BACTERIOLOGY OF FRESH WATER

II. THE DISTRIBUTION AND TYPES OF COLIFORM BACTERIA IN LAKES AND STREAMS

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(With 6 Figures in the Text)

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Introduction

Most of the work on coliform bacteria in relation to public health has hitherto been carried out with the object of finding improved methods for their identification and separation into types. The seasonal distribution of coliform bacteria in the sources of public water supply should also be of interest to water undertakings who are responsible for the quality of the water supplied to the consumer. Few water undertakings, however, examine enough samples during the year to obtain information on the seasonal changes in distribution of coliform bacteria.

Until recent years it was thought that the relative proportions of the various types of coliform bacteria in water in this country were different from those in waters in America and in tropical countries, in that *Bacterium aerogenes* and intermediate types were less prevalent. The reason for this supposition is not clear. It was probably based on results of investigations which were too limited in scope, in that the methods of separation of the bacteria were not entirely satisfactory and the samples of water were representative of only one or two areas. More recent work with full use of differential tests has shown that coliform bacteria other than *Bact. coli* are prevalent in many waters of Great Britain. In the sanitary control of water supplies, however, *Bact. coli*, type I, is regarded as the main indication of animal pollution of recent origin, while the presence of organisms of the intermediate, *aerogenes-cloacae* (I.A.C.), group in water is considered to be indicative of soil

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contamination or of remote animal pollution. This view is the outcome of research by many investigators on the bacteriology of faeces and soil, which showed *Bact. coli* to be predominant in faeces and the I.A.C. group to be equally predominant in soil. (For reviews of literature on coliform bacteria see Malcolm (1938), and Parr (1939).) With the increasing evidence that *Bact. coli* tends to over-grow the I.A.C. group in culture media, Wilson and his colleagues (1935) put forward two modified methods (methods III and IV) for the more accurate differential estimation of *Bact. coli* and the I.A.C. group. With method IV dilutions of the sample to be tested are inoculated into tubes of MacConkey broth and incubated at 37° C. After 48 hr., subcultures are made from all tubes showing the presence of acid and gas into MacConkey broth which is incubated at 44° C. and into citrate medium which is incubated at 37° C. All organisms showing acid and gas at 44° C. are assumed to be of the faecal coli type and all growing in citrate medium to be of the I.A.C. group. Numbers of the respective types present in the original sample are calculated from probability tables. Using this technique Bardsley (1938) was able to show that organisms of the I.A.C. group were more common in faeces than was hitherto believed. They were present in 61% of the samples examined, and in some instances they were the dominant type of coliform bacteria. From the evidence of her results Bardsley recommended that Wilson's method should replace the original test with incubation at 37° C. At present, however, the method employing MacConkey broth is still the test usually recommended in this country, but there is a growing tendency towards the adoption of methods employing incubation at 44° C., as Bardsley (1938) and the Metropolitan Water Board (Harold, 1937) consider this temperature to be almost specific for faecal *Bact. coli*. In this connexion method III, which differs from method IV in that duplicate sets of inoculations into MacConkey broth are made at once, one set being incubated at 44° C. and one at 37° C., is proving the more popular, as it is thought that a count of faecal *Bact. coli* can be obtained after 48 hr. Recently, the Metropolitan Water Board (Mackenzie, 1940), though recognizing the specificity of the temperature, the simplicity of the test, and the time saved, stated "although the number of tests performed is limited, they furnish considerable evidence to the effect that direct inoculation of a sample of water into MacConkey broth followed by incubation at 44° C. gives inferior results as regards the recovery of *Bact. coli*, type I, compared with incubation at 37° C. or 42° C. followed by plating".

Wilson's methods (1935) are open to the objection that an abnormally high count of I.A.C. organisms may be obtained because some species of bacteria other than coliform bacteria are able to grow in citrate medium. This raises some doubts as to whether the I.A.C. group is as prevalent in faeces as Bardsley (1938) reported. In the modified method suggested by the Metropolitan Water Board dilutions of the water in MacConkey broth are first

incubated at 37° C. for 24 hr. Positive tubes are then inoculated into a second series of tubes of MacConkey broth which are incubated at 44° C. Formation of acid and gas at this temperature is regarded as evidence of *Bact. coli*, type I. Atypical coliform bacteria are not considered as important unless *Bact. coli* is absent.

The adoption of any method eliminating the I.A.C. group completely from any consideration would be a bold move, in that it would leave *Bact. coli* as the sole indicator of faecal pollution, and permit the use of waters containing low *Bact. coli* but high I.A.C. numbers.

During the present investigation, samples of water were taken at weekly intervals, at different depths from several lakes in the Lake District, for determination of bacterial counts; there was thus an opportunity to determine the distribution of coliform bacteria. Numbers of this group of washed-in bacteria could be compared with the total number present as determined by plate counts. Methods employed for plate counts, the distribution of bacteria, and the correlation between fluctuations in numbers of bacteria and rainfall, have been recorded previously (Taylor, 1940).

GENERAL CHARACTERISTICS OF LAKES AND STREAMS UNDER INVESTIGATION

General descriptions of Windermere, Thirlmere, and Esthwaite Water have been given in a previous paper (Taylor, 1940), and it suffices here to compare the relative pollution of these waters. "Pollution", in this investigation, is taken to mean the washing in of coliform bacteria from any source and by any means, and it should be pointed out that although no gross pollution, comparable with the discharge of large amounts of crude domestic sewage into estuaries, takes place, there is a marked difference in the degree of pollution of the three lakes.

The most polluted is Esthwaite Water which receives sewage, treated only by means of a septic tank, from 150 houses in the village of Hawkshead, and a smaller amount of crude sewage. As the lake drains an area containing a larger proportion of agricultural land than the areas drained by the other lakes, the pollution from domestic animals is correspondingly higher. Windermere north basin is contaminated chiefly by the River Rothay, which receives the sewage effluent from Ambleside. The degree of pollution by this effluent is variable, but at times of low river flow occasional flocs of sewage fungus may be seen at points below the sewage works. The south basin, which receives the water from the north basin and the outflow from Esthwaite Water, also receives the sewage effluent from the towns of Windermere and Bowness. Thirlmere, which serves as a reservoir to supply drinking water to Manchester, may be considered as the control lake. Human habitations have been removed from the drainage area, a large part of which has been afforested with conifers. The inflows consist of seven streams, supplemented at times of heavy rainfall

by many smaller ones, together with an aqueduct which conveys the joint waters of several streams which would normally have flowed farther down the valley. The inflows originate high up on the mountain sides and fall rapidly down to the lake mostly draining areas of impermeable rock. Three of the streams, Wyth Burn, Birkside Gill, and the Aqueduct, traverse small areas of agricultural land, mainly sheep pasture. The only possible sources of excretal pollution are birds, wild animals, a few cattle, and sheep. Of these, sheep must be by far the most important, for they number more than 5000, graze over land extending from very near the lake to high up on the fells, and have access to some of the inflows.

Water from Thirlmere is drawn off at two depths, 9 and 19 m., and is mixed, screened, and sent down the Manchester aqueduct at an average rate of 40 million gallons per day. In addition, 4·6 million gallons per day are released as compensation water. This steady withdrawal of water causes much greater fluctuation in the level of the lake than would occur under normal conditions. Although water for supply is withdrawn from a depth of 19 m. it has not been found to interfere with the normal summer depletion of oxygen in the hypolimnion.

Pollution by seagulls, which is severe in many parts of the country, is very small in this district, though there are occasional visits by small flocks.

Methods

Samples of water from different depths in the lakes were taken by means of a special apparatus (Mortimer, 1940), and from the rivers by means of a bottle in a clamp fixed on a long rod. All samples were dealt with in the laboratory within 3 hr. of collection. Presumptive counts of coliform bacteria were made by the method advocated by the Ministry of Health (1939), using MacConkey broth and taking as positive all tubes showing acid and 15% or more gas in the Durham tube, after incubation for 48 hr. at 37° C. The most probable numbers of coliform bacteria were calculated from McCrady's tables.

These numbers were not confirmed by plating and identifying the bacteria responsible for the presumptive reaction, so that enumeration of coliform bacteria throughout this survey rests entirely on the formation of acid and gas in the MacConkey broth. An attempt was made, however, to assess the relative numbers of the different types of coliform bacteria. For this purpose tubes of MacConkey broth, containing the highest dilution of lake water which produced a positive reaction after incubation at 37° C. for 48 hr., were selected from a large number of tests. An inoculum from each tube was streaked over the surface of brilliant green bile agar in a petri dish and, after incubation at 37° C., a single colony from each plate was subcultured on a slope of beef peptone agar. The cultures so obtained were identified by a study of their reactions in various test media, which are described later.

NUMBERS OF COLIFORM BACTERIA IN RIVERS AND STREAMS AND THEIR RELATIONSHIP TO PLATE COUNTS AND RIVER LEVELS

Windermere. Samples of water from Trout Beck and from the Rivers Rothay and Brathay were examined to find the presumptive counts of coliform bacteria and the plate counts on sodium caseinate agar incubated at 20° C. The samples were taken at intervals throughout the year and the level of each river was recorded at the same time. The relationship between river level and bacterial counts for each of the three rivers is shown in Figs. 1, 2 and 3 respectively. It may be seen that the relationship is not the same in the case of each river. In Trout Beck (Fig. 1) the counts of coliform bacteria and the plate counts fluctuate in the same direction as the river levels. This suggests that soil, organic matter, detritus, and animal faeces from manured land, *washed in during times of increased river flow*, are *responsible for* increased numbers of bacteria. The increase in plate count during rain, following continued dry weather (July and October), was much greater than the increase following wet weather (early August) as there had been greater opportunity for movable material to accumulate. A very different phenomenon was observed in the Rothay (Fig. 2), where a decrease in coliform bacteria and in plate counts was usually accompanied by an increase in the river level. This is probably explained by the effect of increased river flow in diluting the Ambleside sewage effluent. The counts of bacteria, both coliform and others, in the sewage effluent are so high that the increase caused by washed-in material is masked. The graph for the Brathay (Fig. 3) indicates a third set of conditions. In general, plate counts tended to vary in the same direction as river level but the numbers of coliform bacteria underwent fluctuations which were most often in the opposite direction to river levels, except during parts of August and September when no relationship was apparent. The explanation may be that the river was polluted intermittently by small amounts of crude sewage at a point not far distant from the sampling point, resulting in an addition of coliform bacteria, but at the same time there may have been greater dilution from increased river flow.

Thirlmere. Of the seven inflows studied, two were sampled weekly, and the remainder monthly. Fig. 4 shows the counts of coliform bacteria and the plate counts during 1939 in Fisher Gill and in Birkside Gill. Fisher Gill pours down the rocky slopes to the west of Thirlmere through timbered land. Birkside Gill drains water from the south and south-east, and flows through treeless land which supports numerous grazing sheep. Fluctuations in counts of coliform bacteria were usually accompanied by fluctuations in plate counts and these occurred in general at the same periods in both streams. No records of stream levels are available for these two inflows. It is of interest to note that the greatest counts of coliform bacteria occurred during the period

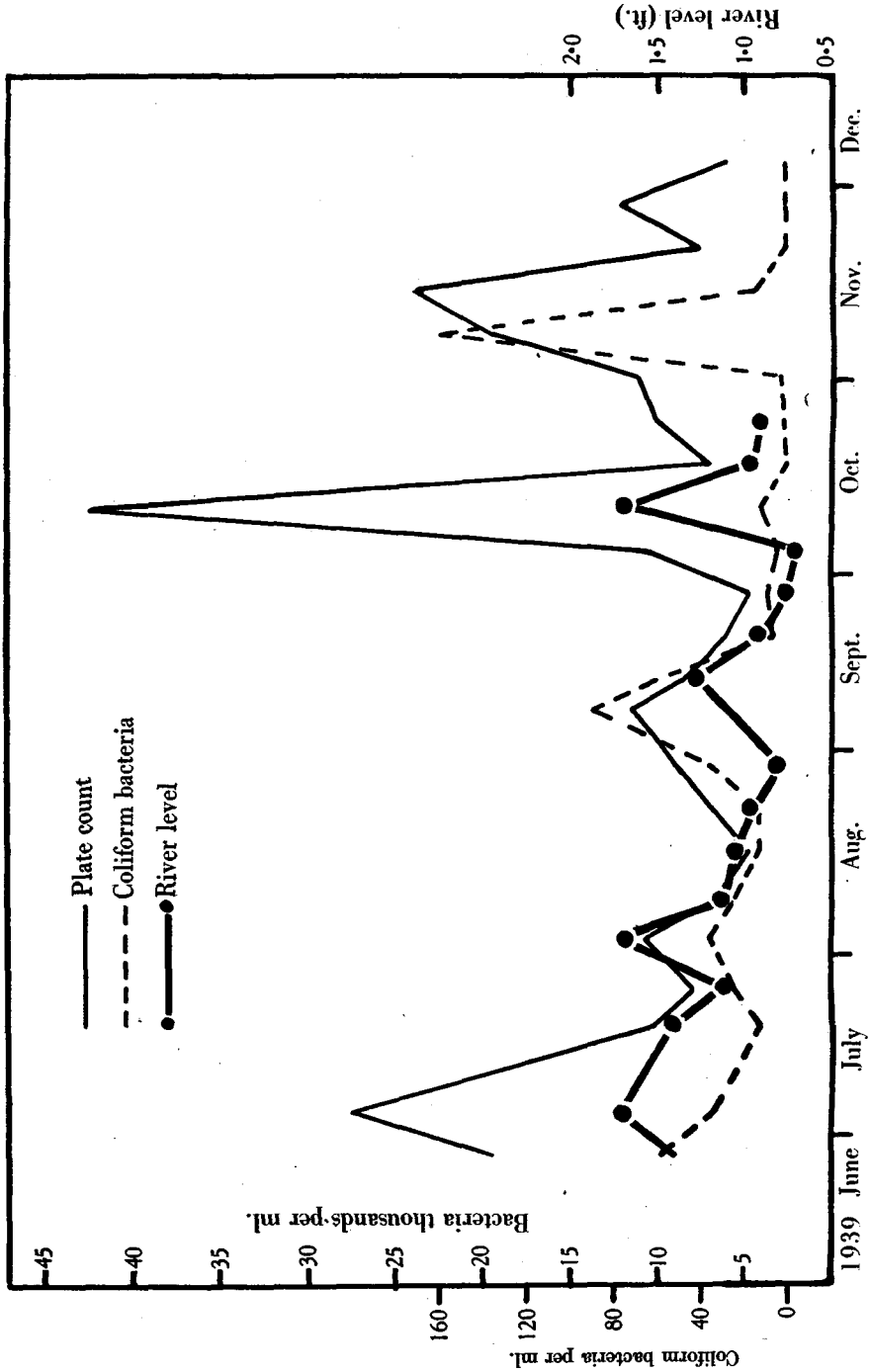

Fig. 1. Plate counts, coliform bacteria and river level, Trout Beck, 1939.

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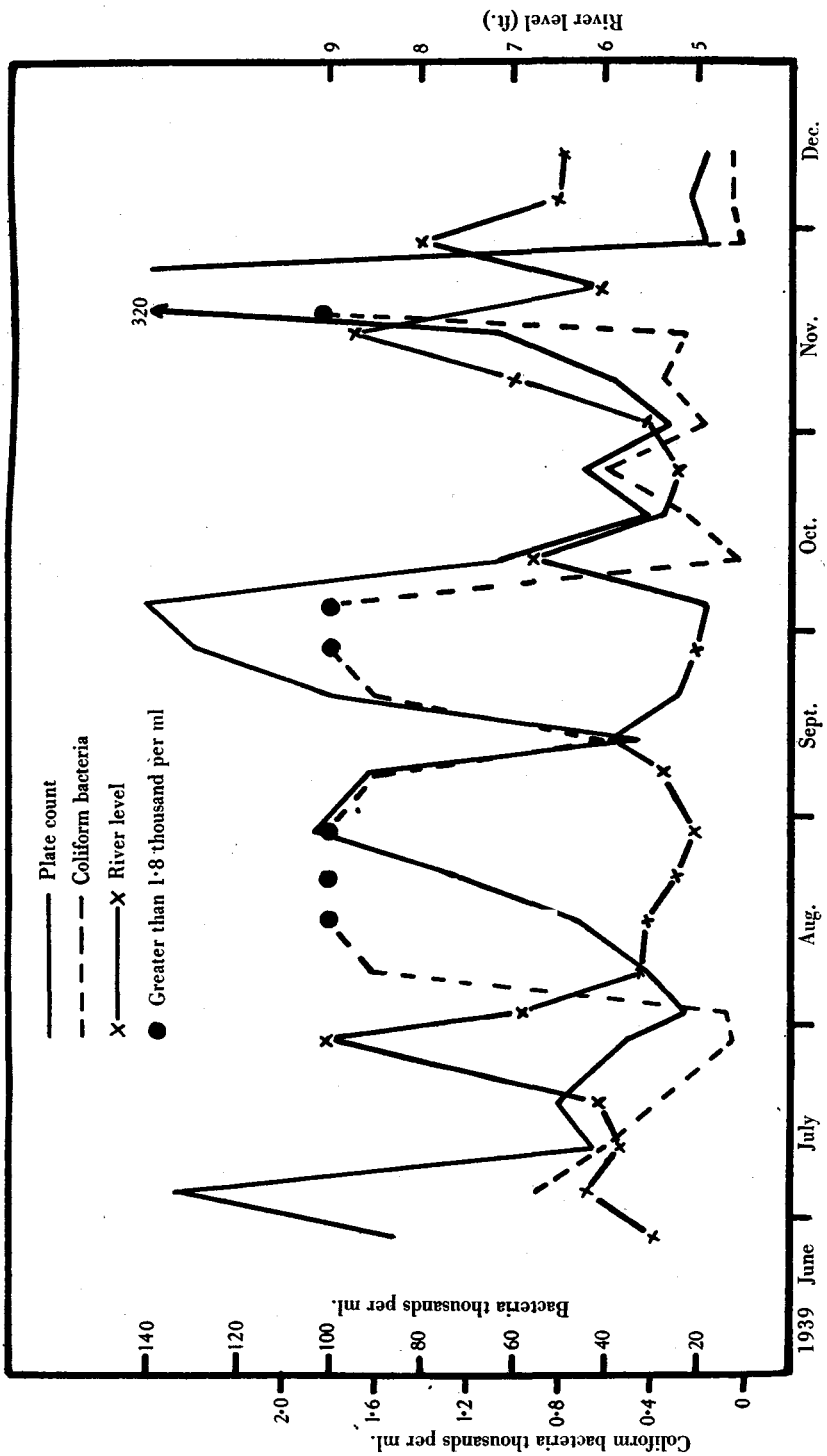

Fig. 2. Plate counts, coliform bacteria and river level, River Rothay, 1939.

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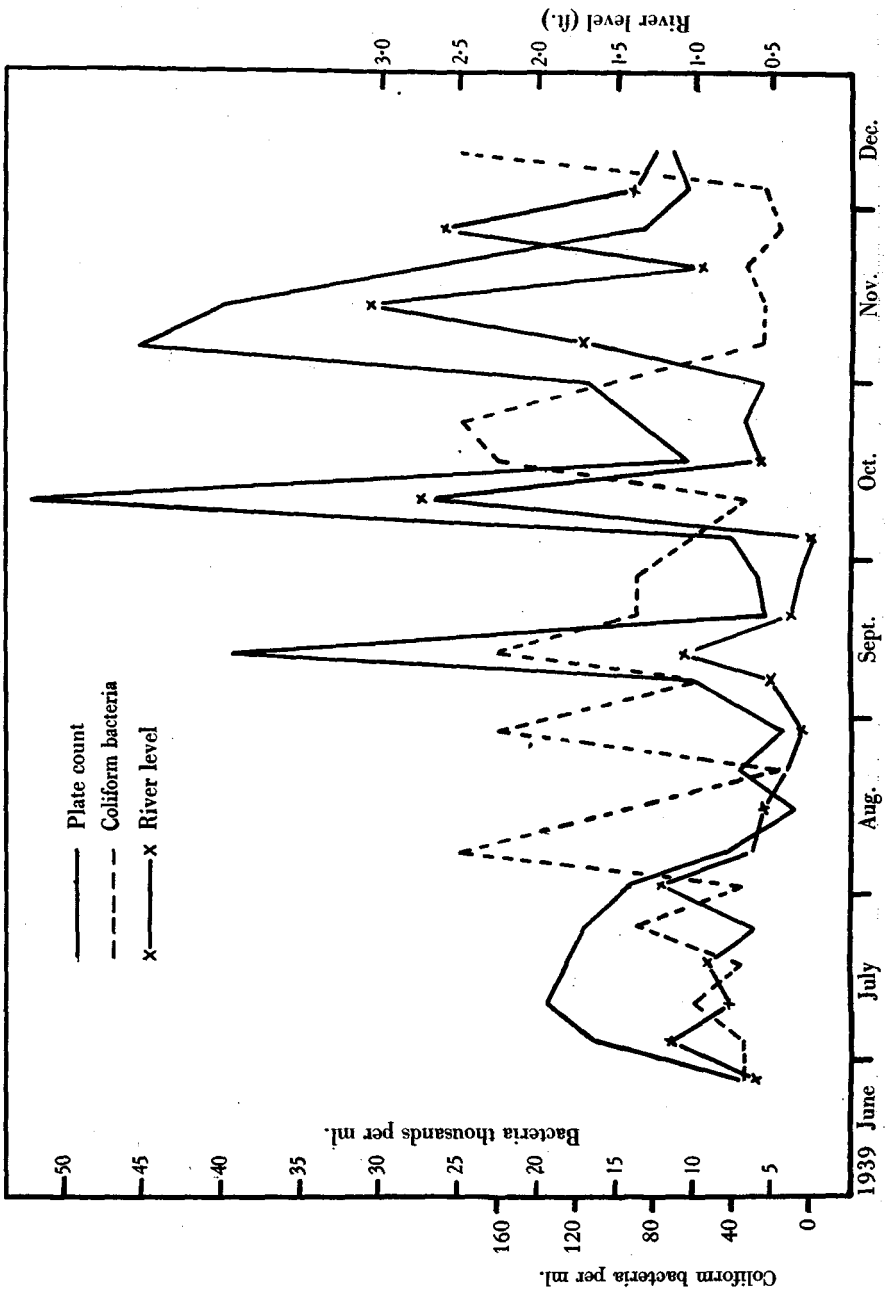

Fig. 3. Plate count, coliform bacteria and river level, River Brathay, 1939.

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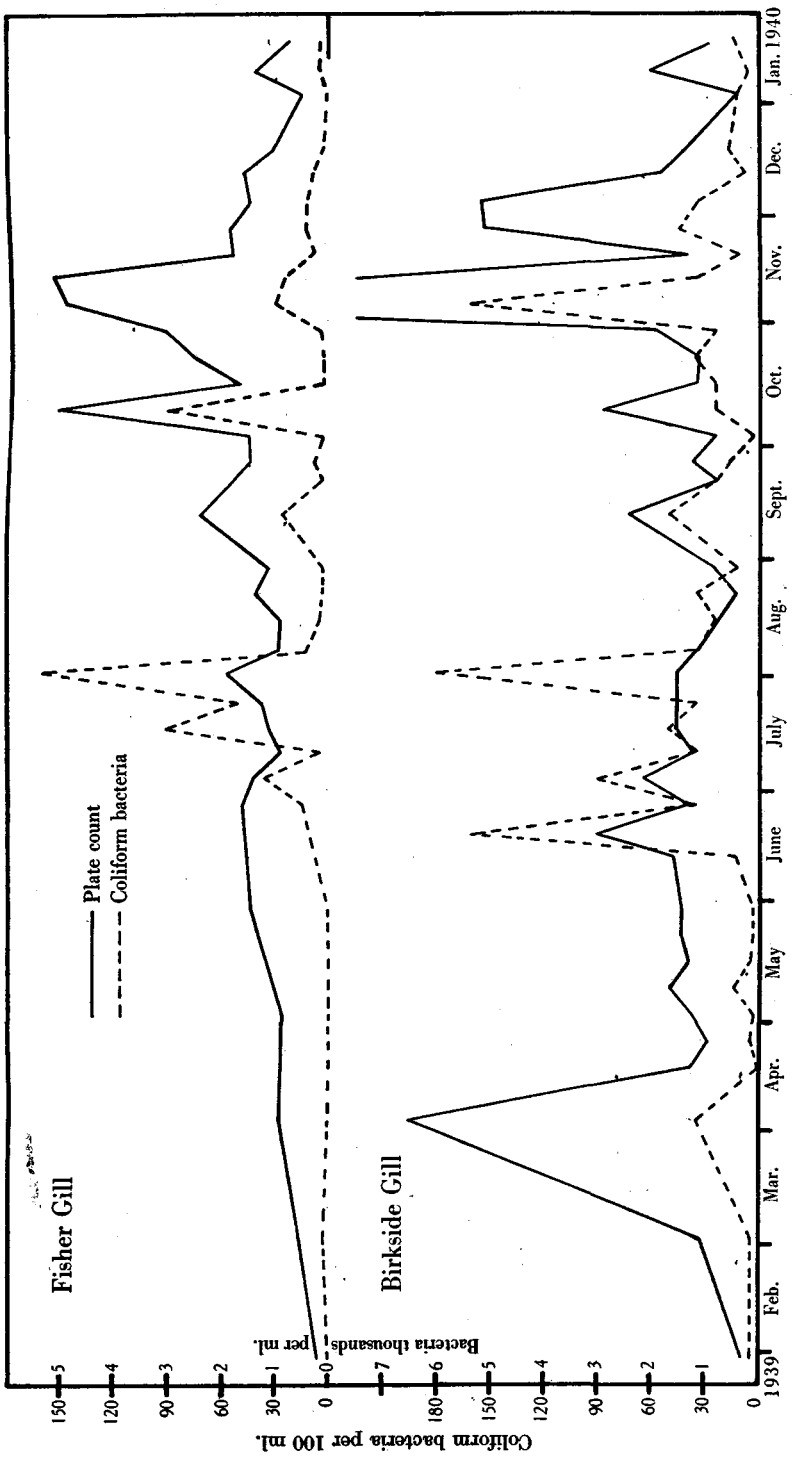

Fig. 4. Plate count and numbers of coliform bacteria, Thirlmere inflows, Fisher Gill and Birkside Gill.

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between mid-June and mid-November; during the winter and spring the numbers were relatively small.

Table 1 shows the averages of coliform and plate counts for all the inflows over the period of sampling.

Table 1. *Averages of plate counts and of counts of coliform bacteria in streams flowing into Thirlmere*

Inflow	Plate count thousands per ml.	Coliform bacteria per 100 ml.
Dob Gill	3·68	6
Wyth Burn	2·49	46
Birkside Gill	2·20	34
Aqueduct	1·89	41
Fisher Gill	1·73	19
Whelpside Gill	1·34	8
Comb Gill	1·09	8

In this table the streams are arranged in descending order of plate counts; with the exception of Dob Gill and the Aqueduct the count of coliform bacteria changes in the same direction. The high plate count in Dob Gill is ascribed to the fact that this stream does not flow directly into the lake but by way of a mountain tarn, which is bordered by large areas of boggy ground, supporting *Sphagnum*. At times of high rainfall organic matter would probably be washed out in sufficient quantity to account for the high ratio of plate count to count of coliform bacteria. The three streams having the greatest average counts of coliform bacteria are those draining areas containing improved pastoral land; hence it is reasonable to suppose that the count of coliform bacteria at any point on the Thirlmere gathering ground is largely dependent on the number of sheep grazing the area.

Discussion. The results obtained agree with those of earlier workers on the effect of rain on the bacterial content of rivers and streams(Boyce, Grünbaum, MacConkey & Hill, 1902). After dry periods and consequent low river level, a sudden spate may wash in detritus and accumulated animal faeces, and may thus increase the bacterial counts. When this material has largely been washed away the counts of bacteria in a polluted river will probably fall with continued rain owing to the effects of dilution with the rain water. In general, increase in river flow will reduce the number of bacteria per unit volume in a polluted river by dilution, and will increase the number in a relatively unpolluted river by the washing-in of extraneous material. When river water is examined at intervals for its potability it is expected that, under normal conditions, plate counts at 20° C. and counts of coliform bacteria will fluctuate in the same direction. If independent fluctuations in counts of coliform bacteria occur the water must be regarded with grave suspicion.

SEASONAL DISTRIBUTION OF COLIFORM BACTERIA AT DIFFERENT DEPTHS

Windermere. The counts of coliform bacteria at depths of 1 and 10 m. in the north and south basins of Windermere between February and October 1939 are shown in Fig. 5. In both basins the count was greatest during the summer and autumn months, when there were large fluctuations from week to week. In general these fluctuations at the two depths in both basins were in the same direction but often the basins behaved independently. From the fact that fluctuations in numbers of coliform bacteria did not occur simultaneously in both basins, it would appear that there was no common causal factor such as rainfall or temperature. Fluctuations occurred after wet and after prolonged dry weather. It had previously been found that during the summer of 1939 rainfall could be correlated with plate counts in the north basin but not in the south basin. It was of interest to determine whether relationships existed between fluctuations in plate counts which represent chiefly, it is believed, the activities of the indigenous flora, and fluctuations in the numbers of coliform bacteria which apparently represent washed-in bacteria. The correlation coefficients between coliform bacteria and plate counts were calculated, and, as can be seen from Table 2, a significant relationship between the two counts was found only at a depth of 10 m. in the south basin.

It would appear that rainfall does not bear the same direct relationship to the incidence of coliform bacteria as it does to plate counts, and that during the summer and autumn months at least, some other factors are responsible for the fluctuations in counts.

Table 2. *Correlations between counts of coliform bacteria and plate counts in samples of water taken weekly or fortnightly at different depths in Windermere, Thirlmere and Esthwaite Water*

Lake	Sampling period	Depth m.	r	P	Remarks
Windermere:					
North basin	Feb.–Dec. 1939	1	0·01	>0·1	Not significant
		10	0·01	>0·1	,,
South basin	Feb.–Dec. 1939	1	0·05	>0·1	,,
		10	0·44	<0·01	Significant
Thirlmere	Feb. 1939–Jan. 1940	1	Neg.	—	Not significant
		10	0·19	>0·1	,,
Esthwaite Water	Apr.–Oct. 1939	1	0·02	>0·1	,,

Thirlmere. Counts of coliform bacteria have at all times been smaller in Thirlmere than in the other lakes under examination. Fig. 6 shows the numbers at depths of 1, 10 and 34 m. (bottom) between February 1939 and January 1940. Only during the months of June, July, August and September, 1939, were coliform bacteria present in any significant numbers and then only at depths of 1 and 10 m. From June to August, numbers were usually much higher at 10 m. than at 1 m. It can be seen from a comparison of Figs. 4 and 6 that relatively high numbers of coliform bacteria appeared in June both in

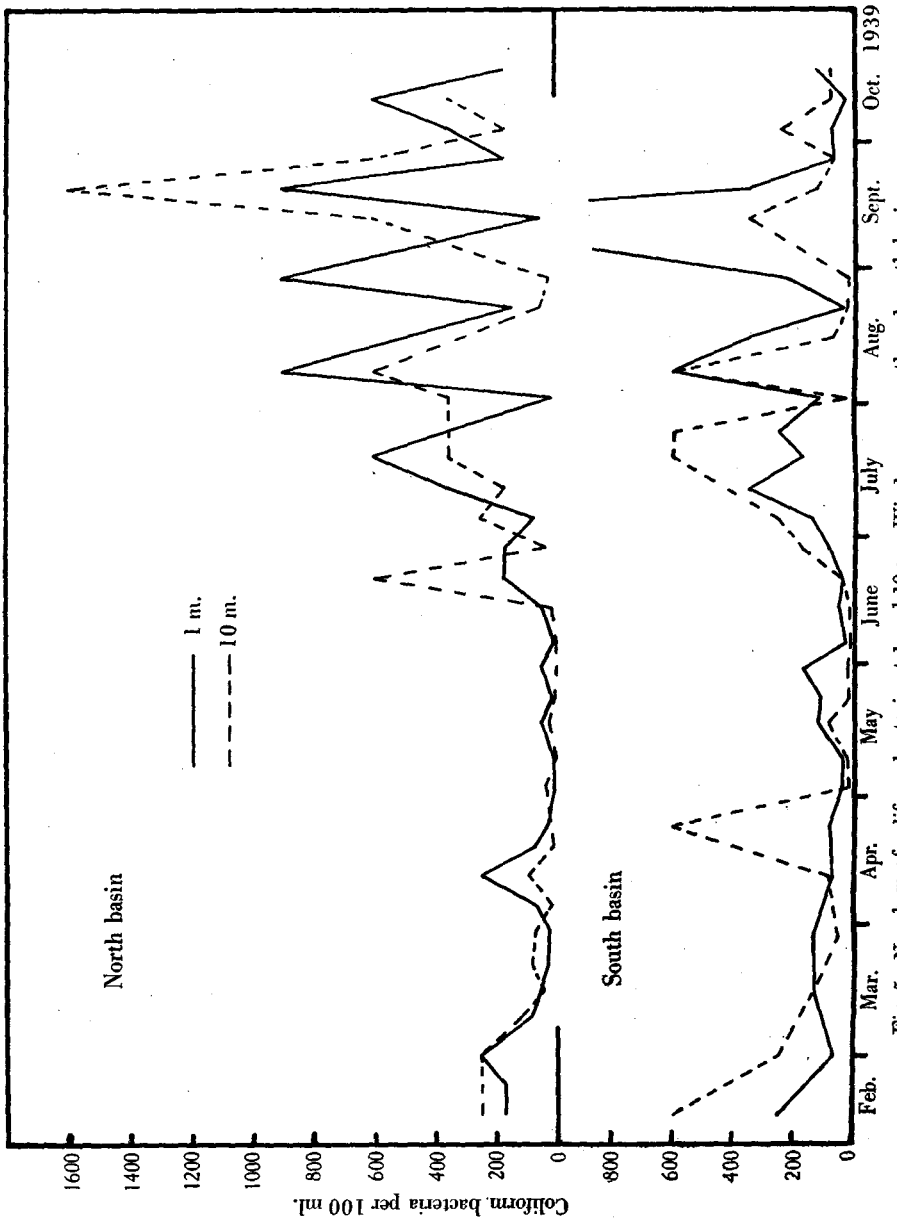

Fig. 5. Numbers of coliform bacteria at 1 and 10 m., Windermere, north and south basins.

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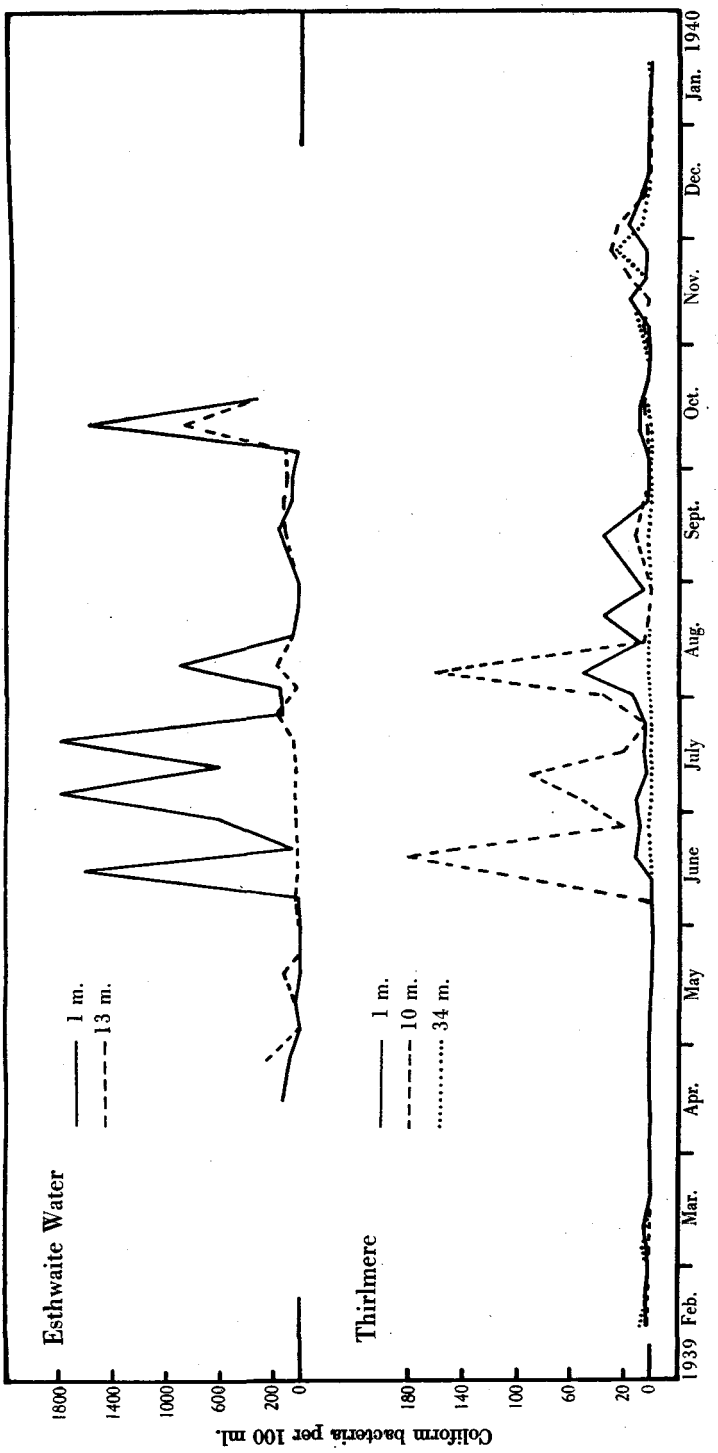

Fig. 6. Numbers of coliform bacteria at different depths, Thirlmere and Esthwaite Water.

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the lake and in Birkside Gill. Correlation coefficients between counts of coliform bacteria and plate counts were not significant (Table 2). The greater numbers of coliform bacteria at 10 m. than at 1 m. during the summer have not yet been explained. Temperature and concentration of dissolved oxygen were approximately the same at the two depths during this period; this indicates that the waters of the epilimnion were in circulation. Bacterial numbers at different depths may have been affected to a different extent by other factors such as sunlight, but it does not appear probable as there was no similar phenomenon in Windermere.

The virtual absence of coliform bacteria in the water near the bottom of the lake during the summer is probably caused by the inability of the inflowing waters to mix with the hypolimnion which is in a state of partial stagnation; hence any coliform bacteria found in that region can only have settled from above or have survived from the pre-stratification period.

Esthwaite Water. Counts of coliform bacteria in the top and bottom layers, 1 and 13 m., of Esthwaite Water between April and October 1939 are also shown in Fig. 6. This period covers the development and duration of stratification until the autumn overturn. Results are similar to those obtained in Thirlmere. Numbers of coliform bacteria at a depth of 1 m. fluctuated from week to week and sometimes reached very high figures, but at the bottom they remained low and showed little tendency to fluctuate. When stratification was destroyed, in October, counts were once more approximately the same at both depths. At a depth of 1 m. no relationship was found between plate counts and counts of coliform bacteria (Table 2). It was noticed that large increases in counts of coliform bacteria sometimes followed periods of heavy rain, but the correlation coefficients between the two factors did not show any significant relationship ($r = 0{\cdot}05$).

Discussion. From the examination of the four bodies of water two main observations can be made; that the incidence of coliform bacteria was most marked in the summer and autumn and that during these periods sharp week to week fluctuations in numbers in the epilimnion took place.

The abundance of coliform bacteria during the summer and autumn has also been reported by Bardsley (1934) during routine examination of samples of surface waters from the north-west of England. She found that the percentage of water samples yielding coliform organisms was greatest between June and November, both for the presumptive count and for the *Bact. coli* count. The reason for this phenomenon is by no means clear and it cannot be explained by any of the available data. Stratification of the lakes may be partially responsible, as the bacteria washed in will be localized in that volume of water which is in circulation, the epilimnion, but as the numbers of coliform bacteria are also greater in free flowing and relatively unpolluted streams, there must be some other major cause. In the streams of the Thirlmere

drainage area the main source of the coliform bacteria must be sheep, the number of which (approximately 5000) is fairly constant throughout the year. There is nothing about the seasonal movements of these animals to suggest that they can contaminate the streams more easily in summer than in winter as, except for a short period during the lambing season in the spring, they are widely distributed on the fells. It appears probable that temperature has an effect on the viability of coliform bacteria, warmer temperature being favourable for prolonged viability. It is significant that greater numbers of coliform bacteria were observed at approximately the same time in all lakes, in June, when the temperature of the water had risen considerably and that temperature change was one of the few factors common to all sampling points. Little evidence on the viability of *Bact. coli* at different temperatures under natural conditions is available; the results of experiments with inoculated and incubated bottles of water under laboratory conditions may be very different from those found under field conditions. It is of interest to note that at Harrisburg, Pa., U.S.A. (1907), a series of examinations for *Bact. coli* made in the midsummer of 1906 showed positive results in 7% of the samples of water entering a storage reservoir and in 27% of the samples leaving it. The storage period was about two days and the temperature of the water was nearly blood heat. Clemesha (1912), in India, studied the question of multiplication of coliform bacteria in warm waters and reported that it was confined to certain members of the coliform group. With pure cultures Haines (1934) found no growth of *Bact. coli* between temperatures of 0 and 5° C. but he did not investigate viability.

The cause of the fluctuations in counts of coliform bacteria in lake waters during the summer months cannot be explained from any of the available data. Large week-to-week changes occur both during wet and dry weather, particularly in the more polluted lakes, and in general the changes are not correlated with changes in the indigenous population as measured by plate counts. In the absence of counts on replicate samples of water taken on the same occasion, and, in view of the large errors involved in the dilution method of counting, it is not known whether these week-to-week fluctuations are significant, but any increase or decrease involving a change of 20–50 times would normally be expected to have some significance.

The effect of stratification on the distribution of coliform bacteria is apparent from the results obtained from Esthwaite Water and from Thirlmere. When stratification had become established, numbers of coliform bacteria near the bottom of Esthwaite Water remained very low and coliform bacteria were usually absent in 100 ml. in samples from near the bottom of Thirlmere. When stratification disappeared and the consequent circulation of the waters was restored, the counts were approximately the same near the bottom and in the surface layer. Low numbers of bacteria in the hypolimnion have

previously been reported for plate counts (Taylor, 1940) and are considered to be a result of the stagnation of that part of the water. This is of practical interest to water undertakings who draw water for supply from the hypolimnion in reservoirs during the summer months; for although the surface waters may contain large numbers of coliform bacteria the numbers of these organisms in the lower layers may be relatively small. Thus any source of pollution might go unnoticed until the autumnal overturn took place.

Seasonal and week-to-week fluctuations and the results of stratification serve to emphasize the risk of drawing conclusions from examinations of samples at only one point and at intervals of time greater than a few days. Where water is used for public supply it is desirable to collect samples for examination frequently from the service main and from the reservoir.

Types of coliform bacteria isolated from lakes and their inflows

A total number of 288 cultures of coliform bacteria were isolated from positive tubes of MacConkey broth by the method outlined previously (p. 20) in the course of examining samples of water from lakes and streams. These were tested for their reactions in the following media: MacConkey broth, tryptone broth for indol production, glucose phosphate broth for the Methyl Red and Voges Proskauer reactions, Koser's citrate, uric acid, and cellobiose, all at 37° C., and nutrient gelatin at 20° C.

Table 3. *Classification of coliform bacteria according to their place of origin*

		Thirlmere		Windermere			Esthwaite Water	Total coliform cultures
				North basin		South basin		
	Type	Lake %	Inflows %	Lake %	Inflows %	Lake %	Lake %	%
Bact. coli	I	87·5	98·2	38·7	58·3	54·5	37·1	66·3
Bact. coli	II	0·0	0·0	6·1	8·3	0·0	7·4	5·2
Intermediate	I	2·5	0·0	16·3	8·3	4·5	14·8	6·6
Intermediate	II	0·0	0·0	10·0	8·3	4·5	14·8	4·7
Bact. aerogenes	I	10·0	1·8	26·5	16·6	22·7	18·5	14·2
Bact. aerogenes	II	0·0	0·0	0·0	0·0	4·5	7·4	0·9
Irregular, other types		0·0	0·0	2·1	0·0	9·6	0·0	1·4
Bact. cloacae		0·0	0·0	0·0	0·0	0·0	0·0	0·0
Atypical (not producing gas in MacConkey broth at 37° C.)		—	—	—	—	—	—	(27·0)
No. of cultures isolated from each source		51	62	96	17	31	31	—

The cultures were classified into types on the basis of these reactions. Table 3 shows the total numbers of cultures isolated from each river or lake and the percentage of the number which each type represented. In the last column is shown the percentage distribution of the different types, referred to the total cultures isolated from all sources. It may be seen that, when inoculated into MacConkey broth and incubated at 37° C., a large number (27% of the 288 cultures isolated) produced either no gas or such quantities

as to be considered negative. These bacteria are discussed in more detail later. The relative proportions of the different types in the total number isolated are in general agreement with those found by previous workers, though the absence of *Bact. cloacae* was surprising. From the results obtained for the different lakes it appears that considerable differences exist between the types present in the relatively polluted and unpolluted waters.

In each of the waters *Bact. coli*, type I, was the organism most frequently isolated. In the pure waters of Thirlmere and its inflows this type almost excluded all others. The polluted waters of Windermere and Esthwaite Water gave higher percentages of *Bact. aerogenes* and contained a variety of intermediate forms.

No additional information was obtained by the use of uric acid and cellobiose media. All *Bact. aerogenes*, types I and II, fermented cellobiose and two cultures of *Bact. coli*, type I, also fermented this carbohydrate. Citrate and uric acid were found to be of equal diagnostic value; only one culture growing in citrate was unable to grow in the uric acid medium. All cultures were tested for growth at 44° C. by inoculating tubes of MacConkey medium. The medium, which had been in a water bath at 44° C. for several hours previously, was inoculated *in situ*, and incubated at that temperature for 48 hr. The bath, which was of the type used by Wilson and his colleagues (1935), had a temperature variation of $\pm 0{\cdot}4°$ C. The results obtained were not quite as specific as those of Bardsley (1938); for although positive results, indicated by the production of acid and gas, were given by 138 of the 140 cultures of *Bact. coli*, type I, six of the 30 cultures of *Bact. aerogenes*, type I, and one Intermediate, type I, also gave positive results. As these seven cultures had been plated out on two successive occasions and the 44° C. test repeated three times on each culture, there seems to be little doubt of the ability of some strains of *Bact. aerogenes* to form acid and gas in MacConkey broth at 44° C.

When the cultures isolated from positive tubes of MacConkey broth were re-inoculated into that medium and incubated at 37° C., it was found that no less than 80 produced either no gas or such small quantities as to be considered negative. As these cultures represented a large fraction (27%) of the total number isolated it was decided to make a further study of their biochemical activities. First, 64 of the cultures were inoculated into MacConkey broth and incubated at 30° C.; 35 readily produced acid and gas; the remainder gave negative results. Fifty-five cultures were then inoculated into tubes of dextrose, sucrose, mannitol, and maltose broths, and incubated at 30° C. The reactions of the lactose-fermenting group are shown in Table 4 and those of the group which did not ferment lactose in Table 5.

Table 4 shows that the large majority of the lactose-fermenting bacteria fell into one group which produced acid and gas in all the media tested and

Table 4. *Reactions of bacteria which fermented lactose at* 30° C. *but not at* 37° C. *(Gram negative non-spore-forming rods)*

Lactose	Dextrose	Sucrose	Mannitol	Maltose	Gelatin liquefaction	No. of cultures
A.G.	A.G.	A.G.	A.G.	A.G.	–	25
A.G.	A.G.	A.G.	A.G.	A.G.	+	3
A.G.	A.G.	A.G.	–	A.G.	–	1
A.G.	A.	A.	A.	A.	+	1

Table 5. *Reactions of bacteria which did not ferment lactose at* 30° C. *(Gram negative non-spore-forming rods)*

Dextrose	Sucrose	Mannitol	Maltose	Gelatin liquefaction	No. of cultures
A.G.	A.G.	A.G.	A.G.	±	8
A.G.	–	A.G.	A.G.	–	6
A.G.	–	A.G.	–	–	1
A.	A.(G.)	A.(G.)	A.(G.)	–	2
A.	–	A.(G.)	A.	–	2
A.	A.	A.G.	A.	–	1
A.	A.	A.	A.	–	1
A.	–	A.	A.	+	1
–	–	–	–	–	1

A. = acid; G. = gas; (G.) = slight gas.

did not liquefy gelatin. Methyl Red, Voges Proskauer, indol production, and citrate utilization tests on these 25 cultures gave varied results and no further differentiation could be made. Following a suggestion by Clegg (1940), a number of these organisms, which fermented lactose at 30° C., were inoculated into MacConkey medium and incubated at 30° C.; after incubation for 48 hr. they were re-inoculated into the same medium and incubated at 35° C. Acid and gas were produced by all cultures. Further inoculations were then made and the cultures incubated at a temperature of 37° C.; all except one culture were found to be positive. Repeated inoculation and incubation at 40° C. gave negative results in all cases. The bacteria which did not ferment lactose belonged to a much larger number of types (Table 5) and resembled species of such genera of intestinal bacteria as *Proteus*, *Salmonella*, *Eberthella* and *Alkaligenes* (Bergey, 1939).

The numbers of cultures which fermented lactose at 30° C. but not at 37° C., and of those which did not ferment lactose are shown in Table 6 as

Table 6. *Origin of cultures fermenting lactose at* 30° C. *but not at* 37° C., *and non-lactose-fermenting cultures. Percentages of total isolated*

	Cultures fermenting lactose at 30° C. but not at 37° C.	Non-lactose-fermenting cultures
Thirlmere, inflow	0·0	11·3
Thirlmere lake	5·9	15·6
Windermere, inflow	23·5	5·9
Windermere, north basin	28·1	20·8
Windermere, south basin	12·9	16·1
Esthwaite Water, lake	9·7	3·2

percentages of the total number isolated from each inflow and lake. The bacteria of the group fermenting lactose at 30° C. were most frequently isolated from Windermere and its inflows, least frequently from Thirlmere, while none were isolated from Thirlmere inflows (0 of 51 cultures). This is interesting in view of the difference in purity of the two lakes. The percentages of non-lactose fermenting bacteria found were roughly the same in Windermere as in Thirlmere but were lower in Esthwaite Water.

Discussion. The classification of nearly 300 cultures of coliform bacteria, isolated from the lakes and their inflows, has shown a relative abundance of different types similar to that found by previous investigators. *Bact. coli*, type I, was by far the most prevalent type and *Bact. aerogenes*, type I, was the next most numerous, in all the waters examined. *Bact. coli* was present in greater proportion in relation to total counts, though in much smaller number in a given volume of water in Thirlmere and its inflows than in the other lakes and rivers examined; only small proportions of *Bact. aerogenes*, type I, and Intermediate, type I, were present. In the relatively impure waters of Windermere, types other than *Bact. coli*, type I, made up from one-half to two-thirds of the number of cultures examined. With the recognized practice of taking *Bact. coli* as the main indicator of faecal contamination, the count of coliform bacteria as measured by the presumptive test at 37° C. should give almost a true index of the pollution of Thirlmere; but the true numbers of *Bact. coli*, type I, in the relatively polluted waters would be much less than the count by the presumptive test. It is generally assumed that the presumptive test at 44° C. will eliminate *Bact. aerogenes* and the Intermediates, thus obtaining the correct index for *Bact. coli*, type I; the presence of *Bact. aerogenes* and the Intermediates in the relatively polluted waters and their scarcity in the almost uncontaminated waters would seem to be of some significance. It may be that the I.A.C. group are of greater importance than is generally supposed in assessing the risks of pollution of sources of water supply; further investigations of the true significance of the I.A.C. group however are required and are being carried out. 27% of the cultures isolated were incapable of forming acid and gas in MacConkey broth at 37° C., though they were isolated from presumptive positive tubes. It is possible that the original reaction had been produced by an organism other than the strain isolated from the brilliant green lactose bile agar medium or that gas formation had resulted from symbiosis. Half the cultures were able to ferment lactose at 30° C. but not at 37° C. when first tested, though it was found that they could be "trained" to cause fermentation at 37° C. by making successive subcultures and incubating at increasing temperatures. The cultures may be compared with those obtained by Levine (1939) from chlorinated water supplies. He found that a large number of these cultures fermented lactose broth very slowly at 37° C. but rapidly at 30° C. and that they generally fermented lactose more

readily in brilliant green bile and formate ricinoleate media at 37° C. than in standard lactose broth. This type of bacterium was most common during the summer months when the temperature of the water was between 12° C. and 20° C. Their sanitary significance has not been established. It may be noticed that a number of workers have reported the isolation of coliform bacteria unable to produce a normal fermentation at 37° C. but able to do so at some lower temperature, usually mentioned as "room temperature" (Parr, 1939; and Stuart, Griffin & Baker, 1938). In this survey bacteria of this type were isolated more frequently from the relatively polluted than from unpolluted waters.

The other half of the atypical cultures proved to be non-lactose-fermenters the reactions of which in carbohydrate media were similar to those of members of the genera *Proteus*, *Salmonella*, *Eberthella* and *Alkaligenes* (Bergey, 1939). The isolation of these organisms from positive tubes may have been a result of their overgrowing the coliform bacteria or else to synergic reactions. Atkinson & Wood (1938) found that the most frequent cause of false positive reactions in the presumptive test was the synergic action of *Bact. coli anaerogenes* and an organism of the *Proteus* type.

Summary

1. The distribution of coliform bacteria in lakes and streams has been studied. Weekly samples have been collected from different depths from the north and south basins of Windermere, from Thirlmere, and Esthwaite Water, and from streams flowing into these lakes. Nearly 300 cultures have been isolated from positive tubes of MacConkey broth and their relationship to the coliform group has been studied and considered.

2. In relatively unpolluted streams the counts of coliform bacteria and the plate counts on sodium caseinate agar tended to fluctuate in the same direction as river level, but in polluted streams the increased flow of water accompanying a higher river level reduced the numbers of bacteria per unit volume. Counts of coliform bacteria were, in general, much higher in summer than in winter, despite the lower rainfall in summer.

3. During the winter period of circulation of the water, samples of water from different depths in the lakes gave approximately the same count for coliform bacteria. When stratification of the water became established numbers of coliform bacteria in the hypolimnion (the lower stratum) dropped to very low figures and remained fairly constant; much higher counts were found in the epilimnion (the upper stratum).

Large fluctuations in numbers of coliform bacteria which occurred from week to week in the lakes were related to fluctuations in plate counts at only one of the seven sampling points, and were not related to previous rainfall.

The count of coliform bacteria was greatest in summer and autumn, a phenomenon that was by no means entirely a result of the bacteria which were washed in being concentrated in the epilimnion. It is suggested that temperature may have affected viability and proliferation.

4. Of the total number of coliform organisms producing acid and gas from lactose at 37° C., approximately 70% were *Bact. coli*, types I and II, and the remainder were members of the I.A.C. group. In the relatively pure waters of Thirlmere and its inflows *Bact. coli*, type I, made up 86 and 98% respectively of the total coliform cultures isolated, whereas in the relatively impure waters of Windermere and Esthwaite Water the percentages were 39 and 37 respectively. The differences were due to the greater proportions of *Bact. aerogenes*, type I, and of intermediate and irregular types in the impure waters. The significance of greater proportions of the I.A.C. group in polluted rather than in unpolluted water is discussed. The actual counts of coliform bacteria were very much lower in Thirlmere than in the other lakes examined.

5. Of the total cultures isolated from positive tubes of MacConkey broth 27% did not produce acid and gas in MacConkey broth at 37° C. Approximately one-half of these cultures, however, fermented lactose at 30° C., and gave varied results with the standard differential tests. The majority of these cultures could, by gradually increasing the incubation temperatures of successive inoculations, be "trained" to ferment lactose at 37° C., but not at 40° C. They were more frequently isolated from the polluted lakes and inflows than from unpolluted waters. The remaining organisms isolated proved to be non-lactose-fermenting species belonging to the genera *Proteus*, *Salmonella*, *Eberthella* and *Alkaligenes* (Bergey).

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