became slower up to the late spring plantings, in which it was slowest.

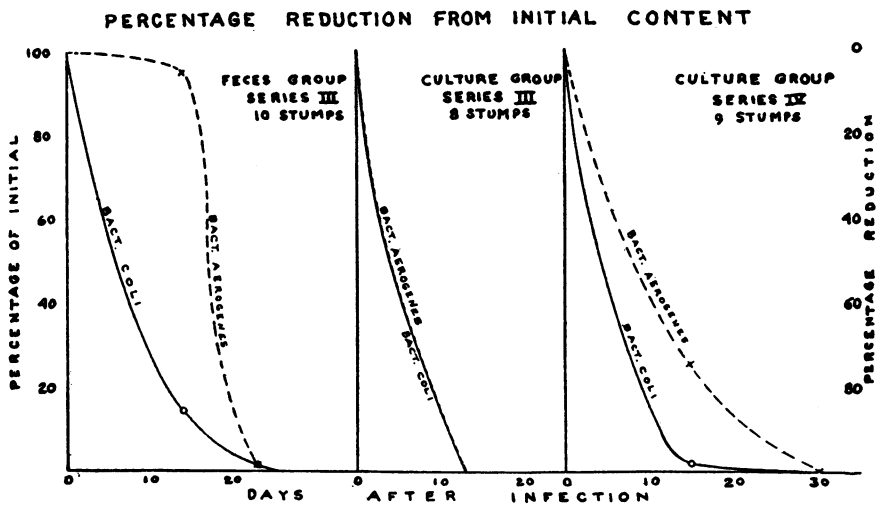


Fig. 4

There was very little difference in the relative rates of reduction throughout the experiments. Reduction occurred consistently but neither organism appeared to gain a significant ascendancy over the other during the period of decline. From 98 to 99 per cent reduction in numbers took place in 21 to 30 days. In figure 4 it is noted that the *Bact. coli* rate of decline in series III of the feces group and series IV of the culture group is somewhat faster than that of *Bact. aerogenes*. The rates of decline were practically the same for the two organisms in series III of the culture group.

*Relative time of survival*

It is seen that the time of survival of both organisms was much shorter in the winter plantings than in the late spring plantings. In the fecal group, *Bact. coli* and *Bact. aerogenes* lived 61 days and 21 days respectively in the winter plantings. In the culture group on the other hand, the corresponding relationships were: In winter, 9 days and 22 days respectively, and late spring, 228 days for both. It was obvious throughout the experiment that there was very little difference in the time of survival of the two organisms.

*Relative rates of growth*

In the case of winter plantings, no significant increase in the numbers of either type of organism occurred at any time in either the fecal or the pure culture group. In the spring and late spring plantings, however, growth of both types of organisms took place in the culture group of plantings. Only very slight growth occurred in the fecal group, appearing in series II of two stumps but not in series III of 10 stumps. In marked contrast to the feces plantings carried over the summer, the pure culture plantings (figure 2 of series III) exhibited a decided increase of *Bact. aerogenes* over *Bact. coli*; the latter increased only slightly in numbers. To a less degree, this was true in figure 3 of series IV. It is noted that the period of this increase is approximately 100 days, beginning about the 75th day and ending on or about the 170th day. The increase in each series began at different times but generally in the warmer months.

The points common to both organisms were: (1) that the growth occurred in the late summer and fall, (2) that the increases began about the 60th day after planting, reached a peak in 120 and 125 days and declined to the 60 day level by about the 170th day, (3) the phenomenon appeared to be independent of the initial magnitude of the numbers planted, and (4) the increased growth was largely confined to *Bact. aerogenes*.

The *Bact. coli* cultures underwent a slight corresponding increase, of which the peak in series IV lagged about 50 days behind that of *Bact. aerogenes*. The strains of *Bact. aerogenes* naturally

present in the feces plantings, did not at any time outgrow the *Bact. coli* in the same series.

#### SUMMARY AND CONCLUSIONS

In these experiments:

1. Under winter conditions, *Bact. coli* and *Bact. aerogenes*, both from fecal material and from cultures, underwent rapid decline, without exhibiting any significant change in relative numbers.

2. At no time was there a material difference in the surviving time of either type of organism in feces.

3. In one of the pure culture series, there was a marked increase in *Bact. aerogenes* beginning about 60 days after infection, accompanied by only a slight increase in *Bact. coli*. The peak of growth was reached about the 125th day and there was subsequent decline to about the 60-day level by the 170th day. Thereafter, relative numbers continued about equal up to the time when both organisms disappeared.

4. The rather marked relative increase in growth of *Bact. aerogenes* over *Bact. coli* which occurred in certain culture plantings suggests the possibility that the commonly observed predominance of *Bact. aerogenes* in nature may be due in part to greater multiplication of certain hardy strains of *Bact. aerogenes*.

5. Under such circumstances, the *Bact. aerogenes* count, if accepted as a quantitative index of fecal pollution, would tend to distort the sanitary picture, yielding no evidence of the recency of the contamination, and giving an exaggerated idea of the amount of pollution after two months, at which time the pollution is less dangerous from the standpoint of water-borne disease than at the outset.

6. The *Bact. coli* count, on the other hand, had a more consistent relation to the initial amount of fecal pollution, and to the time elapsing after its occurrence, both of which factors are of essential importance in judging dangerous pollution.

7. The high resistance of the colon-aerogenes group of organisms to the action of the elements was evident in the tests, and gives further assurance that as test organisms, their survival-time

affords a safe margin over that of the less resistant intestinal pathogens which constitute the real danger from fecal pollution.

8. The plate count method in ferrocyanide-citrate agar was found to be a sensitive measure of changes in numbers of colon-aerogenes organisms occurring under the conditions of this experiment.

9. The fact that *Bact. coli* and *Bact. aerogenes* will live a long time in wood under natural conditions, has a practical bearing on the quality of water from any supply in which the water is in contact with wood. Often wooden storage tanks are used, especially in small supply systems. In older types of private wells, wooden casing is commonly used, and sometimes wooden troughs or conduits as well. There also still remain a few "old oaken buckets." Under such conditions the presence of *Bact. aerogenes* alone is probably of no sanitary significance.

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