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THE BACTERIOLOGICAL EXAMINATION OF SURFACE WELLS.

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THE results of the bacteriological examination of surface wells are more difficult to interpret, in relation to the determination of pollution, than are those obtained with any other class of natural waters.

The influence of immediate local conditions on the bacteriological findings for these water supplies is always considerable and must be taken into account. At the same time the proper determination of the significance of the results of chemical and bacteriological examinations of surface wells is very important, since a considerable portion of the rural population is dependent for its entire drinking water upon shallow wells.

My experience leads me to conclude that chemical analysis is particularly unreliable for this class of waters.

Topographical investigation is of the utmost importance but alone it frequently does not enable a decided reliable opinion to be given whether contamination is, or is not, present, or possible.

The published results of the bacteriological examinations of surface wells are not numerous, while there are none, with which I am acquainted, in which the results are considered in the light of a topographical knowledge of the surroundings of the wells.

The wells included in this paper were all personally examined and the source of the water and its liability to pollution investigated. All but one of them are in the Borough of Colchester, which is very extensive in area and in large part rural. The samples were collected over a space of 3 years, but the great majority within the last 15 months as part of a special investigation. All the samples were personally collected except a few of the duplicate samples from wells already examined, and these were collected with great care and all by

the same inspector. In every case the examination was started within two and a half hours of collection, while for most the interval was much shorter than this.

Nutrient gelatin media of a +1 per cent. reaction was used for cultures placed at 20—21° C., the colonies being counted after 3 days' incubation, for practically all the samples. Nutrient agar, also of a +1 per cent. reaction, was used for cultures placed at 37° C., the colonies being counted after 42 to 48 hours' incubation.

For the *Bacillus coli* estimations some variations of method were employed, but for the great majority of the samples a procedure combining the use of the neutral-red and bile-salt broth methods was used. In every case some of the dilutions were plated, the *B. coli*, or coli-like organisms, isolated, if present, and their characters determined in pure culture.

The *streptococci* enumerations were made by adding varying quantities of the sample, e.g. 0·1, 1, 10 c.c. to tubes of glucose neutral-red broth, while for the larger amount of water a tube of four times strength glucose neutral-red broth was added to this quantity of water in the collecting bottle. The different mixtures were incubated at 37° C. for 48 hours and were then examined, in hanging drop preparations, for streptococci in chains. In only a few instances were the streptococci isolated and their characters determined, but in all doubtful cases the fluid was stained and examined. Only cocci which occurred in definite chains were regarded as streptococci and so recorded.

The results here recorded are based upon the examination of 86 samples of surface well waters obtained from 50 different surface wells.

29	wells	examined	once	=29	samples.
12	,,	,,	twice	=24	,,
3	,,	,,	three times	= 9	,,
6	,,	,,	four ,,	=24	,,

The results and details of an examination upon the effects of a soil environment upon *B. coli*, are also included and considered.

Part I.

The bacterial content of the wells.

The wells and the bacteriological results can be considered in 3 series; from the point of view of the topographical surroundings of the wells.

Series A. Wells which on careful inspection were considered to be free from any risk of contamination.

Series B. Wells which when examined showed obvious possibilities of more or less direct pollution.

Series C. Wells which when examined showed no gross definite evidence of pollution, but which from their proximity to houses, or from being surrounded by manured land, were considered to be probably liable to pollution. In this series are included a number of samples from wells situated in the rear premises of houses in the midst of the town itself, and frequently in populous neighbourhoods. These wells were all covered over and provided with a pump, and for many of them the exact locality of the well could not, with certainty, be ascertained. The houses were all drained and supplied with water-closets, there being no privies or known cesspools in their neighbourhood. The wells were for the most part quite near the houses.

The wells, with two exceptions subsequently indicated, were all, as far as they could be seen, of the same type, i.e. brick lined, but not rendered impervious in any way. Their depth varied greatly in the different parts of the Borough.

The results of the examination of *Series A* wells are given in Table I. This table shows that the bacteriological findings were quite in accord with the topographical inspections, although the latter were noted quite independently, and indeed were recorded at the time the samples were collected. The classification into the three groups was therefore made before any bacteriological results were available, in this way eliminating any unconscious influence upon the topographical classification. Only 8 wells (10 samples) could be classed in Series A.

Excluding one well, the well from which samples 4, 18, and 71 were obtained, all the samples were free from *B. coli* and streptococci in 50 c.c. Larger amounts were not examined.

Bacteriologically I should therefore certainly pass these waters as satisfactory.

The numerical counts are interesting. The enumerations at 37° C. are surprisingly low, considering the possibilities of soil organisms being washed into the wells. The gelatin counts show that enumeration at 20—21° C. is practically valueless for surface wells. Disturbance of the water may produce an enormous increase in the number of these organisms growing on plates, and this without the addition of harmful organisms. This is well shown in sample No. 1.

The well from which samples 4, 18 and 71 were collected is a well which is properly protected from pollution, and is rendered impervious to a considerable depth. It is covered over with a wooden cover provided with a lock and key. The water is pumped by a wind pump up to a tank and then distributed. Sample No. 4 was quite satisfactory. No. 18 is less satisfactory, but is to be accounted for by the disturbance two months previously, when fresh feeders were constructed. Sample 71 is also not perfectly satisfactory, and a slight but sufficient surface contamination due to carelessness when the cover was unlocked was discovered which probably accounts for this.

The results of the examination of *Series B.* wells, shown in Table II, are in marked contrast. In this series 11 wells are included, from which 22 samples were collected. For the majority of the samples, but not all, the bacteriological findings would be sufficient to condemn the supplies, apart from the knowledge of topographical conditions.

Table II shows several points of interest. In the first place in several instances it will be noticed that for the same well at one time *excretal B. coli* were isolated, while on other occasions no such typical organisms were found, but only bacilli which differed more or less widely from the typical *B. coli*. It is probable that, for at least some of these samples, true *B. coli* were present but were not isolated. This is a difficulty not infrequently met with in this class of waters. If these aberrant coli-like organisms are altogether neglected, as some bacteriologists seem to hold should be the case, and the purity of the samples are considered entirely from a *B. coli* standpoint, and from one for which only quite typical organisms are considered, several of the samples from undoubtedly polluted sources would have to be passed as showing no evidence of pollution. Samples 6, 75, 31, 85, 55 illustrate this point.

In the second place the question of the amount of rainfall previous to the sampling seems to play a considerable part in modifying the bacteriological findings.

For example, the well from which samples 31, 37, 48 and 73 were collected was a covered well provided with a pump. The covering over the well was, however, very defective, open joints existing between the stone slab immediately over the well, and the smaller stone slabs surrounding it. The well stood in a yard about 30 feet from the house, while 12 feet away in the opposite direction was a privy and urinal, and next to them a quantity of pigs were kept.

The depth of the well was not actually measured, but from a knowledge of the wells in the immediate neighbourhood it can be stated with considerable certainty that the level of the water at the time of the examinations was not less than 30 feet. The well had been there certainly for more than twenty years and the inside was not rendered impervious or protected in any way.

TABLE I.

No. of sample	Date of examination	No. of organisms per c.c. developing at 37° C.	No. of organisms per c.c. developing at 20° C.	'Excretal' *B. coli* in 0·1	'Excretal' *B. coli* in 1	'Excretal' *B. coli* in 10	'Excretal' *B. coli* in 40 c.c.	Coli-like organisms isolated from 0·1	Coli-like organisms isolated from 1	Coli-like organisms isolated from 10	Coli-like organisms isolated from 40 c.c.	Streptococci in 1	Streptococci in 10	Streptococci in 40 c.c.	Class of Well	Remarks
1	Jan. '04	3	46000		–	–	–					–	–	–	Draw	Recently made.
4	,, ,,	0	2800		–	–	–					–	–	–	Protected well, wooden cover	Fresh feeders recently constructed.
18	Feb. '05	3	1600	–	–	–	+					–	–	–		
71	Aug. '06	33	...	–	–	–	–			+		–	–	+		
10	May '04	5	...		–	–	–					–	–	–	Draw	
11	Aug. '04	5	70	–	–	–	–					–	–	–	Pump	
17	Feb. '05	4	...	–	–	–	–					–	–	–	Draw	
21	Sept. '05	1	46	–	–	–	–					–	–	–	Pump	
45	Nov. '05	24	1970	–	–	–	–					–	–	–	Protected	
61	April '06	6	830	–	–	–	–					–	–	–	Pump	

TABLE II.

6	April '04	96	3500	–	–	–	–			+		–	–	+	The same well. Draw	Heavy previous rainfall.
24	Oct. 4, '05	360	1220	–	+	+	+	+				–	+	+		Very little rainfall for previous two months.
41	Oct. 26, 05	125	6100	–	+	+	+					–	+	+		
75	Aug. '06	1230	...	–	–	–	–	+	+	+		–	+	+		
31	Oct. 12, '05	284	7400	–	–	–	–		+	+		–	+	+	The same well. Pump	Very little previous rainfall.
37	Oct. 18, '05	285	8480	–	+	+	+	+	+			–	–	+		
48	Feb. '06	25	1770	–	–	–	–					–	–	–		Considerable rainfall recently.
73	Aug. '06	810	...	+	+	+	+		+			–	+	+		Extremely little rain for previous two months.
25	Oct. 4, '05	470	2900	–	–	–	–					–	+	+	Pump	
40	Oct. 26, '05	70	48000	–	–	+	+					+	+	+		
83	Sept. '06	1060	...	+	+	+	+					+	+	+		
26	Oct. '05	350	2700	–	+	+	+					+	+	+	Draw	
85	Sept. '06	434	...	–	–	–	–	+				+	+	+		
49	March 6, '06	95	550	–	–	–	–					–	–	–	Open, quite unprotected	
57	April 2, '06	...	...	–	–	–	–					–	–	–		
55	Mar. 22, '06	78	800	–	–	–	–		+		+	–	+	+	Pump	
58	April 4, '06	44	940	–	+	+	+					–	+	+		
12	Sept. '04	2350	over 5000	–	+	+	+					+	+	+	Pump	
19	July, '05	10	...	–	+	+	+					–	–	–	,,	
42	Oct. '05	200	1580	–	–	–	+					–	–	–	,,	
54	March '06	720	8800	+	+	+	+		+			–	+	+	,,	
70	June '04	490	36000	+	+	+	+					+	+	+	Open, unprotected	

This well was obviously liable to marked pollution. Samples 31 and 37 collected within a week of one another and when there had previously been but very little rain for many weeks, show this pollution, while sample No. 73 also shows it very clearly. Sample 48 on the other hand taken after considerable rainfall was very different and did not show pollution. The water was only pumped to waste for 2 to 3 minutes before this sample was collected; probably if prolonged pumping had taken place the contamination would have been evidenced bacteriologically.

Samples 6, 24, 41 and 75 show a somewhat similar condition of affairs. They are from a similar well in the same village.

Samples 49 and 57 are of interest. This well supplies four houses. It is an open hole, rather than a well, about 4 feet deep with a wooden boxing round it and which only partially covers it. It is in a hollow and the water contained in it is apparently derived from a spring.

Behind and rising 6 feet above it is a heavily manured field. On one side about 10 feet away is an ordinary public road, and from the road a cutting conducts all the surface washings from the road direct into the well. With considerable rainfall the washings from the road must run down into the well, but when both the samples were collected the channel from the road was quite dry, and there had not been any rainfall for some time previously. The manured field behind the well is also a source of pollution. With only moderate rainfall the manurial organisms would have to pass through the 6 feet of soil before they could gain access to the water, but with heavy rain the washings would certainly overflow the 6 foot bank which rises steeply up from the well and so gain direct access to the water.

Here the condition of things showed that marked surface pollution was inevitable, but mainly dependent upon the rainfall. Both samples were collected when there had previously been but little rain and showed no streptococci or *B. coli* in either sample, much, I must confess, to my surprise.

This water, really a spring water, is a good illustration of a water supply which would be at once condemned on inspection but the dangers of which were not shown by the two samples bacteriologically examined. I have not found such a condition of affairs one at all commonly met with.

The 37°C. and 20°C. enumerations show extensive variations and cannot be said to be of much assistance in arriving at an opinion. It will be noticed however that the counts at 37°C. are in general high and show a marked difference from those recorded in Table I.

On the whole the *B. coli* and streptococci results show marked agreement. The streptococci results are much more uniform for the same well than are the *B. coli* findings.

The majority of the samples belong to *Series C* and it is for these that bacteriological assistance is most required.

The wells of this series form several distinct groups.

Group α forms the largest class. It consists of draw wells in country districts and villages. All the wells were situated in gardens or patches of ground attached to the houses but often at some little

distance away from the houses. The ground around them was usually cultivated and manured. As a rule they were fairly deep, and without exception they were brick lined without any impervious backing or rendering of the surface in any way. They were either completely open or partially protected by a matchboard covering provided with a hinged door. The brickwork of the well, for the great majority, was continued above the ground effectively preventing the direct access of surface waters. The water was collected in ordinary pails lowered and drawn up by the usual chain and handle.

Wells similarly situated and equally unprotected are commonly met with all over the rural parts of the country. The mouth of the well being unprotected pollution from the pail used, and in other ways, is to some extent inevitable, but apart from this, organisms would have to filter through some depth of earth before they could gain access to the water.

The results of the examination of a number of such wells are shown in Table III.

With these results may be compared those shown in Table IV. This table shows the results of the examination of wells (Group β) quite similarly situated to those of Group α, the essential difference being that they were provided with a pump, the well being otherwise completely covered over and protected from surface pollution.

The comparison between Tables III and IV is very instructive. For example the pump from which samples 29, 36, 47, 82 were collected is in the same village and quite near to the wells from which samples 5, 22, 39, 70 and 7, 23, 38, 79 and 8, 28, 74 were obtained. In other words they have the same subsoil water. Sample No. 30 was from another pump in the same village.

The first-named well (No. 29 etc.) had been much more recently constructed than the others and was in a small grass field, the ground round was not manured. Consequently this well had advantages over the others in addition to being covered, but No. 30 was quite comparable to the others.

The results show what an extensive part surface pollution plays in altering the results of the examination, and probably if many of these open wells had been properly covered in and provided with a pump so that all surface pollution is avoided the bacteriological results would not have shown extensive pollution.

It must be remembered that the soil contamination in these rural areas is comparatively slight as regards human pollution, although

TABLE III.

No. of sample	Date of examination	No. of organisms per c.c. developing at 37° C.	No. of organisms per c.c. developing at 20° C.	'Excretal' *B. coli* in 0·1	'Excretal' *B. coli* in 1	'Excretal' *B. coli* in 10	'Excretal' *B. coli* in 40 c.c.	Coli-like organisms isolated from 0·1	Coli-like organisms isolated from 1	Coli-like organisms isolated from 10	Coli-like organisms isolated from 40 c.c.	Streptococci in 1	Streptococci in 10	Streptococci in 40 c.c.	Class of Well	Remarks
2	Feb. '04	1500	5000	−	−	−	−		+			−	−	−	Draw	
3	March '04	82	5000	−	−	−	−		+			−	−	−		
60	April '06	18	14500	−	−	−	+		+			−	−	−		
5	April '04	186	1700	−	−	−	−					−	−	−	Draw	
22	Oct. 4, '05	1130	2720	−	−	−	−		+	+		+	+	+		
39	Oct. 26, '05	340	8600	−	−	+	+					−	+	+		
78	Sept. '06	450	...	+	+	+	+					+	−	+		
7	April '04	206	2600	−	−	−	−					+	+	+	Draw	
23	Oct. 4, '05	900	2800	−	+	+	+					+	+	+		
38	Oct. 18, '05	2080	13700	−	−	−	−		+			+	+	+		
79	Sept. '06	2320	...	+	+	+	+					−	+	+		
8	April, '04	44	1600	−	−	−	−		+			−	+	+	Draw	
28	Oct. '05	110	5170	−	−	−	−	+				+	+	+		
74	Aug. '06	370	...	−	−	−	−		+			−	−	+		
9	May '04	...	2500	−	+	+	+					−	−	−	Open; draw	
16	Feb. '05	220	over 50000	−	−	−	−					−	−	−	Draw	Recently cleaned out.
20	July, '05	30	...	−	+	+	+					−	−	−	,,	
27	Oct. 9, '05	415	5460	−	+	+	+					+	+	+	Draw	
35	Oct. 18, '05	645	13400	−	+	+	+					−	−	+		
80	Sept. '06	3250	...	−	−	−	−					+	+	+		
32	Oct. '05	305	14100	−	+	+	+					+	+	+	Draw	
81	Sept. '06	950	...	−	+	+	+					+	+	+		
33	Oct. '05	272	6440	−	−	+	+		+			+	+	+	Draw	
76	Aug. '06	1300	...	−	−	+	+		+			+	+	+		
34	Oct. '05	134	3220	+	+	+	+					−	−	−	Draw	
84	Sept. '06	296	...	−	+	+	+					+	+	+		
43	Nov. '05	27	3300	−	−	−	−		+			−	−	−	Draw	
62	April '06	165	14000	−	−	−	−					−	−	−	,,	
15	Dec. '04	3420	over 50000	−	−	−	−		+			−	−	+	Protected	Recently disturbed.

TABLE IV.

No. of sample	Date of examination	No. of organisms per c.c. developing at 37° C.	No. of organisms per c.c. developing at 20° C.	'Excreta' *B. coli* in 0·1	1	10	40 c.c.	Coli-like organisms isolated from 0·1	1	10	40 c.c.	Streptococci in 1	10	40 c.c.	Class of Well	Remarks
29	Oct. 9, '05	0	350	–	–	+	+					–	–	–	Pump	
36	Oct. 18, '05	5	530	–	–	–	–			+		–	–	–	Pump	
47	Feb. '06	4	530	–	–	–	–					–	–	–	Pump	
82	Sept. '06	6	...	–	–	–	–					–	–	–	Pump	
46	Nov. '05	9	2020	–	–	–	–					–	–	–	Pump	Same well as No. 12 Table.
30	Oct. '05	78	1060	–	–	–	–					–	–	+	,,	
44	Nov. '05	20	4260	–	–	–	–					–	–	–	,,	
63	April '06	5	505	–	–	–	–				+	–	–	–	,,	
56	March '06	...	1170	–	–	–	–				+	–	–	+	Pump	
59	April '06	6	1330	–	–	–	–				+	–	–	–	Pump	
72	Aug. '06	12	...	–	–	–	+					–	–	+	,,	
77	Nov. '06	143	5300	–	–	+	+		+			–	+	+	,,	
86	Sept. '06	4	...	–	–	–	–		+			–	–	–	,,	

TABLE V.

13	Dec. '04	30	480	–	–	–	+					–	–	–	Pump	
14	Dec. '04	0	40	–	–	–	–					–	–	–	,,	
50	March '06	4	150	–	–	–	+					–	+	+	Pump	
68	April '06	8	700	–	–	–	–			+		–	–	–	Pump	
51	March '06	10	220	–	–	–	–					–	–	–	Pump	
52	March '06	9200	innumerable	–	+	+	+					–	–	+	Pump	
69	April '06	112	7700	–	–	–	–		+			–	–	–	Pump	
53	March '06	38	570	–	–	–	–					–	–	+	Pump	
64	April 11, '06	72	2300	–	+	+	+		+			–	–	–	Pump	
67	April 24, '06	16	1340	–	+	+	+	+	+			–	–	–	Pump	
65	April '06	38	5300	–	–	–	–			+	+	–	–	–	Pump	
66	April '06	26	5200	–	–	+	+					–	–	–	,,	

sometimes the ground round, or at least near, the wells is extensively manured. As a rule, however, this does not extend up to within several yards of the well so that all the organisms applied to the surface would have to pass through a good many feet of soil before they could gain access to the water supply. The filtering power of the soil is great and frequently much underrated, and the results here recorded lend much support to the view that if such wells are properly covered and provided with pumps, and particularly if, in addition, they are rendered impervious to a depth of a few feet, they form, in really rural districts, a reasonably safe source of supply. A depth of 10 to 12 feet is sufficient for the well to be imperviously lined. Failing this a definite area round the well should be rigidly protected.

The difference between the open draw wells and the pump provided wells is clearly shown by the *B. coli* and streptococci results, but it is even more marked in the enumerations, especially the 37° C. determinations.

A comparison between the two samples 62 (Table III) and 64 (Table IV) brings this out very clearly. Both samples were collected the same day, the two wells are quite close to one another, and if proximity to houses and possible sources of pollution is a test of purity, No. 62 is the better sample. As regards *B. coli* and streptococci both are satisfactory, but the difference between the open well and the surface protected well with proper pump is very obvious for both the 37° and 20° enumerations.

Samples 12 and 46 both from the same well are very instructive.

Sample 12 (Table II) was collected Sept. 1904. When then seen the well was covered and provided with a pump, but the covering was defective and less than 10 feet away was a cesspool which was leaking into a field adjacent to the well. The well was a very shallow one and not rendered impervious in any way, but was lined with bricks. The well was condemned, and to meet my requirements was deepened, rendered impervious by a cement lining and a thick backing of clay, to a depth of 10 feet, while it was properly covered, so that surface water could not gain access. At the same time the drains were relaid and an impervious cesspool provided.

The second sample (No. 46, Table IV), collected November, 1905, about a year after the alterations, showed no *B. coli* or streptococci in 50 c.c. and may be considered satisfactory.

An entirely fresh source of drinking water was not available, but the above shows that it may be possible by suitable alterations to convert an initially highly contaminated source of supply into one which may be considered satisfactory and free from risk.

The chemical analysis, as far as it was carried out, for seven of these wells is given in Table VI. These wells were all in the same village. The chief interest of the figures is that the results do not show the

differences met with on bacteriological examination, or certainly not to anything like the same extent. Compare for example the chemical and bacteriological analyses of the wells from which samples 27 and 29 were respectively taken.

The remaining wells of *Series C* can be included in a third group—Group γ. This group consists of wells situated in the town itself and usually surrounded with houses. The houses supplied have for one reason or another avoided taking the public supply of the town, remaining content to use the old well water. Without exception they were covered and provided with pumps. The houses surrounding them were all drained and provided with water-closets, no privies or known cesspools being in their neighbourhood. The soil round should therefore be free from pollution. They are all old wells and, although not examined, it can be assumed as almost certainly true that none of them would have their interior rendered at all impervious.

The position of this group of wells would certainly render them liable to suspicion on inspection, but if a properly constructed and tight drainage system is present, and the coverings are water-tight, such wells may really not be liable to dangerous pollution. Bacteriological examination is here particularly valuable to say whether they are actually being contaminated or not.

The results of the examination of a number of such wells are shown in Table V.

Samples 13 and 14 are taken from wells surrounded by houses which tap a considerable supply of water in the middle of the town of Colchester, water which finds an outcrop as a spring about a quarter of a mile away on the side of a narrow valley of considerable depth. It is evidently a pure supply as shown by sample 14 although it flows under a highly populated area.

Sample 13, a well quite close to No. 14, is rather less satisfactory, apparently because the water is stored in a large tank, and the sample was collected after storage in the tank.

Samples 50, 68, 51, 52 and 69 are from wells supplied with pumps, all of which tap this same underground water. On the whole, except No. 52 well, they are not unsatisfactory. For this well the contamination was probably from surface water gaining access.

Mere inspection of these wells is insufficient to determine if they are contaminated or not. Thus the well from which samples 64 and 67 were collected, was on topographical inspection certainly as favourably situated as wells 13, 14, 51, 53, etc. and it was impossible from inspection alone to say that it was contaminated. The bacteriological results were however conclusive, and the well was closed.

TABLE VI.

Chemical Analyses figures. Samples all collected Oct. 1905.

Source of the samples	Physical characters (in 2 foot tube)	Reaction	In parts per 100,000: Chlorine	Total solids	Nitrogen as nitrates and nitrites	Saline ammonia	Albuminoid ammonia	Sediment
The same well as 27, 35, 80	Slightly turbid, colourless	Neutral	4·3	34	0·64	0·007	0·008	Abundant débris, some animalculae.
32, 81	Clear, colourless	Faintly acid	5·0	—	0·36	0·012	0·019	Slight, a few animalculae.
29, 36, 47, 82	Quite clear, colourless	Neutral	5·9	62	0·84	0·008	0·006	Nil.
7, 23, 38, 79	Somewhat turbid, colourless	Faintly acid	4·0	—	0·54	0·01	0·016	Considerable, some animalculae.
25, 40, 83	Somewhat turbid, yellow brown	Faintly acid	4·8	—	0·84	0·01	0·0196	Slight débris and a few animalculae.
5, 22, 39, 78	Turbid, slightly yellow	Faintly acid	4·7	—	0·59	0·008	0·013	Considerable. Vegetable débris, animalculae.
6, 24, 41, 75	Quite clear, colourless	Faintly acid	4·1	—	0·48	0·01	0·015	Slight. Nematode worms found.

TABLE VII.

	Unpolluted (Series A)	Obviously liable to pollution (Series B)	Doubtful (Series C)	Results stated as percentage: Total	Unpolluted	Obviously liable to pollution	Doubtful
Number of samples examined	10	22	54	86	—	—	—
'Excretal' *B. coli* present in 0·1 c.c.	0	4	3	7	0	18	6
,, ,, ,, 1·0 ,,	0	11	14	25	0	50	26
,, ,, ,, 10 ,,	0	12	20	32	0	55	37
,, ,, ,, 40 ,,	1	13	24	38	10	59	44
,, ,, absent in 50 ,,	9	9	30	48	90	41	56
Streptococci present in 1 c.c.	0	6	13	19	0	27	24
,, ,, 10 ,,	0	15	17	32	0	68	31
,, ,, 40 ,,	1	17	26	44	10	77	48
,, absent in 50 ,,	9	5	28	42	90	23	52

Dealing with the results of all the analyses one fact stands out strikingly, and this is the close relationship between the *B. coli* and the streptococci results.

This is brought out in Table VII. For *Series A* the results are identical. For *Series B* they are very similar, while for *Series C* they

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are almost identical. Thus for *Series C, B. coli* and streptococci are present in 1 c.c. in 26 and 24 per cent.; in 10 c.c. in 37 and 31 per cent., and in 40 c.c. in 44 and 48 per cent. respectively.

This table also enables a rough comparison between the topographical and bacteriological results to be made.

PART II.

The characters of the coliform organisms isolated from surface wells with a consideration of their significance and origin.

Excluding a large number of organisms isolated but only incompletely investigated, 86 organisms were isolated and their characters determined to the extent shown in Table VIII. They can be conveniently classified into the following groups:

Group	Number isolated	Percentage	
1. 'Excretal' *B. coli*	23	29·5	55·1
2. 'Excretal' *B. coli* modified as indicated	20	25·6	
3. Modified *B. coli*-like organisms	10	12·8	
4. Non-lactose fermenters	17	21·8	
5. Considerably variant organisms	8	10·3	
6. Non-glucose fermenters	8	—	

The percentages are calculated after exclusion of the non-glucose fermenters, as only a few of these were worked out, generally for some special reason. The percentages refer only to glucose fermenters.

In Groups 1 and 2 the term *excretal B. coli* is used in the sense suggested by me in a previous paper[1] for an organism which is a short bacillus with rounded ends, producing a translucent non-corrugated appearance on gelatin slope; non-liquefaction of gelatin (2 weeks); permanent acid production in litmus milk with clotting of the milk within two weeks; lactose and glucose fermentation with both acid and gas production; a positive neutral-red reaction, and production of indol. Only 20 organisms conformed in every respect to the above characters, but with these may be included two organisms which were typical except that the neutral-red reaction was only partial, and a third which produced a rather thicker and whiter growth on the gelatin slope but was otherwise typical.

[1] Savage, W. G. (11. 1905). "The Characters of the *Bacillus coli* as an Indicator of Excretal Contamination," *Lancet*, vol. 1., p. 284.

TABLE VIII.

Characters of the organisms isolated.

No.	Source. Sample	Motility	Gelatin slope	Liquefaction of gelatin	Litmus milk: Acid	Litmus milk: Coagulation	Litmus milk: Time in days taken to coagulate milk	Lactose fermentation: Present	Lactose fermentation: Rate	Glucose fermentation	Saccharose fermentation	Dulcite fermentation	Indol fermentation	Neutral-red reaction	Starch fermentation	Remarks
1	1	–	Yellow growth	+	+	+	6	+	ss	+	–		+	+		Liquefies gelatin slowly (4 weeks).
2	2	+	Smooth bluish translucent growth	+	+	–		+	ss	+	–		–	+	–	,, ,, ,, (2 months).
3	3	+	,, ,, ,, ,,	+	+	–		+	ss	+	–		–	+	–	,, ,, ,, ,,
4	6	+	,, ,, ,, ,,	–	+	–		–		+	–		+	+		
5	8	+	,, ,, ,, ,,	–	+	–		ac		+	–		+	+		
6	9	+	,, ,, ,, ,,	–	+	+	7	+	slow	+	–	–	+	+	–	
7	70	+	,, ,, ,, ,,	–	+	+	1	+	r	+	+	–	+	p	–	
8	12	+	,, ,, ,, ,,	–	+	+	2	+	r	+			+	+		
9	13	+	,, ,, ,, ,,	–	+	+	5	+	r	+			+	+		
10	15	+	,, ,, ,, ,,	–	+	–		+	ss	+	–	–	+	+		
11	18	–	,, ,, ,, ,,	–	+	+	3	+	r	+	+	–	+	+		
12	19		,, ,, ,, ,,	–	+	+	1	+	r	+	–		+	+		
13	20	+	,, ,, ,, ,,	–	+	+	1	+	r	+			+	+		
14	22	+	Yellow slightly wrinkled growth	+	+	+	6	ac		+	+	+	–	+		Liquefies gelatin only after 3 months. Atypical gelatin colonies.
15	22	+	Yellow semi-translucent growth	+	+	+	7	+	ss	+	ac	+	+	+		Liquefies gelatin only after 4 months.
16	23	–	Smooth bluish translucent growth	–	+	+	3	+	r	+			+	–		
17	24	+	,, ,, ,, ,,	–	+	+	6	ac		+	–		–	+		
18	24	–	,, ,, ,, ,,	–	+	+	9	+	r	+			+	–		
19	26		,, ,, ,, ,,	–	+	+	2	+	r	+			+	+		
20	27	+	,, ,, ,, ,,	–	+	+	7	+	ss	+	+	–	+	+		
21	28	+	,, ,, ,, ,,	–	+	–		–		+	+	–	+	+		
22	29	–	Smooth semi-translucent white growth	–	+	+	2	+	r	+	–	–	+	–		
23	31	+	Translucent, yellow growth	–	+	+	7	+	slow	+	ac	+	+	–		On re-examination showed slight gelatin liquefaction after 1 month.
24	31	–	Smooth bluish translucent growth	–	+	–		ac		+	–	–	+			
25	31	+	Yellow, semi-translucent growth	–	+	+	6	+	slow	+	+			+		
26	32	+	Smooth bluish translucent growth	–	+	+	13	+	r	+			+	–		
27	33	–	,, ,, ,, ,,	–	+	+	7	+	r	+	+	+	+	+		
28	33		,, ,, ,, ,,	–	+	+	7	+	ss	+	–	–	+	+		
29	34	+	,, ,, ,, ,,	–	+	+	6	+	r	+	–	+	+	–		
30	35	+	,, ,, ,, ,,	–	+	+	7	+	r	+	+	–	+	+		
31	36	–	,, ,, ,, ,,	–	+	–		+	r	–	ac	–	+	–		Produces acid but no gas in mannite and maltose media.
32	37	+	Transl. growth : later distinctly yellow	–	+	+	6	+	slow	+	+	+	+	+		
33	37	+	Smooth bluish translucent growth	–	+	+	6	+	r	+			+	+		
34	38	–	White, opaque growth	–	+	+	13	+	slow	–			+	–		
35	38	–	,, ,, ,,	–	–	–		+	slow	–			+	–		
36	39	–	Smooth bluish translucent growth	–	+	+	1	+	r	+	–	+	+	+		
37	40	+	,, ,, ,, ,,	–	+	+	1	+	r	+	–	+	+	+		
38	41	–	,, ,, ,, ,,	–	+	+	2	+	r	+	–	–	+	+		
39	42	+	,, ,, ,, ,,	–	+	+	10	+	r	+			+	+		

40	43	+	Smooth very translucent growth				−	+	−		+	r	+	−	+	+	+	−
41	50	−	Smooth bluish translucent growth				−	+	+	12	+	r	+	−	+	+	−	
42	50	−	,,	,,	,,	,,	−	+	+	11	+	r	+	−	+	+	p	
43	51	+	,,	,,	,,	,,	−	alk	−		−		−	−	−	+	−	
44	52	−	,,	,,	,,	,,	−	+	+	5	+	ss	+	+	−	+	+	
45	54	+	,,	,,	,,	,,	−	+	+	20	+	r	+	+	+	+	+	
46	54	+	,,	,,	,,	,,	−	+	−		+	slow	+	+	−	+	p	
47	55	−	,,	,,	,,	,,	−	+	−		+	ss	+	−	−	+	−	
48	55	+	,,	,,	,,	,,	−	alk	−		−	−	+	−	−	+	+	
49	55	−	Smooth white semi-translucent growth				−	+	−		+	r	−	−	−	+	−	
50	56	−	,,	,,	,,	,,	−	+	−		+	r	−	−	−	+	−	
51	58	+	Smooth bluish translucent growth				−	+	+	2	+	r	+	+	+	−	+	
52	59	+	,,	,,	,,	,,	−	+	−		ac		+	+	+	−	p	
53	59	+	,,	,,	,,	,,	−	alk	−		−		−			+	−	
54	60	+	,,	,,	,,	,,	−	+	−		−		+	+	−	+	−	
55	60	−	,,	,,	,,	,,	−	+	+	2	+	r	+	−	+	+	+	
56	62	+	,,	,,	,,	,,	−	+	+	8	ac		−	−	−	+	+	
57	63	+	,,	,,	,,	,,	−	+	−		−		+			+	p	
58	64	+	,,	,,	,,	,,	−	+	−		ac		+	−	−	+	+	
59	64	−	Smooth white & somewhat opaque growth				−	+	+	2	+	r	+	+	−	−	+	−
60	64	+	Smooth bluish translucent growth				−	+	+	5	+	slow	+	+	−	+	+	
61	65	+	,,	,,	,,	,,	−	+	−		+	r	+	−	−	+	+	
62	66	+	,,	,,	,,	,,	−	+	+	1	+	r	+	−	+	+	+	
63	67	+	,,	,,	,,	,,	−	+	+	21	+	r	+	−	−	+	+	
64	67	−	,,	,,	,,	,,	−	+	+	3	+	r	+	+	−	+	−	
65	67	+	,,	,,	,,	,,	−	+	−		+	ss	+			−	−	
66	68	+	,,	,,	,,	,,	−	+	−		ac		+	−	−	−	−	
67	69	+	,,	,,	,,	,,	−	+	−		−		+			+	p	
68	71	+	,,	,,	,,	,,	−	+	+	12	−		+	−	+	+	−	
69	72	+	,,	,,	,,	,,	−	+	+	7	+	r	+	−	−	+	−	
70	73		,,	,,	,,	,,	−	+	+	8	+	r	+	+	+	+	−	
71	73	+	,,	,,	,,	,,	−	+	+	11	+	r	+	−	+	−	−	
72	74	+	,,	,,	,,	,,	−	+	+	5	+	r	+	−	+	−	−	
73	75		,,	,,	,,	,,	−	+	−		−		+			−	−	
74	75	+	Smooth rather white growth				−	+	+	9	+	slow	+	+	+	−	−	−
75	75	+	Smooth bluish translucent growth				−	+	−		+	slow	+	−	−	−	+	
76	76	+	,,	,,	,,	,,	−	+	−		−		+	−	−	tr	−	
77	76	+	,,	,,	,,	,,	−	+	+	3	+	r	+	−	+	+	+	
78	78	−	,,	,,	,,	,,	−	+	+	5	+	r	+	+	−	+	−	
79	79	+	,,	,,	,,	,,	−	+	+	9	+	r	+	−	+	+	+	
80	81	−	,,	,,	,,	,,	−	+	+	3	+	r	+	−	−	+	+	
81	83	+	,,	,,	,,	,,	−	+	+	3	+	r	+	−	+	+	+	
82	84	+	,,	,,	,,	,,	−	+	+	21	+	r	+			+	+	
83	85	+	,,	,,	,,	,,	−	+	−		ac		+	−	−	−	+	
84	86	+	,,	,,	,,	,,	−	+	−		ac		+	−	−	−	p	
85	77	+	,,	,,	,,	,,	−	+	−		+	ss	+			+	−	
86	77	+	,,	,,	,,	,,	−	+	+	1	+	r	+	−	+	+	−	

ss = slow and slight lactose fermentation as measured by the amount of the gas produced.
r = rapid gas production in lactose media.
ac = acid only, no gas.
p = partial neutral-red reaction.

As regards the 20 organisms of Group 2, for 11 of them the only difference was that no neutral-red reaction was given, for 5 the lactose fermentation was delayed and in several of these cases the gas produced was only slight in amount, for 3 the clotting of the milk was delayed, while for the remaining organism no indol was produced, but on retesting after a week's isolation indol was found to be present in slight amount. The variations from the 'excretal' type form are here very slight, and these two groups, forming rather over one half of the glucose fermenters, are included in Tables I to V as *excretal B. coli.*

Group 3 includes 10 organisms which differ in several particulars from *excretal B. coli,* for example in not coagulating milk. Group 4, that of non-lactose fermenters, includes 9 organisms which produced neither acid nor gas in lactose and 8 which produced a little acid but no gas. Group 5 includes organisms considerably modified, 6 of them producing a yellow growth on gelatin slopes while the other two, apparently identical organisms, slowly liquefied gelatin.

Only 29·5 per cent. were quite typical, and a characteristic feature of the bacteriology of surface well water is the large percentage of atypical organisms met with. In many of the samples from undoubtedly contaminated wells, no quite typical *B. coli* could be found, but organisms were isolated which deviated in several particulars from the accepted excretal type. In other cases the typical and the atypical coli were found side by side.

The origin and significance of these variant forms are of great interest and practical importance.

Three suppositions are possible as to their origin :—

(1) That they are derived from normal typical *B. coli* of faeces, which have become atypical by the loss of certain attributes owing to unfavourable environment. (2) That they are derived from identical atypical organisms in faeces or sewage, which, owing to greater hardiness and adaptability, have flourished better than the typical varieties, and have thus become relatively more abundant. (3) That they are true saprophytes, natural to water or soil, and are not of excretal origin.

The last supposition seems improbable, since if this were the true explanation we should expect to meet with such organisms equally in pure and impure soil, in pure water equally with polluted water. This, at least for the forms only slightly differing from *B. coli* as met with in the intestine, is not found to be the case, and these organisms are undoubtedly more prevalent in polluted than in pure

sources. Indeed they cannot be isolated from quite uncontaminated sources. It is highly probable therefore that their primitive origin is to be found in excretal matter.

If these organisms were derived from typical forms, two questions obviously arise: (*a*) Can such atypical organisms be converted into typical forms by artificial laboratory methods? (*b*) Can typical organisms, by cultivation in soil or water, be made to become atypical?

A summary of the experimental work of other observers in these two directions is given in my book on *The Bacteriological Examination of Water Supplies* (London, 1906), pages 145 to 153. Here I only wish to record and consider some additional experiments recently made in these directions.

As regards the first question (*a*) only two points were dealt with. The organisms which ferment lactose slowly might be considered to be typical *B. coli* which had lost the power to split up lactose vigorously owing to their saprophytic environment. The three organisms which were retested did not altogether bear out this view: No. 32 tested after 5 months in the laboratory (on gelatin slope) still showed only slow lactose fermentation. No. 44 retested after 2 months in the laboratory, showed now no gas production, but it formed acid. No. 28 retested after 6 months showed no change in the lactose medium, neither acid nor gas being formed.

The other character investigated was milk coagulating power:—

Nine organisms, which when isolated did not coagulate milk, were inoculated into litmus milk containing solid calcium carbonate. Four of the same organisms were also reinoculated into ordinary litmus milk. They were all incubated at 37°C. and were not disturbed or re-examined for a month. They were subsequently re-examined at intervals with the following results:

None of the 4 ordinary milk tubes showed any coagulation after one month and the two which were kept for two months also showed no coagulation. They all produced acid.

Six of the chalk-milk tubes, after one month, showed complete coagulation; 3 showed acid only. Of these 3, two, re-examined after 38 days, were completely clotted, while the other was alkaline and not coagulated. Coagulation was obtained therefore in 8 out of 9 instances in the chalk-milk.

These experiments would seem to show that the coagulation in these late cases is not due to the acid produced but rather to a ferment.

The calcium carbonate by neutralizing part of the acid produced allowed the organism to continue its growth until a sufficient amount of ferment was produced to coagulate the milk. This hypothesis is quite in accord with results obtained by me in 1904[1], but I hope again to obtain the results with other strains of this organism.

As regards the second question (*b*) more extended work was carried out. It is obvious that sojourn of *B. coli* is likely to be more prolonged in soil than in water, and if alteration of character is to be traced it is more likely to be met with soil as the saprophytic environment than if water is the medium selected. Houston watered soil with sewage, but few experiments have been carried out with typical *B. coli* added to non-sterile soil and under quite natural conditions.

In the experiments carried out by me a number of carefully selected *B. coli* strains were added to natural soils and their fate studied through prolonged periods.

Two separate series of experiments were carried out with two different patches of soil which were free from *B. coli*. The patches were formed by removing the covering turf with a sterile spade, each was about 18 × 18 inches. The two soil areas were fairly near one another and consisted of a brown sandy soil, which had not been manured for many years, certainly not within 3 years. It was ordinary grass pasture land. Samples of the soil were removed by a sterile spatula from the surface from time to time, placed in sterile bottles and at once transmitted to the laboratory and there examined without delay. All the samples were personally collected by the writer.

Experimental Patch, A.

Three preliminary samples were collected before inoculation, *i.e.* on March 26th and 28th and April 4th, 1906. On *March* 26th two organisms P_1 and P_2 were isolated, but on the occasion of the other two examinations no organisms at all like *B. coli* were isolated although as much as 3 grammes of soil were examined on each occasion. P_1 and P_2 certainly were not *B. coli* (their characters are dealt with later on) so that it may be said with considerable confidence that *B. coli* was absent from this experimental soil.

On *April* 9th, the patch was watered with a mixture of *B. coli* races. For this purpose five strains of *B. coli*, obtained, two from polluted soil and three from cow manure, and one strain of *Bacterium lactis aerogenes* (derived from milk), were subcultivated on gelatin slope. From this slope each was inoculated into

[1] Savage, W. G. (1904). "The coagulation of milk by *Bacillus coli communis*," *Journ. of Path. and Bacteriol.*, vol. x. p. 90.

broth and 1 c.c. of the two days' broth culture of each was added to a litre flask of sterile water.

On *April* 9th this mixture was distributed over the patch. The six organisms had their characters re-determined immediately before being selected for the inoculation. They were all completely typical, and indeed were selected on this account.

Also the five *B. coli* strains from their action on saccharose and dulcite could be divided into three groups. Their characters are given in Table IX. The five coli strains are $S_1 S_2 S_3 S_4 S_5$ and the aerogenes S_6. The patches were left exposed and under perfectly natural conditions throughout, except that Patch A, between 9th and 19th April, received daily a quart of sterile water, since the weather was extremely dry.

On *April* 19th three samples were collected from Patch A. Ten organisms were isolated and their characters determined from the three different soils. Of these organisms four proved to be *B. coli* and with characters quite unchanged. The other six were all *Bact. aerogenes* and were quite unchanged from the original except that five now gave no neutral-red reaction and the other a partial reaction.

For the actual isolation of these organisms and for the examination of other samples, plates of Drigalski-Conradi medium and glucose neutral-red bilesalt agar were employed. In all cases the subcultivations were made in the first place into glucose neutral-red broth. If this was not fermented the organisms were not further examined, so that this investigation does not deal with organisms which have lost (if such be the case) the power to ferment glucose.

On *May* 9th (one month from seeding), three further soil samples from different parts of the patch were collected. In the same way 13 organisms were fully investigated. Of these two were *B. coli*. They both were perfectly typical. The remaining 11 were *Bact. aerogenes*. They were quite typical and like the original strain, except that six gave no neutral-red reaction and one a partial reaction. The aerogenes organism had evidently greatly outgrown the other five strains added.

On *June* 19th (ten weeks from seeding) three additional samples were collected. Only six of the isolated organisms fermented glucose. Of these two were *B. coli* and four *Bact. aerogenes*, although as far as possible *B. coli* colonies were selected for subcultivation.

Both the *B. coli* subcultivations isolated showed some deviation from the original forms. They belonged to separate strains (*a* and *b*), since one fermented dulcite, but not saccharose, while the second fermented both these substances. The one (*a*) only differed from *B. coli* in that it fermented lactose but slowly and to a slight extent. Instead of producing at least 1 inch of gas in the inner tube within 24 to 48 hours, this organism after 48 hours formed $\frac{1}{3}$ inch gas and acid and after a week's incubation at 37° C. not more than $\frac{1}{2}$ inch of gas was produced. The other (*b*) *B. coli* strain in its action towards lactose was identical, producing, if anything, even less gas. This organism also exhibited no motility (24 hours' gelatin slope growth in broth in hanging-drop preparation) while the neutral-red reaction was incomplete.

The four aerogenes organisms were typical, except that one gave only a partial neutral-red reaction and the other three no trace of reaction.

On *June* 23rd, one week later, fresh samples were collected. Although 18 different organisms were subcultivated only five fermented glucose, and all these were *Bact. aerogenes*.

TABLE IX.

Number	Source	Motility	Gelatin slope	Liquefaction of gelatin	Litmus milk	Lactose fermentation		Glucose	Saccharose	Dulcite	Indol	Neutral red reaction	Starch
						+	r	+	+	+	+	+	
S_1	Cow excreta	+	Smooth bluish translucent growth	–	Acid and clot within two days								
S_2	Soil	+	,, ,, ,,	–	,, ,,	+	r	+	+	+	+	+	
S_3	,,	+	,, ,, ,,	–	,, ,,	+	r	+	+	–	+	+	
S_4	Cow excreta	+	,, ,, ,,	–	,, ,,	+	r	+	–	+	+	+	
S_5	,, ,,	+	,, ,, ,,	–	,, ,,	+	r	+	–	+	+	+	
S_6	Milk	–	Thick, white growth	–	,, ,,	+	r	+	+	–	+	+	+
S_7	Cow excreta	+	Smooth bluish translucent growth	–	,, ,,	+	r	+	+	+	+	p	
S_8	Soil	+	,, ,, ,,	–	,, ,,	+	r	+	+	+	+	p	
S_9	Surface well	+	,, ,, ,,	–	,, ,,	+	m	+	–	+	+	p	
S_{10}	Soil	+	,, ,, ,,	–	,, ,,	+	r	+	–	+	+	p	
S_{11}	Surface well	+	,, ,, ,,	–	,, ,,	+	r	+	+	+	+	–	
P_1	Soil	+	,, ,, ,,	–	Acid no clot	–		+	–	–	–	–	
P_2	,,	+	,, ,, ,,	–	,, ,,	+	slow	–	–	–	–	–	

r = rapid gas production.
m = moderate and not abundant gas production.
p = partial neutral-red reaction.

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All five fermented lactose slowly and with little gas production. Four produced only a partial neutral-red reaction. One produced no indol. These particular strains when re-examined after being kept on gelatin slope for two months gave still a delayed lactose fermentation, yielding practically no gas after two days but a normal amount after a week, while the strain which gave no indol, now produced it in large amount.

On *Oct.* 19th, the last sample was collected, *i.e.* 6½ months from the original seeding. Very few glucose fermenters could be isolated, only seven organisms being found which fermented glucose. No glucose fermenters were present in 0·1 gramme.

These seven organisms were all identical. They differed widely from *B. coli.* They agreed in that the growth on the gelatin slope was typical, they were actively motile coli-like bacilli, produced indol, and rapidly fermented glucose. Neither dulcite nor saccharose was fermented.

The points of difference were: no trace of neutral-red reaction was produced; although acid was formed in litmus milk no coagulation took place even after three weeks' incubation; lactose was only slightly fermented. In all acid was produced but either no gas or only a bubble or two.

The point at once arises, are these organisms altered *B. coli*?

The patch was protected from artificial contamination, but to be certain that they were not derived from adventitious pollution the surface soil just by the side of Patch A was examined, but no organisms of this or the *B. coli* group were found in as much as 3 grammes of it.

It may be assumed that these organisms either were derived from the *B. coli* (and aerogenes) strains added or were derived from organisms present in the soil at the time of inoculation. The two organisms P_1 and P_2 were isolated from the patch before inoculation. Their characters are given in Table IX. The only differences from the above are as regards glucose and lactose fermentation. Thus P_1 differs only in that there is no trace of lactose fermentation. P_2 differs only in that there is slow but considerable gas production in lactose media, while glucose is not at all fermented.

Two of the above organisms were re-investigated as regards their action on lactose after subcultivation in broth, gelatin slope, and broth and then into lactose media. They only fermented lactose with acid but no gas production, thus making them nearly indistinguishable from P_1.

In view of these facts it is not justifiable to say that these atypical organisms were derived from the typical *B. coli* with which the soil was inoculated.

Apparently all the *B. coli* had died out or become non-glucose fermenters.

Experimental Patch, B.

This patch was similar in character to Patch A. No *B. coli* or coli-like organisms were found in it before inoculation.

On *July* 8th, 1906, the patch was watered with five strains of *B. coli* (S_7 S_8 S_9 S_{10} S_{11} Table IX) in the same way as Patch A. They were all quite typical except that one produced no neutral-red reaction and the other four a partial reaction only. These organisms differed in two particulars from those used for Patch A in that, in the first place no *Bacterium aerogenes* was used, and in the second, several of the strains were organisms which showed some instability of character.

Thus S_9 when isolated did not coagulate milk until after 11 days' incubation, while it then showed no motility. Also S_{11} when isolated yielded no indol and gave a complete neutral-red reaction.

On *August* 16th, the first samples after inoculation were collected (*i.e.* 40 days after seeding). Eight glucose fermenting organisms were studied. All failed to give a neutral-red reaction, two gave no indol and two gave a delayed time for milk coagulation (7 and 12 days respectively). Otherwise quite typical.

Sept. 13th further samples collected, 9½ weeks after inoculation. Nine glucose fermenters were studied. Most of them showed no neutral-red reaction, while for all but two milk coagulation was markedly delayed. The actual times taken to coagulate milk were 6, 22, 11, 21, 9, 21, 4, 20, 19 days respectively. They all fermented dulcite like the originals and seven fermented saccharose and two not.

Part of the original batch of milk media tubes was used for the inoculations, while five were reinoculated into freshly prepared milk tubes with almost identical results.

Oct. 19th (after 15 weeks) a fresh sample was collected. No glucose fermenters could be isolated.

Oct. 25th (after 15½ weeks) a fresh sample collected. Six glucose fermenting organisms isolated. Except for absent or incomplete neutral-red reaction and a slightly delayed time for milk coagulation for two of the isolated forms, they were all completely typical.

It is to be noticed that all six fermented both dulcite and saccharose, while the organism which coagulated milk in four days from the Sept. 13th sample also fermented both these substances. The alteration in coagulation time may possibly be explained by the fact that this strain had multiplied or happened only to be present where the actual sample was collected.

Nov. 12th (after 18 weeks) a final sample was collected. Very difficult to find any glucose fermenters at all and only three were met with. They varied as regards their action on neutral-red but otherwise were identical and quite typical. They all fermented both saccharose and dulcite.

The results of the two series of soil investigations are not altogether easy to explain. In the first place they show that the neutral-red reaction is an unstable character for these organisms kept in soil. As regards the other characters indol producing power remained stable, the gelatin slope characters were unaltered and the motility was unimpaired.

In no case was definite loss of power to coagulate milk met with, but on one occasion organisms were observed which showed a marked delay in coagulating milk.

The delayed coagulation was noted in a number of instances in organisms isolated from the surface wells.

Also in several instances the organisms isolated had a diminished power of breaking up lactose. The action on lactose was delayed and in some instances only a little gas was produced.

This diminished lactose fermentating power has been already noticed as a characteristic of a number of the organisms isolated from the surface wells, while greater lactose splitting power could not be re-acquired for the three organisms retested. In an earlier paper[1] already referred to I drew attention to the existence of such organisms, and described the characters of 12 such bacilli. The fact that in these soil inoculation experiments such slow and diminished lactose fermenting bacilli were met with on several occasions and that they were undoubtedly derived from typical *B. coli* affords an additional support to the opinion I have expressed that these organisms are true *B. coli* altered by environment.

SUMMARY.

1. Numerical counts on gelatin media are of very limited value for surface well examinations and are quite useless for open draw-wells. The blood-heat enumeration is of use, but still of but limited value.

2. The influence of rainfall and local conditions generally is marked for this class of waters with regard to their bacterial content.

Whether the well is uncovered, or covered and provided with a pump has a very important influence upon the bacteriological results. For a good many wells which show very bad bacteriological findings the cause of pollution is frequently due to surface pollution rather than to pollution of the ground water through the soil. If these wells are properly protected they will become suitable sources of drinking supply.

3. The results obtained confirm the value of *B. coli* and streptococci estimations as the best tests by which to judge the freedom from pollution of such waters.

The streptococci results are of great value, only second in importance to the *B. coli* determinations. In numerical distribution these two

[1] See Footnote, p. 489.

organisms closely agree. The presence of streptococci is more reliable as evidence to deduce pollution than their absence is to exclude it.

4. An opinion regarding the freedom of surface wells from pollution is best formed on the basis of combined topographical and bacteriological investigation.

5. Inspection alone is frequently quite insufficient for the purpose of determining if a well is contaminated and if it should be closed.

Of the 50 wells examined no less than 31 had to be classed as doubtful (Series C), topographical examination alone affording insufficient data for the expression of a decided opinion.

In view of the fact that pure water supplies are not easy to obtain in many rural districts, a water supply, which may have been in use for many years, cannot be condemned in the absence of clear evidence that it is unsatisfactory and dangerous.

6. In surface well waters a large proportion of the coli-like organisms isolated are atypical in one or more particulars.

7. Typical *B. coli* implanted into soil showed some alteration of character, but the changes were not extensive and no evidence was obtained that the widely aberrant organisms met with in different soils and waters ever represent typical *B. coli* altered by unfavourable environment.

8. Organisms closely allied to *B. coli*, but differing in one or more characters, possess significance as indicators of faecal contamination.

The more nearly the organism isolated resembles an "excretal" *B. coli* the greater is its significance as an indicator of pollution. Consequently the fewer the number required to condemn a sample water in which they occur. Stated as a working proposition, the more the characters of the coli-like organisms deviate from that which for convenience may be spoken of as the typical form, the greater the proportionate number of them required to condemn the water.

9. The presence of 'excretal' *B. coli* in 10 c.c. or less of a surface well water points to undesirable pollution and is sufficient to condemn the water.