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THE BACTERIAL CONTAMINANTS of MILK and the VALUE of the VARIOUS METHODS for DETERMINING THEIR PRESENCE.

by

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C O N T E N T S

S E C T I O N I.

The PLATE METHOD of ESTIMATING the BACTERIAL CONTENT of MILK - the LIMITATIONS of PROCEDURE in COMMON USE with SUGGESTED IMPROVEMENTS.

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The plating method of estimating the bacterial content of milk is a development of the procedure introduced by Koch in 1881 for the isolation of pure cultures of bacteria by means of solid nutrient media. (1) It is the most important method of determining the number of living organisms present and is the one most frequently employed. It is well recognised, however, that accurate counts cannot be obtained by this method, chiefly for the following reasons:-

(1) Some of the bacteria present are unable to grow or to form colonies in the medium employed and under the conditions provided, and therefore are not counted. (2-14)

(2) Where a number of organisms occur together as, for example, in a chain or cluster, they may not be isolated by the manipulations of the test, with the result that only one colony is formed instead of a number of colonies corresponding to the actual number of organisms. (2 and 13-17)

(3) Some bacteria may be prevented from growing and forming colonies owing to the presence of others. Such

"antagonism" is most likely to occur on crowded plates, or where there are "spreading" colonies. (2 and 18)

Further, it has been found that in many instances there is a great variation in the results obtained from duplicate examinations of the same sample of milk, especially where the tests are performed by different workers and in different laboratories. This is undoubtedly due in part to differences in the technique and the medium employed, and also to differences in manipulative skill. (2, 17 and 19-21)

Apart from such disturbing factors a very considerable variation may occur owing to the fact that milk is not a homogeneous fluid. This is dealt with in Section II. (See page 80).

The plating method of counting bacteria was first used extensively for the grading of market milk in America, where in order to obtain more uniform results a standard procedure was adopted by The American Public Health Association. (14) A similar method was adopted for the same purpose in Great Britain by the Ministry of Health. (22) Such methods have not proved entirely satisfactory and have been the subject of a considerable amount of criticism, although not on very well defined grounds.

Accordingly the objects of the present investigation are as follows:-

(1) To determine whether it is possible to obtain

equally satisfactory results by means of a modification of the usual plating method which involves a marked saving in materials and also a distinct reduction in the time taken.

(2) To examine the general problem of the reliability of a limited number of plate estimations as an index of the bacterial content of samples of milk. This involves the question of the extent of variation between individual counts where large numbers of plates are prepared from the same sample under similar conditions.

(3) To suggest a procedure for increasing the accuracy of the method.

Throughout the work, except where otherwise stated, the pipettes used were calibrated to <u>deliver</u> 1 cc., and plates of standard size (10 cm. diameter) were employed. The counts were made, after incubation at 37°C for 48 hours, by naked eye examination in a good window light.

A simplification of the Official Method of Plating.

The procedure prescribed by the Ministry of Health (22) for the estimation of the bacterial content of milk by the plating method is to make dilutions of (a) 1/10, (b) 1/100 and (c) 1/1000. Dilution (a) is prepared by adding 10 c.c. of milk to 90 c.c. of sterile water; dilution (b), by adding 10 c.c. of dilution (a) to 90 c.c. of sterile water; and dilution (c), by adding 10 c.c. of

dilution (b) to 90 c.c. of sterile water. Agar plates are then poured from the respective dilutions, 1 c.c. of inoculum being used for each plate. Where a large number of samples are tested, as in the Bacteriology Department of the West of Scotland Agricultural College which receives annually over 10,000 samples, this method entails the use of large quantities of media and much apparatus. The question therefore arose as to whether equally satisfactory results could be obtained using a simpler and less expensive method and the following considerations regarding the dilutions presented themselves.

In making the plate or colony counts, plates prepared from dilution 1/10, are only employed in the case of samples containing less than 3,000 bacteria per c.c. But these samples are of such a high standard of purity that it is questionable whether, in the routine examination of milk, there is any practical advantage to be gained by a more accurate count than that afforded by the 1/100 dilution plates. Consequently the 1/10 dilution plates are as a rule unnecessary

Most of the milk samples received in the writer's laboratory are less than 24 hours old when tested, the age of the evening milk samples being 20 to 22 hours and that of the morning milk samples, 8 to 10 hours. In many cases they come from farms where great care is taken in the production and treatment of milk. The majority of the samples therefore

contain less than 30,000 bacteria per c.c. and so plates prepared from the 1/100 dilution are generally employed in making the counts. In samples containing a greater number of bacteria even although there are so many colonies on these plates as to make only an approximate estimate possible, the plate counts in conjunction with the coliform test, give a very good indication of the degree to which milk of this age has been contaminated and the temperature at which it has been kept.

It was evident then, that the existing method would be simplified and the amount of work reduced if the 1/10 dilution were omitted and the 1/100 dilution made by adding 1 c.c. of the sample of milk to 99 c.c. of sterile water. A number of samples were therefore examined by both methods, 5, and in a few samples 10, parallel plates being employed. The milk and the 1/100 dilution bottle were subjected to the same amount of agitation as in the official method, i.e. they were shaken 25 times, but with a number of the later samples the 1/100 dilution bottle was shaken 40 and in a few cases, 100 times. The object of this additional shaking was to ensure thorough mixing of the inoculum with the sterile water. A later experiment, however, proved that this was unnecessary. (See p.7).

The results obtained by the two methods correspond very closely (See Table 1, pages 25 to 31). The differences

between the mean plate counts are slight and in most cases less than the standard deviations. They are therefore of no significance.

The proposed method has the advantage of being not only simpler, but requires less apparatus (fewer dilution bottles and pipettes), and results in a saving of time and labour. Less time is spent in performing the necessary manipulations, and in cleaning, preparing and sterilizing apparatus, of which much less is required. The simplicity of the method renders it less liable to error. Finally, a considerable reduction in the cost is effected.

Before adopting the proposed method, it was considered desirable to determine whether an agitation of 25 shakes was sufficient to ensure a uniform distribution of the organisms throughout the suspension.

In the following experiment 25 samples of milk were employed. Each sample was shaken 25 times as usual, and the 1/100 dilution prepared by adding 1 c.c. to 99 c.c. of sterile water. The 1/100 dilution was then agitated and a set of 20 parallel plates poured after the suspension had been shaken 25, 40 and, in the case of the last 12 samples, 50 times. The plates were numbered in sequence as they were poured, so that a comparison could be made of the results obtained from the first five and from the 20

parallel plates.

It will be seen from an examination of Table 2 (pages 32 and 33), in which the results are recorded, that an increase in the amount of shaking has had no appreciable influence on the bacterial count, for the differences are, almost without exception, less than the standard deviations. Further, the differences are not in all cases increases. In several instances the count has been decreased by the additional shaking, although the decrease is too small to be of any significance. The additional shaking does not appear to have distributed the organisms more uniformly throughout the suspension, for the standard deviation, both in the case of the counts of the first 5 and of the 20 parallel plates, has not been consistently reduced. In many samples there has been an increase. The agreement between the counts obtained from the 5 and from the 20 parallel plates appears to be quite satisfactory.

As regards the higher dilutions, in the official method for the estimation of the bacterial content of milk, the plates for the 1/1000 dilution are prepared by taking as the inoculum for each plate 1 c.c. of dilution (c) 1/1000. It would considerably simplify the method and reduce the work and the amount of apparatus required, if the preparation of dilution (c) 1/1000 were omitted and the

1/1000 dilution plates poured by using as the inoculum 0.1 c.c. of dilution (b) 1/100. An experiment was therefore carried out to determine whether as satisfactory results could be obtained in this way as by the official method. In this experiment the 1/100 dilution was prepared by the modified method previously discussed. Five parallel plates from the 1/100 and 1/1000 dilutions were poured, using the procedure prescribed in the official method. Then other 5 plates from the 1/100 dilution were poured using 0.1 c.c. for each plate, the liquid being measured by means of a 1 c.c. pipette, graduated in hundredths. The results of this experiment are given in Table 3 (page 34).

Considering the 1/1000 dilution results by the two different methods, it is seen that in 13 samples out of 20, the corresponding means agree fairly closely, the differences being less than the standard deviations. In the remaining samples, however, they differ widely, these of the plates prepared by the new method being in some cases greater, and in others less, than those of the plates prepared by the official method. In 11 samples the mean counts of the 1/100 dilution plates agree fairly closely with those of the 1/1000 dilution plates prepared by the official method, but only in 8 samples do they agree with those of the 1/1000 dilution plates prepared by the proposed method.

This method of preparing the 1/1000 dilution plates was therefore not so satisfactory as the official one. It was thought that this might be due to variations in the amount of inoculum added to the plate, caused by the use of an ordinary 1 c.c. pipette, graduated in hundredths, which might be insufficiently exact for the purpose.

To test this supposition the experiment was repeated using a pipette which is designed to deliver 0.1 c.c. the last drop being expelled. Sets of 5 parallel plates were poured as before. The results are given in Table 4 (page 35). It will be seen that in all samples there is very close agreement between the corresponding means and standard deviations of the 1/1000 dilution plate counts. Further, in 18 cases out of 25, the mean of the 1/100 dilution plate counts correspond very closely with those of the two sets of 1/1000 dilution plate counts.

It is concluded that the pipette was responsible for the unsatisfactory results obtained in the previous experiment and that the proposed technique is satisfactory.

The method proposed above is simpler than the official one and requires fewer dilution bottles and pipettes. At the same time the results show a close correspondence with those given by the official method. When estimating the bacterial content of milk, it is therefore possible to

effect a great saving in time, labour and cost, by omitting the 1/10 dilution bottles, and preparing by the proposed methods the 1/100 and 1/1000 dilution plates.

The Reliability of the Official Method.

In estimating the bacterial content of milk by the plating method, if the organisms are unevenly distributed in the milk and in the dilution water, there will be a variation in the number present in each cubic centimetre of inoculum used for the plates and in the number of colonies formed on the plates. An error will, therefore, occur which will vary according to the degree of uniformity in which the organisms are distributed. The error will be less if a number of parallel plates are made, so that the final estimate of the bacterial content is based, not on the results obtained from one plate, but from two or more.

Numerous experiments have been carried out to determine the extent of this error. In many cases parallel plates from the same sample were made, five, ten or twenty parallel plates being prepared. The plates were numbered in sequence as they were poured, so that a comparison could be made of the colony counts of the first two, the first five, and so on. In carrying out these experiments 1/100

dilution plates were examined, prepared at first by the official method, and latterly by the new method previously discussed. The inoculum was placed directly in the Petri plate, agar was added and the two then mixed. The distribution of the colonies on the plates showed that the agar and the inoculum had been satisfactorily mixed. Care was taken to maintain uniformity in the manipulations. The time that elapsed from start to finish did not exceed 15 minutes. A fresh pipette was taken for each dilution and also for each set of parallel plates prepared from any one dilution. It is noteworthy that the first plate count of any one set of parallel plates did not differ significantly from the successive plate counts, nor was there any correlation between the individual plate counts, and the order in which they were poured as would have been found if the pipette effect, emphasised by Albus (20) in his criticism of Prescott and Parker (23), was of real importance.

The plates were examined after 48 hours incubation at 37°C. Where less than five hundred colonies were found on a plate, all the colonies were counted; where more than five hundred colonies were found, the plate was divided into segments, and the colonies on two or more of these counted. The size of the segments depended on the number of colonies on the plate, but generally the colonies on a half or a

quarter of the plate were counted. A few plates on which a very large number of "pin point" colonies appeared were discarded.

In order to compare the variations in bacterial count in a number of parallel plates prepared from the same sample, use has been made of the Arithmetic Mean of the parallel plate counts and of the Standard Deviation (24) as calculated by means of the formula:-

$$S.D. = \sqrt{\frac{\Sigma d^2}{n}} \times f,$$

where d = the deviation from the arithmetic mean,

n = the number of observations, and

f = the correcting factor for low values of n. (25)

In order to compare the variation in sets of parallel plates, each set prepared from a different sample of milk, it is clear that absolute figures such as the mean and the standard deviation are useless because different samples may differ widely in their bacterial content. Accordingly for this purpose it is necessary to express the variation of each set as a function of the mean. Use was therefore made of a measure of relative dispersion Pearson's Coefficient of Variation, (26) which is the ratio of the standard deviation to the arithmetic mean, expressed as a percentage, and calculated by means of the formula:-

$$\text{Coefficient of Variation} = \frac{100 \times \text{Standard Deviation}}{\text{Arithmetic Mean}}$$

The coefficient of variation obviously cannot be employed for the comparison of the variations in duplicate plate counts, as in such cases the standard deviation is in-determinate.

Samples with less than 50 colonies per plate may give very high coefficients of variation as a result of the low values of the means. For this reason, in preference to the coefficients of variation for these samples, the standard deviations have been used as a basis for comparison.

The results of these experiments are given in Tables 5, 6 and 7 (pages 36 to 44). The means of the first 2, 5 and 10 and the 20 parallel plate counts have been calculated.+ In the series of 2 plate counts the actual deviation from the mean is given, but in the remaining series the standard deviation and the coefficient of variation are reported. Tables 8, 9 and 10 (pages 45 to 47) are frequency tables of the standard deviations of the means of 5, 10 and 20 plate counts respectively of samples giving less than 50 colonies per 1/100 dilution plate. These tables are combined in Table 11 (page 48) where the frequency is expressed as a percentage. The benefit obtained by the use of a larger number of parallel plates is clearly shown in part (2) of Table 11, for the greater the number of plates, the lower the percentage of high standard deviations.

+In the case of samples for which 20 parallel plates were poured.

It will be seen from part (1) of Table 11, (in which the results of all the samples are included), that in all three series (5, 10 and 20 plate) the standard deviation in over 50 per cent of the samples was less than 5.0, in over 90 per cent it was less than 10.0, and only a few exceeded 15.0. This demonstrates the marked uniformity in the plate counts of samples with a mean count of less than 50 colonies per plate. This is corroborated by the mean standard deviation in each series. See Table 12, (page 49). These results show that in samples which give mean colony counts of less than 50 on the 1/100 dilution plates, there is fairly close agreement between the parallel plate counts, the standard deviation seldom exceeding 15, and being on an average about 5. Such deviations are not high from the biological standpoint. They represent variations in bacterial counts which are of no practical significance in milk analysis.

Tables 13, 14 and 15, (pages 50 to 52), are frequency tables of the coefficients of variation of the counts of 5, 10 and 20 plates respectively in the case of samples giving 50 or more colonies per 1/100 dilution plate. These tables are combined in Table 16 (page 53) where the frequency is expressed as a percentage. It will be seen from the Tables that in the 5 plate series, 22.4 per cent of the samples had coefficients of variation of over 24.4 per cent; in the 10

plate series, 37.3 per cent of the samples; and in the 20 plate series 50.8 per cent. If the results of the first five, the first 10 and the 20 plate counts for each of the same group of samples are considered, (see Table 16, part 2), in the 5 plate series, 30.4 per cent of the samples had coefficients of variation of over 24.4 per cent; in the 10 plate series, 40.5 per cent; and in the 20 plate series, 50.8 per cent. The magnitudes of the coefficients of variation have risen to some extent according to the number of plate counts from which their estimation has been made. It would therefore appear that the correcting factor, f (see page 12), has not been sufficiently great to compensate for the lower number of observations in the 5 and the 10 plate sets of counts. The values of the coefficients of variation for the 20 plate sets are the most reliable, as they have been estimated from the largest number of plate counts.

Considering therefore the results of the series of 20 plate counts, the coefficient of variation exceeds 24.4 per cent, in 50.8 per cent of the samples giving 50 or more colonies per 1/100 dilution plate. The parallel plate counts of such samples vary considerably. This variation is so great, even in the case of a set of plate counts with a coefficient of variation of only 25 per cent, that it constitutes a serious source of error in the estimation of the bacterial content of milk by the official method, even where two plates are used for

each dilution. For example, individual plates belonging to a parallel set with a mean count of 250 and a coefficient of variation of 25 per cent will yield counts ranging from 188 to 312 so that if only two plates have been used and their colony counts are at the upper limit of the range the bacterial content may be estimated as 31,200 instead of 25,000. Similarly if the colony counts are at the lower limit of the range the bacterial content may be estimated as 18,800. In the case of samples with higher coefficients of variation the range of variation of parallel plate counts is much greater.

The wide range of variation between parallel plate counts which may occur in the case of samples whose mean plate counts are 50 or over, can be clearly brought out as follows. The percentage difference between the mean of the two lowest plate counts and that of the two highest for each sample is calculated by means of the formula -

$$\text{Percentage difference} = \frac{(X_1 - X_2) \times 100}{Y}$$

Where X_1 = the mean of the two highest plate counts

X_2 = " " " " lowest plate counts, and

Y = the mean of the 20 plate counts.

The results so obtained from samples whose mean plate counts are 50 or more are given in Tables 17 and 18 (pages 54 to 57), the latter being a frequency table of these percentage

differences. It will be seen from Table 18 that in 69.4 per cent of these samples the percentage difference is 50 per cent or more, in 26.1 per cent of the samples it is 100 per cent or more. The significance of such differences between plate counts in the case of samples of which the average bacterial content is 32,000 or 100,000 is shown in Table 19 (page 58). This Table gives the numbers of bacteria estimated from plate counts which differ by 25, 50 and 100 per cent. It is evident from Tables 17, 18 and 19, that if only two plates are poured for each dilution and the average bacterial content of the milk is over 5,000 there is a possibility of a serious error arising when making bacterial estimations of approximately 70 per cent of the samples, due to the fact that both plate counts may happen to fall at the higher or the lower limit of the range of variation. The probability of this occurring is 1 in 95. A still greater error would be possible if bacterial counts were to be judged by single plates of each dilution.

From a consideration of the various points discussed above it therefore appears clear that in a very high proportion of samples, of which the average bacterial content is 5,000 or more (i.e. the mean plate count is 50 or more), the plating method of estimating the bacterial content of milk may yield highly misleading results if only one, or even two, plates are poured for each dilution.

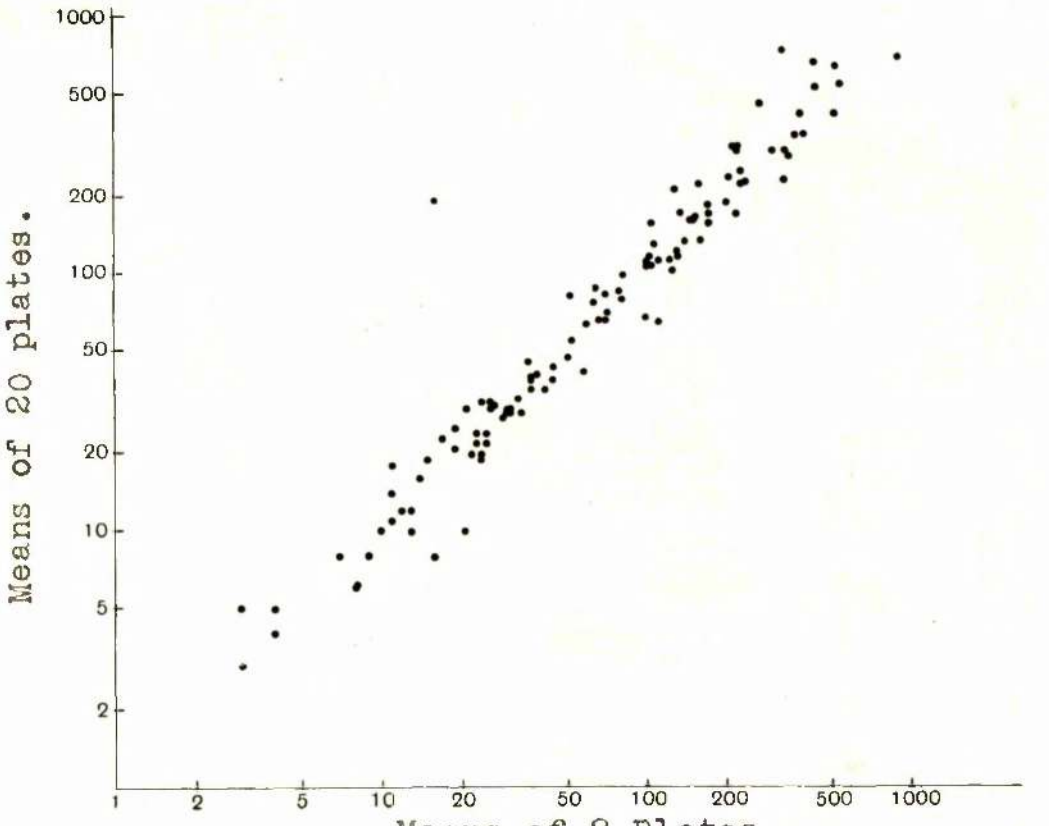
The question arises as to whether more reliable results can be obtained by the use of more than 2 plates for each dilution. As it was the practice to number the plates in sequence as they were poured, it was possible to calculate the means of the first 2, 5 and 10 and the 20 parallel plate counts (See Table 5, pages 36 to 39). A comparison of these means, obtained from 120 samples, is expressed graphically in Figures 1, 2 and 3. A logarithmic scale has been employed so as to cover the range satisfactorily. If the mean count of 20 plates is taken as the standard, it is evident that the results of 5 and 10 plate counts are much more reliable than those of 2.

A comparison of these means is also given in Tables 20, 21 and 22 (pages 59 to 67), the means of the 20 plate counts being again taken as standard.

The actual differences between the mean of the first 2, 5 and 10 plate counts respectively and that of the 20 plate counts are given and also the percentage differences, the latter being determined from the formula:-

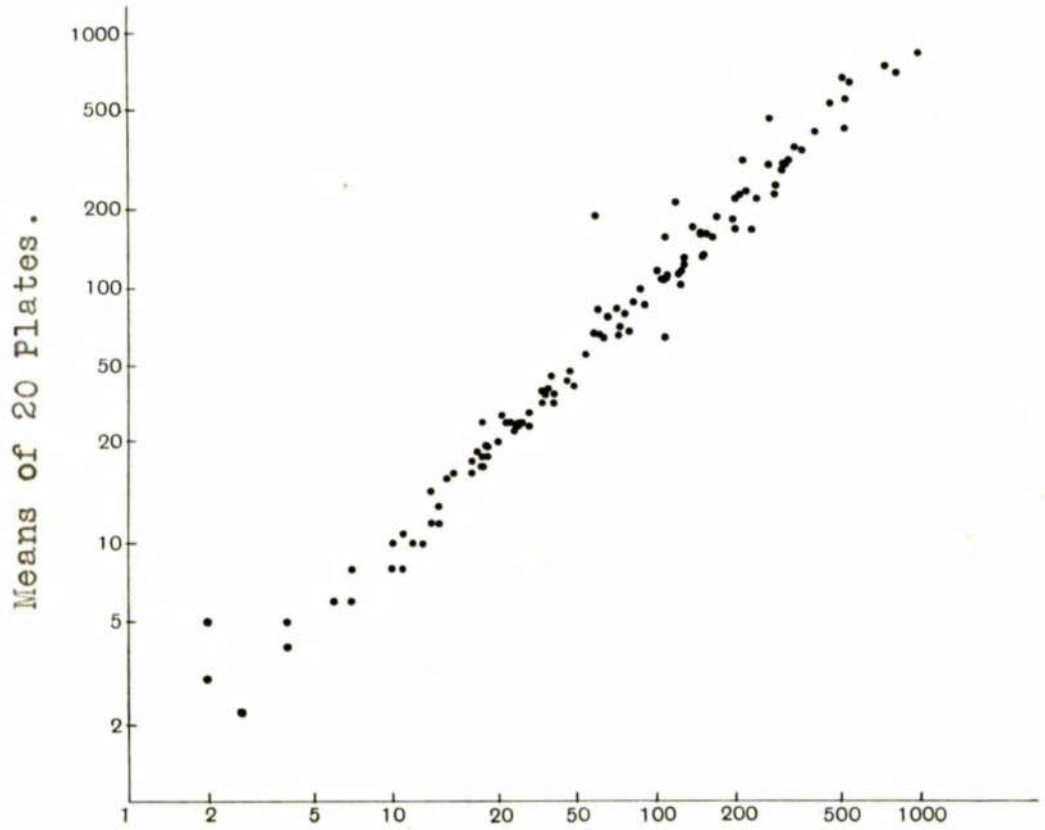
$$\text{Percentage difference} = \frac{(M_{20} - M_x) \times 100}{M_{20}}$$ where M_{20} equals the mean of the 20 plate counts, and M_x equals the mean of the first 2, 5 or 10 plate counts respectively. The percentage difference may be either positive or negative

F I G U R E I.



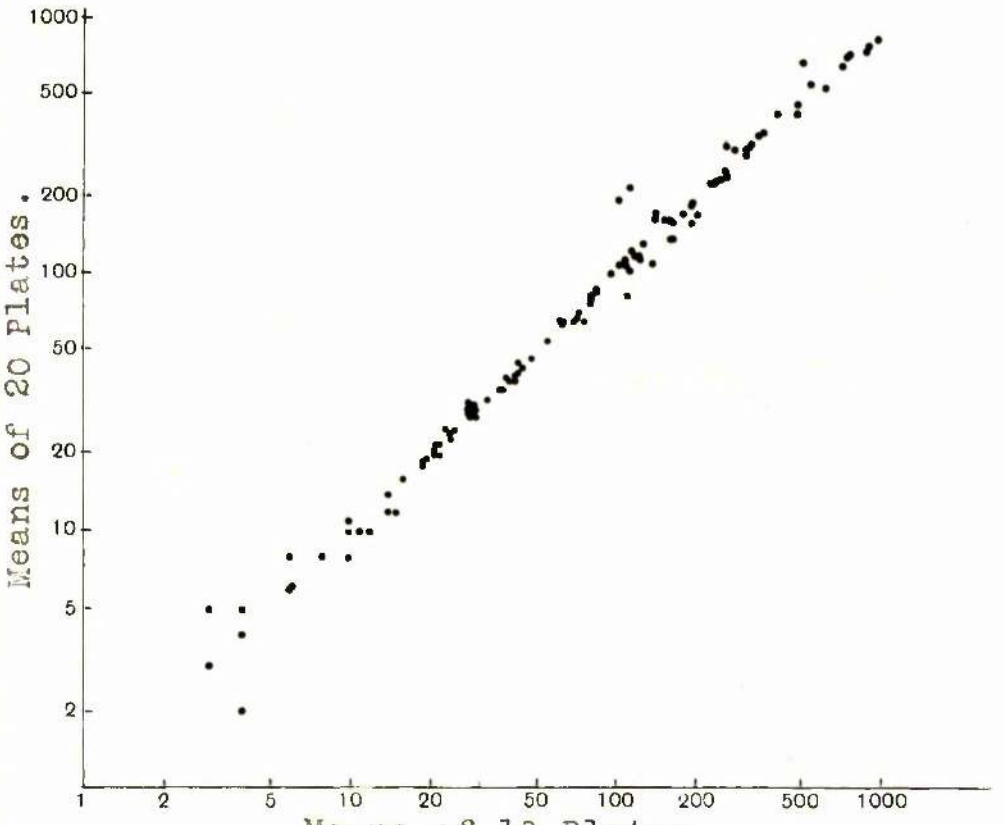
Correlation diagram showing relation between mean counts of two plates and of 20 plates.

F I G U R E II.



Correlation diagram showing relation between mean counts of five plates and of 20 plates.

F I G U R E III.



Correlation diagram showing relation between mean counts of ten plates and of 20 plates.

according to the value of M_x being less or greater than the value of M_{20}. Tables 23, 24 and 25 (pages 68 to 70) give the frequency of these percentage differences, a separate table being employed for each series of comparisons. These three tables are combined in Table 26 (page 71), where the frequencies are expressed as percentages of the number of samples, viz 120. Fifty-one samples with mean counts of less than 50 colonies on the 1/100 dilution plates have been included in the tables. In such samples the actual differences between the mean counts obtained from the 2, 5 or 10 plates and from the 20 plates are generally very small but owing to the effect of the low value of the means, the percentage differences between the mean plate counts are in some instances high. The marked uniformity of the parallel plate counts of such samples is clearly shown by Tables 27, 28 and 29 (pages 72 to 74) which give the frequencies of the actual differences between the mean colony counts of 2, 5 and 10 plates, and of the corresponding 20 plates. In only a very small proportion of the samples is the difference between the means greater than 5.0. These tables are combined in Table 30, (page 75) where the frequencies are expressed as percentages of the number of samples.

Table 23 (page 68) shows that in 36.5 per cent of the samples, the mean colony counts of the first 2 plates

differ by more than 19.4 per cent from those of the corresponding 20 plates. The percentage of samples, however, is reduced to 21.5 per cent when a deduction of 15 is made as a percentage allowance for 18 samples having mean plate counts of less than 50. In the case of these 18 samples with low mean counts, although the percentage difference between the means of the first 2 and the 20 plate counts is over 19.4 the actual difference is of no practical importance. If the mean of the 20 plate counts is taken as a standard, it is obvious that in a large proportion of the samples, viz; 21.5 per cent, the mean count of 2 plates is not satisfactory. For example, in the case of one sample, No.56 (Tables 5 and 20, pages 38 and 61), the mean of the 2 plate counts was 339, and the mean of the 20 plate counts 240, the percentage difference between the means being 41.3 per cent. Using only 2 plates, therefore, the bacterial content of this sample would be estimated as 33,900 instead of 24,000.

It is evident from Tables 21 and 24 (pages 62 to 64 and page 69) that the results obtained from the use of 5 parallel plates are more satisfactory. In 49.3 per cent of the samples the difference between the mean count of 5 plates and that of 20 plates does not exceed 9.4 per cent. In 19.1 per cent of the samples, i.e. 23 samples, the difference exceeds 19.4 per cent. These 23 samples may be divided into three groups.

(1) Samples, 9 in number, which gave mean plate counts

of less than 50. (See Table 31, page 76). It will be seen that the actual differences between the means of the 5 and of the 20 plate counts are of no practical significance. The high values of the percentage differences are due to the low values of the means.

(2) Samples, 4 in number, with very high mean plate counts (See Table 32, page 77). Owing to the large number of colonies on the plates, only an approximate count could be made, and this may be partly responsible for the large differences between the mean counts.

(3) Samples, 10 in number, giving irregular plate counts (See Table 33, page 78). In only 5 of these samples, viz: Numbers 218, 14, 66, 198 and 23, are the differences between the mean counts so great as to give misleading results where only 5 plates are used.

With the exception, therefore, of about 4 per cent of the samples, of which the plate counts are highly irregular, reliance can be placed on the mean counts obtained from the five 1/100 dilution plates, provided that the bacterial content of the milk does not exceed 50,000 or 60,000 organisms per c.c., i.e. that the number of colonies on the plates does not exceed 500 or 600.

Tables 22 and 25 (pages 65 to 67, and page 70) show that there is a very close agreement between the mean

counts of 10 and 20 parallel plates. In only 9.9 per cent of the samples does the difference between the mean counts exceed 19.4 per cent, and if allowance is made for the samples with mean plate counts below 50, then only 4.1 per cent, i.e., 5 samples, exceed this difference. It will be seen from Table 34 (page 79) that in only 2 of these 5 samples, viz; Numbers 14 and 23, are the differences between the mean counts so great as to give misleading results, where only 10 plates are employed. It should be noted that where the mean plate count is not above 50, a very close agreement exists between the means obtained from 10 and from 20 parallel plates, the actual difference between the means in all instances being less than 4.5.

It is apparent that the results obtained from 10 plate counts are more trustworthy than those obtained from 2 or 5. The 10 plate method, however, suffers from the disadvantage that the requirements in media and apparatus are high and apart from the question of cost, the method is consequently not suitable for the routine examination of milk. Further, it has been shown that the 5 plate method yields results which appear to be sufficiently reliable for this purpose.

Suggested procedure for the more accurate estimation of the bacterial content of Milk.

In the routine examination of milk for bacterial content, the following method is recommended. Omit the 1/10 dilution bottle and 1/10 dilution plates, and prepare the 1/100 and 1/1000 dilution plates by the modified methods previously discussed. Use 5 parallel plates for the 1/100 dilution and 2 plates for the 1/1000 dilution.

As has already been shown, in samples whose counts amount to as much as 50,000 or more organisms per c.c., the above method yields reliable results. When the bacterial content exceeds 50,000 organisms per c.c., e.g. up to 100,000, a reasonable degree of reliance can be placed on the results obtained, owing to the large number of 1/100 dilution plates, which can be used to some extent to confirm the results of the two 1/1000 dilution plates. But if it is desired to obtain fairly accurate estimates when the counts are in the neighbourhood of 100,000 organisms per c.c. at least 5 plates must be used for the 1/1000 dilution.

Nevertheless the possibility of the occurrence of a serious error is not completely eliminated even where 5 plates are used, for in 4 per cent of the samples the estimates may be highly misleading. Therefore in grading

milk by this method, where the bacterial count exceeds the count which is the limiting standard for the grade, the results should be confirmed by means of a second test upon another sample. While this is advisable where five plates are poured for each dilution, it is essential where only one or two plates are poured, otherwise a milk supply may be condemned unjustly. It must, in fact, always be borne in mind that the most reliable information in regard to the degree of purity of any given milk supply is obtained not from single tests, but from a series of tests performed at intervals over a period of several months, or preferably over a full year.

TABLE 1.

(Individual plates in order of inoculation)

Sample No.	OFFICIAL METHOD: No. of Colonies per plate	Mean	S.D.*	C.V.+ (per cent)	PROPOSED METHOD: No. of Colonies per Plate	Mean	S.D.*	C.V.+ (per cent)
83.	43				43			
	43				41			
	41	42	1.5	3.6	42	42	1.4	3.4
	42				40			
	40				42			
84.	579				644			
	589				599			
	544	570	47.4	8.3	584	595	35.1	5.9
	516				566			
	622				604			
85.	146				118			
	144				117			
	143	144	2.4	1.7	119	117	5.3	4.6
	141				121			
	146				109			
86.	84				85			
	85				85			
	87	86	1.8	2.1	81	85	2.8	3.3
	86				87			
	88				87			
87.	171				155			
	223				177			
	187	189	23.7	12.5	170	156	22.9	14.7
	175				150			
	189				126			

S.D.* - Standard Deviation corrected (See page 12)

C.V.+ = Coefficient of Variation (See page 12)

TABLE 1. (Contd)

Sample No.	OFFICIAL METHOD: No. of Colonies per plate	Mean	S.D.*	C.V.+ (per cent)	PROPOSED METHOD: No. of Colonies per plate	Mean	S.D.*	C.V.+ (per cent)
88.	1024 1128 1172 992 1024	1068	69.1	6.5	960 928 1018 1112 1056	1015	85.0	8.4
89.	68 66 67 64 68	67	2.0	2.9	87 94 88 85 85	88	4.3	4.9
90.	106 109 104 103	106	3.0	2.9	116 109 104 109 124	112	9.0	8.0
91.	51 53 71 46 52	55	11.0	20.1	48 63 51 58 49	54	7.5	13.8
93.	9 12 9 12 10	10	1.8	18.3	3 7 16 10 9	9	5.5	60.8
94.##	624 555 621 576 583	592	34.5	5.9	602 494 584 568 551	560	47.8	8.5

1/100 dilution shaken 50 times.

TABLE 1. (Contd)

Sample No.	OFFICIAL METHOD No.of Colonies per plate	Mean	S.D.	C.V. (p.cent)	PROPOSED METHOD No. of Colonies per plate	Mean	S.D.	C.V. (per cent)
95. ++	55 55 57 58 65	58	4.8	8.2	65 63 61 51 54	59	7.0	11.8
96. ++	24 17 16 18 15 17 17 14 11 16	17	3.6	20.9	10 14 15 13 13 20 16 19 16 14	15	3.1	20.8
97. ++	52 48 60 48 64 59 52 60 61 51	56	6.2	11.2	54 53 57 49 64 48 56 54 52 62	55	5.4	9.9
98.	123 102 119 168 104	123	3.1	2.5	141 143 113 121 104	124	19.8	16.0

++ 1/100 dilution shaken 50 times.

TABLE 1. (Contd)

Sample No.	OFFICIAL METHOD: No. of Colonies per plate	Mean	S.D.	C. V. (per cent)	PROPOSED METHOD: No. of Colonies per plate	Mean	S.D.	C. V. (per cent)
99.	167 170 170 170 171	170	2.9	1.7	103 108 114 119 110	111	7.0	6.3
100.	23 20 17 21 17 18 19 18 17 18	19	2.1	11.2	19 18 16 13 13 16 16 14 17 104	25	30.0	120.0
101.	7 9 5 8 7 7 10 11 11 6	8	2.0	23.8	3 3 5 4 4 10 6 6 7 4	5	2.3	45.8
102.	133 132 135 137 122	132	6.8	5.1	100 127 134 106 108	115	16.9	14.7
103.	66 77 68 73 63	69	6.5	9.3	110 78 65 64 56	75	24.6	32.8

TABLE 1. (Contd)

Sample No.	OFFICIAL METHOD No. of Colonies per plate	Mean	S.D.	C.V. (per cent)	PROPOSED METHOD No. of Colonies per plate.	Mean	S.D.	C.V. (per cent)
	23				24			
	26				23			
	20				25			
	27				19			
104.	21	23	2.3	9.9	24	23	6.3	27.2
	22				11			
	23				35			
	24				24			
	22				24			
	22				22			
	215				186			
	213				184			
105.	230	213	16.9	7.9	196	191	6.5	3.4
	190				196			
	219				193			
	159				151			
	154				139			
106.	177	155	16. 2	10.4	162	159	16.2	10.2
	142				171			
	144				172			
	137				125			
	174				128			
108.	138	151	22.8	15.1	155	139	16.0	11.5
	136				134			
	172				152			
	19				26			
	24				29			
	23				22			
	24				22			
109.	35	23	6.2	27.0	24	24	4.8	20.0
	29				19			
	18				21			
	24				16			
	14				29			
	21				29			

TABLE 1. (Contd)

Sample No.	OFFICIAL METHOD No. of Colonies per plate	Mean	S.D.	C.V. (per cent)	PROPOSED METHOD No. of Colonies per plate	Mean	S.D.	C.V. (per cent)
	11				23			
	14				22			
	17				22			
	24				22			
110.	12	13	4.8	37.0	19	23	4.6	20.0
	9				25			
	10				21			
	9				33			
	13				17			
	14				21			
	11				16			
	37				17			
	24				25			
	17				23			
111.	15	22	7.8	35.2	19	20	3.0	15.2
	17				20			
	26				17			
	22				18			
	18				21			
	22				20			
	31				47			
	33				45			
	38				52			
	36				38			
112.	35	35	3.4	9.5	38	41	5.9	15.5
	38				38			
	38				33			
	31				39			
	31				39			
	38				44			
	15				12			
	25				16			
	27				12			
113.	18	22	5.5	25.1	11	13	2.4	17.5
	30				11			
	22				16			
	24				15			
	18				12			

TABLE 1. (Contd)

Sample No.	OFFICIAL METHOD: No. of Colonies per plate	Mean	S.D.	C.V. (per cent)	PROPOSED METHOD: No. of Colonies per plate	Mean	S.D.	C.V. (per cent)
249.	36 33 36 37 35 34 38 37 37 45	37	3.5	9.4	- 27 32 33 33 35 39 37 39 37	35	4.2	11.9

TABLE 2 (1)

COUNTS of FIRST FIVE PARALLEL PLATES

Sample No.	25 Shakes		40 Shakes		50 Shakes	
	Mean Count	S.D.	Mean Count	S.D.	Mean Count	S.D.
142	20	4.6	15	5.5		
143	174	29.4	204	54.7		
144	294	57.7	323	40.6		
145	82	43.9	60	8.7		
146	8	3.4	12	2.7		
147	27	8.1	27	9.0		
148	148	22.1	157	24.9		
149	113	18.0	127	10.8		
150	189	62.9	210	52.6		
151	149	36.3	129	23.3		
152	442	639.6	826	745.4		
153	420	108.5	659	206.9		
154	108	16.6	103	34.1		
208	22	4.0	20	4.5	24	4.4
209	108	5.5	112	4.0	122	9.4
210	42	7.3	38	5.1	41	4.2
211	31	3.2	41	3.9	35	1.3
213	37	2.9	37	3.5	36	3.1
214	29	5.6	31	.8	30	2.1
215	33	4.1	33	3.1	33	2.2
216	67	8.6	72	2.2	71	4.9
217	49	1.5	40	6.1	43	5.5
218	111	10.4	105	15.8	59	20.9
221	21	2.2	23	2.1	26	5.5
222	18	2.3	22	5.1	21	1.8

TABLE 2. (11)

COUNTS of TWENTY PARALLEL PLATES

Sample No.	25 Shakes Mean Count	25 Shakes S.D.	40 Shakes Mean Count	40 Shakes S.D.	50 Shakes Mean Count	50 Shakes S.D.
142	13	7.2	14	4.1		
143	163	25.7	176	58.0		
144	306	50.2	326	29.0		
145	94	31.4	84	23.2		
146	9	2.5	10	3.1		
147	25	5.5	30	5.8		
148	144	19.3	139	18.9		
149	108	32.8	120	12.3		
150	211	57.8	238	68.1		
151	132	26.3	135	18.5		
152	548	468.9	729	480.5		
153	485	110.3	743	200.3		
154	119	59.3	113	32.4		
208	21	2.8	21	3.6	23	2.6
209	99	12.4	116	12.7	121	8.2
210	40	5.2	39	6.6	40	5.4
211	34	3.7	36	3.6	36	4.2
213	36	3.3	36	4.8	36	3.4
214	30	2.7	30	1.3	30	2.3
215	33	2.9	33	3.1	33	2.5
216	65	7.7	67	7.0	73	5.8
217	49	4.1	46	8.8	46	7.4
218	103	24.6	66	28.4	66	22.0
221	23	2.5	24	2.6	24	1.5
222	19	3.6	20	4.3	20	2.5

TABLE 3.

FIVE PLATE COUNTS

Sample No.	Dilution 1/100. Mean Count	S.D.	C.V. (per cent)	Dilution 1/1000. Official Method Mean Count	S.D.	C.V. (per cent)	Proposed Method Mean Count	S.D.	C. V. (per cent)
186	18	4.4	24.5	2	1.6	7.5	1	.8	77.5
194	7	1.5	21.8	1	1.0	10.3	1	.8	77.5
195	16	3.7	23.4	4	1.7	4.2	4	2.5	61.3
189	29	3.7	12.6	6	1.0	17.2	8	2.8	35.5
177	44	30.2	68.7	16	3.6	22.6	9	6.5	71.8
175	92	4.1	4.4	7	1.4	20.3	10	4.0	40.0
193	30	3.0	9.9	10	1.2	11.6	11	2.7	24.7
176	111	6.7	6.1	14	2.7	19.2	12	4.8	39.8
172	91	31.2	34.3	8	1.3	16.1	14	2.2	15.9
191	75	1.6	2.2	13	1.4	10.9	18	9.7	53.8
188	145	5.6	3.8	17	6.3	37.2	20	2.6	12.9
178	251	8.7	3.5	21	4.3	20.3	22	3.0	13.5
187	533	9.8	1.8	28	4.0	14.3	24	3.7	15.6
174	119	21.9	18.4	10	4.4	43.9	26	7.1	27.3
190	539	24.5	4.5	59	16.7	28.2	61	14.2	23.3
181	651	198.8	30.5	58	2.2	3.8	109	22.3	20.5
192	879	8.7	1.0	178	11.2	6.3	129	31.7	24.6
179	1081	87.0	8.1	148	28.5	19.3	130	12.3	9.4
185	1714	1382.7	8.1	232	58.2	25.1	161	12.9	8.0
173	1335	888.3	66.5	522	127.3	24.4	402	93.3	23.2

TABLE 4.

FIVE PLATE COUNTS

Sample No.	Dilution 1/100. Mean	S.D.	Coefficient of Variation %	Dilution 1/1000 Official Method Mean	S.D.	Coefficient of Variation %	Dilution 1/1000 Proposed Method Mean	S.D.	Coefficient of Variation %
225	10	.8	7.8	1	0.6	64.5	1	0.8	77.5
233	20	1.6	7.8	2	3.1	154.9	2	1.9	97.0
226	37	2.6	7.0	3	2.5	81.7	3	2.1	68.8
236	54	2.5	4.6	7	1.2	16.6	7	0.8	11.1
238	52	3.0	5.7	6	0.8	12.9	7	1.2	16.6
240	91	2.7	3.0	9	1.3	14.3	8	1.2	14.5
239	83	1.7	2.0	8	1.2	14.5	8	1.7	21.0
224	91	3.4	3.7	10	0.8	7.8	10	0.8	7.7
241	125	4.8	3.8	11	0.6	5.9	11	0.8	7.0
230	132	5.9	4.5	12	0.8	6.5	12	0.8	6.5
246	110	5.0	4.6	14	1.5	11.1	13	2.3	17.8
223	68	12.1	17.9	14	2.2	15.7	14	2.6	18.4
244	118	4.4	3.7	14	1.8	12.9	15	1.0	7.0
245	146	4.5	3.1	15	2.6	17.2	15	1.5	10.3
231	227	8.1	3.6	14	0.8	5.5	16	1.2	7.3
242	266	13.6	5.1	23	1.0	4.5	22	1.0	4.7
248	262	12.2	4.6	27	1.8	6.8	26	2.5	9.7
229	338	17.8	5.3	32	1.2	3.6	31	0.6	2.1
237	360	7.1	2.0	38	1.7	4.4	38	1.5	4.1
243	354	13.2	3.7	51	3.4	6.6	48	1.7	3.5
227	338	17.8	5.3	51	1.2	2.3	50	2.2	4.4
234	442	8.5	1.9	71	1.0	1.5	71	1.2	1.6
228	813	39.0	4.8	92	2.3	2.5	93	2.5	2.6
232	1495	70.6	4.7	118	3.1	2.6	121	1.7	1.4
247	1024	8.0	.8	121	3.4	2.8	124	4.3	3.5
235	4497	100.4	2.2	718	39.0	5.4	733	44.2	6.0

-35-

TABLE 5.

SAMPLES for which 20 PLATES were poured

RESULTS OBTAINED from the USE of 2, 5, 10 and 20 PARALLEL PLATES
in ASCENDING ORDER of MEANS of 20 PLATE SERIES.

	First 2 Plates		First 5 Plates			First 10 Plates			20 Plates		
Sample No.	Mean Plate Count	Deviation	Mean Plate Count	Standard Deviation.	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)
204x	1	0	3	2.8	92.5	4	2.1	52.3	2	2.3	116.5
205x	3	0	2	1.2	57.8	3	1.3	44.0	3	1.2	38. 7
164	4	0	4	1.4	35.5	4	1.1	26.5	4	1.1	27.7
170	4	0	4	1.9	47.8	4	1.4	34.4	5	2.7	54.0
204	3	3.0	2	3.1	153.0	3	2.2	72.7	5	4.2	83.1
80	8	.5	7	1.2	16.5	6	1.6	27.0	6	1.8	30.4
163	8	3.0	6	3.3	54.4	6	2.3	38.2	6	2.0	33.7
159	9	1.5	10	2.2	21.6	8	3.0	37.7	8	2.8	35.6
162	16	9.5	11	9.5	86.6	10	6.2	61.5	8	4.8	60.1
199x	7	3.0	7	2.5	36.0	6	3.7	60.9	8	5.2	64.4
19	10	5.5	10	5.4	54.2	10	4.1	40.8	10	3.7	37.0
146	13	2.5	12	2.7	22.6	12	2.8	23.4	10	3.1	31.0
196	21	9.5	13	11.3	86.5	11	7.4	67.4	10	5.2	52.3
202x	11	2.0	11	2.6	23.5	10	2.7	27.4	11	3.4	30.5
160	12	1.5	15	5.5	36.8	15	3.8	25.5	12	4.8	40.2
204y	13	2.0	14	3.1	21.9	14	3.1	21.9	12	4.3	35.5
142	11	2.0	15	5.5	36.5	14	4.0	28.9	14	4.1	29.1
81	14	3.5	14	3.4	24.1	16	4.8	29.8	16	4.5	28.0
200x	11	0	16	11.9	74.2	19	12.2	64.1	18	10.2	56.8
155	24	1.5	20	6.3	31.5	19	7.1	37.4	19	5.8	30.4
70	15	.5	17	3.6	21.2	19	4.1	21.8	19	3.7	19.5
39	22	.5	22	1.8	8.3	21	2.9	13.6	20	3.7	18.4
222	24	2.5	22	5.1	23.3	22	3.8	17.4	20	4.3	21.5
208	19	1.0	20	4.5	22.5	21	4.3	20.6	21	3.6	17.2
52	23	4.0	23	5.0	21.6	21	3.6	17.0	22	3.4	15.3
201x	25	.5	22	3.9	17.6	22	3.9	17.8	22	3.3	14.9
71	17	.5	21	6.5	31.0	24	7.0	29.2	23	5.0	21.9
41	25	1.5	23	3.8	16.7	24	4.0	16.9	24	4.3	17.8
221	23	2.0	23	2.1	9.1	24	2.4	10.1	24	2.6	10.9
45	19	2.0	25	10.4	41.5	23	7.2	31.4	25	6.2	24.7
171	29	.5	29	2.2	7.7	30	4.4	14.8	28	4.4	15.6
40	30	8.0	33	9.1	27.5	29	8.4	28.8	29	7.7	26.4

TABLE 5. (Contd)

SAMPLES for which 20 PLATES were poured.

RESULTS OBTAINED from the USE of 2, 5, 10 and 20 PARALLEL PLATES in ASCENDING ORDER of MEANS of 20 PLATE SERIES.

	First 2 Plates		First 5 Plates			First 10 Plates			20 Plates		
Sample No.	Mean Plate Count	Deviation	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation. (per cent)
42	30	2.5	30	5.9	19.5	29	6.7	22.9	29	6.4	22.0
219	34	8.0	30	8.1	26.9	28	6.2	22.2	29	5.9	20.5
82	30	5.0	30	6.1	20.4	29	4.9	16.7	30	4.4	14.8
147	21	6.0	27	9.0	33.3	28	6.7	24.1	30	5.8	19.3
182	26	1.0	28	4.0	14.3	28	6.4	22.9	30	7.8	26.1
206	26	7.5	22	7.5	34.2	29	10.4	35.7	30	7.9	26.4
214.	31	.5	31	.8	2.6	30	1.1	3.7	30	1.3	4.4
212.	24	1.0	26	4.2	16.0	28	5.6	20.1	32	6.8	21.4
215	33	1.0	33	3.1	9.3	33	2.4	7.4	33	3.1	9.5
211	42	4.0	41	3.9	9.5	38	4.1	10.7	36	3.6	10.1
213	37	.5	37	3.5	9.5	37	2.8	7.7	36	4.8	13.2
171x	45	6.5	41	10.3	25.1	40	8.7	21.9	39	7.2	18.5
210	37	.5	38	5.1	13.4	42	6.8	16.1	39	6.5	16.8
55	37	5.5	37	6.3	17.1	39	6.1	15.8	40	6.8	17.1
72.	39	2.0	39	1.8	4.7	42	6.6	15.8	41	6.1	14.9
48.	59	4.5	49	10.9	22.3	43	9.9	23.0	42	8.4	20.0
197	45	6.0	46	5.4	11.8	45	4.3	9. 6	44	4.8	11.0
217	36	3.5	40	6.1	15.2	43	8.4	19.5	46	8.8	19.1
75	51	6.5	47	9.6	20.4	48	6.4	13.4	48	8.7	18.1
203x	53	4.0	54	6.6	12.2	56	5.8	10.3	56	5.1	9.1
44	60	1.5	63	6.5	10.4	63	6.2	9.9	65	9.2	14.1
218	113	2.5	105	15.8	15.1	77	36.5	47.4	66	28.4	43.1
78.	67	1.0	61	12.7	20.8	64	11.6	18.1	67	12.0	18.0
216	71	2.0	72	2.2	3.0	70	5.2	7.5	67	7.0	10.4
53	60	8.0	58	7.0	12.2	62	10.8	17.5	68	12.2	17.9
51	101	32.5	79	35.5	44.9	72	23.9	33.3	69	19.4	28.1
79	72	20.0	73	17.9	24.5	73	12.9	17.6	72	23.6	32.8
166	64	17.5	65	18.2	28.0	81	26.2	32.4	79	21.3	26.9
199	82	16.0	76	14.8	19.5	81	12.6	15.6	81	15.1	18.7
145	52	0	60	8.7	14.6	81	29.3	36.2	84	23.2	27.6
161	71	3.5	71	35.9	50.6	115	72.6	63.1	85	59.8	70.3
168	80	4.0	91	17.4	19.1	85	14.8	16.7	88	15.2	17.3
43	65	1.0	81	17.4	21.5	85	12.1	14.3	90	12.5	13.9
74	83	.5	88	11.0	12.5	97	13.2	13.6	101	16.1	15.9
11	128	24.0	125	19.9	15.9	114	25.8	22.6	106	21.7	20.5
203	107	4.5	108	4.1	3.8	110	7.3	6.6	110	6.8	61.7

TABLE 5. (Contd)

SAMPLES for which 20 PLATES were poured.

RESULTS OBTAINED from the USE of 2. 5. 10 and 20 PARALLEL PLATES in ascending ORDER of MEANS of 20 PLATE SERIES.

	First 2 Plates		First 5 Plates			First 10 Plates			20 Plates		
Sample No.	Mean Plate Count	Deviation	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)
38	104	4.5	106	4.9	4.6	104	11.0	10.6	111	32.2	29.0
183	103	3.5	111	10.8	9.7	119	15.7	13.2	112	12.3	11.0
49	125	15.5	123	13.7	11.1	124	19.7	15.9	116	20.4	17.6
209	113	.5	112	4.0	3.5	109	10.4	9.5	116	12.7	10.9
47	105	49.0	102	69.4	68.1	123	52.9	43.0	120	41.6	34.6
149	135	3.0	127	10.8	8.5	119	13.4	11.2	120	12.3	10.3
15	133	9.0	130	10.5	8.1	116	23.6	20.4	126	27.1	21.5
151	109	1.0	129	23.3	18. 1	128	19.2	15.0	135	18.5	13.7
148	163	28.5	157	24.9	15.9	146	21.6	14.8	139	18.9	13.6
165	145	10.0	154	26.1	16.9	142	22.1	15.5	139	20.1	14.5
65	107	4.0	110	12.1	11.0	196	213.1	108.7	161	152.6	94.8
220	176	6.0	167	11.4	6.8	165	16.5	10.0	162	14.4	8.9
10	153	37.0	150	69.9	46.6	141	50.8	36.0	166	50.7	30.6
59	155	14.5	158	12.6	8.0	162	10.2	6.3	166	22.1	13.3
62	136	16.5	149	20.5	13.8	155	39.2	25.3	166	34.9	21.0
157	234	22.0	233	47.6	20.4	206	42.9	20.8	175	43.9	25.1
143	176	35.5	204	54.7	26.8	182	52.5	28.8	176	58.0	33.0
16	137	17.0	140	31.5	22.5	146	23.0	15.8	178	46.5	26.1
18	175	23.0	197	34.9	17.7	195	32.7	16.8	191	49.6	25.9
65	205	70.0	172	77.3	44.9	197	119.4	60.6	195	85.8	44.0
14	16	2.0	59	110.6	187.4	103	114.5	111.2	198	178.5	90.1
23	131	10. 5	120	14.4	12.0	114	12.3	10.8	221.	249.1	112.7
207	161	12.5	203	50.6	24.9	229	46.4	20.3	230	38.0	16.5
7	232	0	245	29.7	12.1	239	58.3	24.4	232	59.1	25.5
150	241	.5	210	52.6	25.0	241	79.1	32.8	238	68.1	28.6
56	339	93.5	275	108.3	39.4	251	84.3	33.6	240	73.2	30.5
57	210	5.5	224	41.9	18.7	267	67.2	25.2	246	51.9	21.1
169	231	21.0	289	177.3	61.3	260	181.5	69.8	260	152.7	58.7
6	356	12.0	308	138.7	45.0	316	91.5	29.0	299	89.0	29.8
200	225	15.0	273	67.6	24.8	282	67.9	24.1	312	100.2	32.1
20	306	38.0	306	43.4	14.2	313	55.3	17.7	314	58.3	18.6
46	344	13.5	315	42.9	13.6	313	31.5	10.1	314	28.6	9.1
66	216	29.0	219	40.6	18.5	265	65.9	24.9	326	96.7	29.7
144	327	20.5	323	40.6	12.6	313	30.4	9.7	326	29.0	8.9
26	377	4.5	363	15.6	4.3	349	28.3	8.1	359	26.7	7.4
8	402	58.0	343	41.8	12.2	367	85.7	23.3	367	84.0	22.9

TABLE 5 (Contd)

SAMPLES for which 20 PLATES were poured.

RESULTS OBTAINED from the USE of 2, 5, 10 and 20 PARALLEL PLATES in ascending ORDER of MEANS of 20 PLATE SERIES.

	First 2 Plates		First 5 Plates			First 10 Plates			20 Plates		
Sample No.	Mean Plate Count	Deviation	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)
201	391	37.5	408	61.8	15.1	414	71.1	17.2	435	73.8	17.0
64	527	8.5	534	21.2	4.0	431	112.1	23.3	437	142.2	32.5
198	278	13.0	271	27.8	10.2	495	334.3	67.5	475	236.6	49.8
60	449	32.5	463	117.0	25.3	622	1185.0	190.2	553	121.0	21.8
17	561	33.5	524	49.8	9.5	545	58.3	10.7	573	63.5	11.1
37	337	5.0	551	26.7	4.8	721	346.3	48.0	674	244.7	36.3
183x	442	220.5	519	202.4	39.0	515	126.8	24.6	698	381.3	54.6
152	923	393.5	826	745.4	90.2	758	553.1	73.0	729	480.5	65.9
197x	1780	132.0	1031	625.6	60.5	774	576.5	74.5	751	494.4	65.8
21	337	13.0	758	469.0	61.9	884	348.6	39.4	772	292.0	37.8
196x	1069	227.5	1120	210.8	18.8	916	285.0	31.1	815	238.6	29.3
51	1055	111.0	1000	140.5	14.0	979	192.9	19.7	866	189.8	21.9
202	1956	124.0	2045	203.3	9.9	1812	552.0	30.5	1628	513.8	31.6
54	1076	590.0	1433	755.5	52.7	1779	680. 3	38.2	1724	670.6	38.9
205	2127	1.0	2189	313.1	14.3	2225	255.6	11.5	2242	207.3	9.3
203y	2992	112.0	2906	228.4	7.9	2871	284.7	9.9	3045	485.3	15.9

TABLE 6.

SAMPLES for which only 10 PARALLEL PLATES were poured.

RESULTS OBTAINED from the USE of 5 and 10 PARALLEL PLATES in ASCENDING ORDER of MEANS of 10 PLATE SERIES.

Sample No.	First 5 Plates: Mean Plate Count	First 5 Plates: Standard Deviation	First 5 Plates: Coefficient of Variation (per cent)	10 Plates: Mean Plate Count	10 Plates: Standard Deviation	10 Plates: Coefficient of Variation (per cent)
122	2	1.0	49.7	2	.9	45.0
138	2	1.0	49.7	2	.8	39.7
127	2	1.1	57.5	2	.9	47.0
101	4	1.0	24.9	5	2.3	45.8
137	5	1.1	23.0	6	1.4	23.7
136	6	1.8	30.3	7	2.4	35.0
128	7	2.5	35.0	7	1.8	26.2
125	13	2.8	21.8	12	2.0	16.4
121	15	5.1	34.0	12	4.9	40.4
115	13	2.5	18.9	13	2.1	16.5
113	12	2.5	20.4	13	2.4	17.5
140	12	2.2	18.0	14	3.5	24.8
96	13	2.2	16.6	15	3.1	20.8
119	14	3.6	25.7	16	3.7	23.3
133	17	2.5	14.4	18	2.0	11.3
139	17	1.8	10.7	18	2.8	15.7
131	19	3.7	19.7	20	3.6	17.8
111	20	4.5	22.3	20	3.0	15.2
104	23	2.7	11.8	23	6.3	27.2
110	22	1.8	8.3	23	4.6	19.9
109	25	3.5	13.8	24	4.8	20.0
126	27	4.4	16.3	24	4.8	20.1
100	20	3.1	15.3	25	29.7	11.2
116	30	5.6	18.7	28	4.9	17.6
130	29	6.1	20.9	28	5.0	17.8
141	30	1.7	5.8	28	5.2	18.7
132	26	4.3	16.5	28	5.9	21.0
182	28	4.0	14.3	28	6.4	22.9
167	40	42.7	106.8	31	29.8	96.1
114	30	4.7	15.8	31	3.8	12.2
249	32	3.5	10.8	35	4.2	11.9

TABLE 6. (Contd)

SAMPLES for which only 10 PARALLEL PLATES were poured.

RESULTS OBTAINED from the USE of 5 and 10 PARALLEL PLATES in ASCENDING ORDER of MEANS of 10 PLATE SERIES.

Sample No.	First 5 Plates: Mean Plate Count	First 5 Plates: Standard Deviation	First 5 Plates: Coefficient of Variation (per cent)	10 Plates: Mean Plate Count	10 Plates: Standard Deviation	10 Plates: Coefficient of Variation (per cent)
118	34	12.1	28.4	36	9.5	24.5
120	26	6.3	24.2	36	32.4	89.9
123	39	6.3	16.1	37	5.8	15.8
112	44	7.0	15.8	41	5.9	15.5
129	53	7.3	13.7	48	9.0	18.8
97	55	6.5	11.8	55	5.4	9.9
134	81	14.4	17.7	75	16.2	21.6
199	76	14.8	19.5	81	12.6	15.6
135	102	12.7	12.4	95	17.8	18.7
50	93	11.0	11.8	99	14.4	14.6
77	101	35.4	35.0	107	25.7	24.0
154	103	34.1	33.1	109	25.5	23.4
124	124	12.3	9.9	112	16.5	14.7
67	167	49.0	29.4	168	33.2	19.7
58	203	13.2	6.5	175	90.7	51.8
117	200	12.9	6.5	181	30.1	16.6
69	194	52.7	26.6	225	55.6	24.7
200	273	67.6	24.8	282	67.9	24.1
76	306	22.9	7.5	287	3.5	1.2
156	459	226.8	49.4	526	195.1	35.2
153	659	206.9	31.4	729	199.2	27.3
158	1808	44.2	2.4	2398	33.1	1.4

TABLE 7.

SAMPLES for which only 5 PARALLEL PLATES were poured.

RESULTS OBTAINED from the USE of 5 PARALLEL PLATES, in ASCENDING ORDER of MEANS.

5 PLATES

Sample No.	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent.)
3	4	3.3	81.7
194	7	1.5	21.8
22	9	2.5	27.3
93	9	5.5	60.8
225	10	.8	7.8
195	16	3.7	23.4
27	16	4.0	25.3
186	18	4.4	24.5
2	18	8.5	47.3
28	20	3.2	15.8
233	20	1.6	7.8
92	26	2.9	11.2
189	29	3.7	12.6
193	30	3.0	9.9
30	32	6.0	18.7
226	37	2.6	7.0
83	42	1.4	3.4
177	44	30.2	68.7
32	45	6.7	14.8
29	50	10.1	20.2
238	52	3.0	5.7
236	54	2.5	4.6
91	54	7.5	13.8
95	59	7.0	11.8
25	59	4.0	6.7
223	68	12.1	17.9
34	69	10.2	14.8
33	71	5.6	7.9
191	75	1.6	2.2
103	75	24.6	32.8
239	83	1.7	2.0
86	85	2.8	3.3

TABLE 7 (Contd)

SAMPLES for which only 5 PARALLEL PLATES were poured.

RESULTS OBTAINED from the USE of 5 PARALLEL PLATES in ASCENDING ORDER of MEANS

5 PLATES

Sample No.	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)
89	88	4.3	4.9
240	91	2.7	3.0
224	91	3.4	3.7
172	91	31.2	34.3
175	92	4.1	4.4
31	99	13.5	13.7
36	100	14.8	14.8
246	110	5.0	4.6
99	111	7.0	6.3
176	111	6.7	6.1
90	112	9.0	8.0
102	115	16.9	14.7
85	117.	5.3	4.6
244	118	4.4	3.7
174	119	21.9	18.4
98	124	19.8	16.0
241	125	4.8	3.8
230	132	5.9	4.5
1	132	90.2	68.4
108	139	16.0	11.5
188	145	5.6	3.8
245	146	4.5	3.1
87	156	22.9	14.7
106	159	16.2	10.2
105	191	6.5	3.4
35	195	42.7	21.9
231	227	8.1	3.6
24	242	47.4	19.6
178	251	8.7	3.5
248	262	12.2	4.6
242	266	13.6	5.1
107	290	54.3	18.7
227	338	17.8	5.3
229	338	17.8	5.3
243	354	13.2	3.7

TABLE 7. (Contd)

SAMPLES for which only 5 PARALLEL PLATES were poured.

RESULTS OBTAINED from the USE of 5 PARALLEL PLATES in ASCENDING ORDER of MEANS.

5 PLATES

Sample No.	Mean Plate Count	Standard Deviation	Coefficient of Variation (per cent)
237	360	7.1	2.0
234	442	8.5	1.9
4	504	435.3	86.4
187	533	9.8	1.8
190	539	24.5	4.5
94	560	47.8	8.5
84	595	35.1	5.9
180	633	64.7	10.2
181	651	198.8	30.5
228	813	39.0	4.8
192	879	8.7	1.0
88	1015	85.0	8.4
247	1024	8.0	.8
179	1081	87.0	8.1
173	1335	888.3	66.5
232	1495	70.6	4.7
5	1574	91.6	5.8
185	1714	138.3	8.1
235	4497	100.4	2.2

TABLE 8.

FREQUENCY TABLE of the STANDARD DEVIATIONS of the MEANS of SAMPLES giving colony counts of less than 50 on the 1/100 dilution Plates.

FIVE PLATE SERIES

(first 5 plates of 10 and 20 plate series included)

Standard Deviations	Frequency	Frequency, expressed as a percentage of Number of Samples.
0 - 4.4	65	61.9
4.5 - 9.4	30	28.6
9.5 - 14.4	8	7.6
14.5 - 19.4	-	-
19.5 - 24.4	-	-
24.5 - 29.4	-	-
29.5 - 34.4	1	.9
34.5 - 39.4	-	-
39.5 - 44.4	1	.9
Over 44.4	-	-

Total Number of Samples - 105.

Mean of Standard Deviations 4.9

TABLE 9.

FREQUENCY TABLE of the STANDARD DEVIATIONS of the MEANS of SAMPLES giving colony counts of less than 50 on the 1/100 dilution Plates.

TEN PLATE SERIES

(first 10 Plates of 20 plate series included)

Standard Deviations	Frequency	Frequency, expressed as a percentage of Number of Samples.
0 - 4.4	48	55.2
4.5 - 9.4	32	36.8
9.5 - 14.4	4	4.6
14.5 - 19.4	-	-
19.5 - 24.4	-	-
24.5 - 29.4	-	-
29.5 - 34.4	3	3.4
Over 34.4	-	-

Total Number of Samples - 87

Mean of Standard Deviations - 5.5

TABLE 10.

FREQUENCY TABLE of the STANDARD DEVIATIONS of the MEANS of SAMPLES giving colony counts of less than 50 on the 1/100 dilution Plates.

TWENTY PLATE SERIES

Standard Deviations	Frequency	Frequency, expressed as a percentage of the Number of Samples
0 - 4.4	26	51
4.5 - 9.4	24	47
9.5 - 14.4	1	2
Over 14.4	-	-

Total Number of Samples - 51.

Mean of Standard Deviations - 4.8

TABLE 11.

FREQUENCY TABLE of the STANDARD DEVIATIONS of the MEANS of SAMPLES giving colony counts of less than 50 on the 1/100 dilution plates.

The Frequencies are expressed as percentages of the Number of Samples.

Standard Deviations	Part I. All Samples			Part 2. In one Group of Samples		
	5 Plates	10 Plates	20 Plates	5 Plates	10 Plates	20 Plates
0 - 4.4	61.9	55.2	51	51	56.9	51
4.5 - 9.4	28.6	36.8	47	35.3	37.3	47
9.5 - 14.4	7.6	4.6	2	13.7	5.9	2
14.5 - 19.4	-	-	-	-	-	-
19.5 - 24.4	-	-	-	-	-	-
24.5 - 29.4	-	-	-	-	-	-
29.5 - 34.4	.9	3.4	-	-	-	-
34.5 - 39.4	-	-	-	-	-	-
39.5 - 44.4	.9	-	-	-	-	-
Over 44.4	-	-	-	-	-	-

T A B L E 12.

The Mean Standard Deviation of the Mean Counts of Samples giving colony counts of less than 50 on the 1/100 dilution plates.

Series of Parallel Plate Counts	ALL SAMPLES		IN ONE GROUP OF SAMPLES	
	Number of Samples	Mean Standard Deviation	Number of Samples	Mean Standard Deviation
5 plates	105	4.9	51	5.0
10 plates	87	5.5	51	4.9
20 plates	51	4.8	51	4.8

TABLE 13.

FREQUENCY TABLE of the COEFFICIENTS of VARIATION of the SAMPLES giving colony counts of 50 and over, on the 1/100 dilution Plates.

FIVE PLATE SERIES

(first 5 Plates of 10 and 20 Plate series included)

Coefficient of Variation (per cent)	Frequency	Frequency, expressed as a percentage of the Number of Samples.
0 - 4.4	26	17.0
4.5 - 9.4	34	22.1
9.5 - 14.4	30	19.5
14.5 - 19.4	21	13.6
19.5 - 24.4	9	5.4
24.5 - 29.4	10	6.5
29.5 - 34.4	5	3.3
34.5 - 39.4	3	2.0
39.5 - 44.4	-	-
44.5 - 49.4	5	3.3
49.5 - 54.4	2	1.3
54.5 - 59.4	-	-
59.5 - 64.4	3	2.0
64.5 - 69.4	3	2.0
69.5 - 74.4	-	-
Over 74.4	3	2.0

Total Number of Samples - 154.

TABLE 14.

Frequency Table of the Coefficients of Variation of the Samples giving colony counts of 50 and over, on the 1/100 dilution Plates.

Ten Plate Series

(first 10 plates of 20 plate series included)

Coefficient of variation per cent	Frequency	Frequency, expressed as a percentage of the number of Samples
0 - 4.4	2	2.3
4.5 - 9.4	4	4.7
9.5 -14.4	16	18.6
14.5 -19.4	18	20.9
19.5 -24.4	14	16.3
24.5 -29.4	8	9.3
29.5 -34.4	6	7.0
34.5 -39.4	5	5.8
39.5 -44.4	1	1.2
44.5 -49.4	2	2.3
49.5 -54.4	1	1.2
54.5 -59.4	-	-
59.5 -64.4	2	2.3
64.5 -69.4	1	1.2
69.5 -74.4	2	2.3
Over 74.4	4	4.7

Total Number of Samples 86.

TABLE 15.

TWENTY PLATE SERIES.

Frequency Table of the Coefficients of Variation of the Samples giving colony counts of 50 and over, on the 1:100 dilution plates.

Coefficients of Variation per cent.	Frequency	Frequency, expressed as a percentage of the number of Samples.
0 - 4.4	-	-
4.5 - 9.4	6	8.7
9.5 - 14.4	10	14.5
14.5 - 19.4	11	15.9
19.5 - 24.4	7	10.1
24.5 - 29.4	10	14.5
29.5 - 34.4	9	13.0
34.5 - 39.4	4	5.8
39.5 - 44.4	2	2.9
44.5 - 49.4	-	-
49.5 - 54.4	1	1.5
54.5 - 59.4	2	2.9
59.5 - 64.4	1	1.5
64.5 - 69.4	2	2.9
69.5 - 74.4	1	1.5
Over 74.4	3	4.3

Total Number of Samples - 69.

TABLE 16.

Frequency Table of the Coefficients of Variation of the Samples giving colony counts of 50 and over, on the 1/100 dilution Plates. The Frequencies are expressed as percentages of the Number of Samples.

Coefficients of Variation per cent.	PART 1. ALL SAMPLES			PART 2. IN ONE GROUP OF SAMPLES		
	5plates	10plates	20plates	5plates	10plates	20plates
0 - 4.4	17.0	2.3	-	7.2	-	-
4.5 - 9.4	22.1	4.7	8.7	10.1	5.8	8.7
9.5 -14.4	19.5	18.6	14.5	27.5	21.7	14.5
14.5 -19.4	13.6	20.9	15.9	17.4	18.8	15.9
19.5 -24.4	5.4	16.3	10.1	7.2	13.0	10.1
24.5 -29.4	6.5	9.3	14.5	10.1	8.7	14.5
29.5 -34.4	3.3	7.0	13.0	-	8.7	13.0
34.5 -39.4	2.0	5.8	5.8	2.9	5.8	5.8
39.5 -44.4	-	1.2	2.9	-	1.4	2.9
44.5 -49.4	3.3	2.3	-	5.8	2.9	-
49.5 -54.4	1.3	1.2	1.5	2.9	-	1.5
54.5 -59.4	-	-	2.9	-	-	2.9
59.5 -64.4	2.0	2.3	1.5	4.3	2.9	1.5
64.5 -69.4	2.0	1.2	2.9	1.5	1.4	2.9
69.5 -74.4	-	2.3	1.5	-	2.9	1.5
Over 74.4	2.0	4.7	4.3	2.9	5.8	4.3

TABLE 17.

The percentage difference between the mean of the 2 lowest and that of the 2 highest counts of each set of parallel plate counts, in the case of samples giving colony counts of 50 and over on the 1/100 dilution plates.

The samples have been arranged in ascending order of means of 20 plate series.

Sample No.	Mean of 20 plate Counts	Mean of the 2 lowest Plate Counts	Mean of the 2 highest Plate Counts	Difference between the mean of the 2 lowest and that of the 2 highest plate counts	Difference between the mean of the 2 lowest and that of the 2 highest plate counts expressed as a percentage.
203x	56	48	63	15	26.7
44	65	52	83	31	47.7
218	66	36	113	77	116.6
78	67	50	89	39	58.3
216	67	52	74	22	32.8
53	68	53	87	34	50
51	69	54	111	57	82.6
79	72	52	90	38	52.8
166	79	51	115	64	81
199	81	67	107	40	49.4
145	84	52	124	72	85.7
161	85	31	194	163	191.7
168	88	66	116	50	56.8
43	99	65	109	44	48.9
74	101	75	130	55	54.5
11	106	75	144	69	65.1
203	110	102	123	21	19.1
38	111	87	194	107	96.4

TABLE 17. (Contd)

Sample No.	Mean of 20 plate Counts	Mean of the 2 lowest plate counts	Mean of the 2 highest plate counts	Difference between the mean of the 2 lowest and that of the 2 highest plate counts	Difference between the mean of the 2 lowest and that of the 2 highest plate counts expressed as a percentage.
183	112	95	142	47	41.9
49	116	83	153	70	60.3
209	116	94	135	41	35
47	120	56	183	127	105.8
149	120	102	137	35	29.2
15	126	86	178	92	73.0
151	135	108	164	56	41.5
148	139	122	178	56	40.3
165	139	110	177	67	48.2
63	161	89	568	479	297.5
220	162	14	19	5	3.1
10	166	95	246	151	91
59	166	127	204	77	46.5
62	166	115	239	124	74.7
157	175	126	266	140	80
143	176	122	197	75	42.6
16	178	114	269	155	87.1
18	191	136	290	154	80.6
65	195	120	374	254	130.2
14	198	13	543	530	267.7
23	221	101	843	742	335.7
207	230	161	286	125	54.3
7	232	96	300	204	87.9
150	238	131	345	234	98.3
56	240	144	386	242	100.1
57	246	203	357	154	62.6
169	260	60	523	463	17.8

TABLE 17. (Contd)

Sample No.	Mean of 20 plate counts	Mean of the 2 lowest plate counts	Mean of the 2 highest plate counts	Difference between the mean of the 2 lowest and that of the 2 highest plate counts	Difference between the mean of the 2 lowest and that of the 2 highest plate counts expressed as a percentage.
6	299	109	400	291	97.3
200	312	199	517	318	101.9
20	314	211	413	202	64.3
46	314	270	361	91	29
66	326	192	492	300	92
144	326	284	370	86	26.4
26	359	308	397	89	24.8
8	367	306	859	553	150.7
201	435	305	583	278	63.9
64	437	160	558	398	91.1
198	475	249	1054	805	169.4
60	553	383	1286	903	163.3
17	573	472	663	191	33.3
37	674	288	1276	988	146.3
183x	698	316	1438	1122	160.7
152	729	100	1516	1416	194.2
197x	751	350	1780	1430	190.4
21	772	337	1264	927	120.1
196x	815	506	1260	754	92.5
61	866	600	1242	642	74.1
202	1628	977	2480	1503	92.3
54	1724	415	2600	2185	126.7
205	2242	2016	2644	628	28.0
203y	3045	2340	3888	1548	50.8

TABLE 18.

FREQUENCY TABLE of the PERCENTAGE DIFFERENCE between the MEAN of the 2 LOWEST and that of the 2 HIGHEST COUNTS of each SET of 20 PARALLEL PLATE COUNTS.

SAMPLES with MEAN PLATE COUNTS of 50 and OVER.

Percentage Difference between the Mean of the two lowest Plates and that of the two highest.	Frequency	Frequency, expressed as a percentage of the Number of Samples.
0 - 9.4	1	1.5
9.5 - 19.4	2	2.9
19.5 - 29.4	6	8.7
29.5 - 39.4	3	4.3
39.5 - 49.4	9	13.0
49.5 - 59.4	7	10.1
59.5 - 69.4	5	7.2
69.5 - 79.4	3	4.3
79.5 - 89.4	7	10.1
89.5 - 99.4	8	11.6
99.5 - 109.4	3	4.3
109.5 - 119.4	1	1.5
119.5 - 129.4	2	2.9
129.5 - 139.4	1	1.5
139.5 - 149.4	1	1.5
149.5 - 159.4	1	1.5
159.5 - 169.4	3	4.3
169.5 - 179.4	-	-
179.5 - 189.4	-	-
189.5 - 199.4	3	4.3
Over 199.4	3	4.3

Total Number of Samples - 69.

TABLE 19.

Percentage Difference between the Mean of the 2 Lowest Plates and that of the 2 Highest.	Average Bacterial Content	Mean of 2 Lowest Counts.	Mean of 2 Highest Counts.
25	32,000	28,000	36,000
	100,000	87,500	112,500
50	32,000	24,000	40,000
	100,000	75,000	125,000
100	32,000	16,000	48,000
	100,000	50,000	150,000

TABLE 20.

COMPARISON of MEAN COUNTS USING 2 and 20 PLATES, in ASCENDING ORDER of MEANS of 20 PLATE SERIES.

Sample No.	First 2 Plates Mean Plate Count.	20 Plates Mean Plate Count.	Difference	Percentage Difference.
204x	1	2	1.0	50.0
205x	3	3	0	0
164	4	4	0	0
170	4	5	1	20.0
204	3	5	2	40.0
80	8	6	2	33.3
163	8	6	2	33.3
159	9	8	1	12.5
162	16	8	8	10
199x	7	8	1	12.5
19	10	10	0	0
146	13	10	3	30.0
196	21	10	11	110.0
202x	11	11	0	0
160	12	12	0	0
204y	13	12	1	8.3
142	11	14	3	21.4
81	14	16	2	12.4
200x	11	18	7	38.9
155	24	19	5	26.3
70	15	19	4	21.1
39	22	20	2	10.0
222	24	20	4	20.0
208	19	21	2	9.5
52	23	22	1	4.6
201x	25	22	3	13.6
71	17	23	6	26.1
41	25	24	1	4.2
221	23	24	1	4.2
45	19	25	6	24.0
171	29	28	1	3.6
40	30	29	1	3.5
42	30	29	1	3.5
219	34	29	5	17.2
82	30	30	0	0

TABLE 20. (Contd)

Sample No.	First 2 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
147	21	30	9	30.0
182	26	30	4	13.3
206	26	30	4	13.3
214	31	30	1	3.3
212	24	32	8	25.0
215	33	33	0	0
211	42	36	6	16.7
213	37	36	1	2.8
171x	45	39	6	15.4
210	37	39	2	5.1
55	37	40	3	7.5
72	39	41	2	4.9
48	59	42	17	40.5
197	45	44	1	2.3
217	36	46	10	21.7
75	51	48	3	6.3
203x	53	56	3	5.4
44	60	65	5	7.7
218	113	66	47	71.2
78	67	67	0	0
216	71	67	4	6.0
53	60	68	8	11.8
51	101	69	32	46.4
79	72	72	0	0
166	64	79	15	19.0
199	82	81	1	1.2
145	52	84	32	38.1
161	71	85	14	16.5
168	80	88	8	9.1
43	65	90	25	27.8
74	83	101	18	17.8
11	128	106	22	20.8
203	107	110	3	2.7
38	104	111	7	6.3
183	103	112	9	8.0
49	125	116	9	7.8
209	113	116	3	2.6
47	105	120	15	12.5
149	135	120	15	12.5
15	133	126	7	5.6
151	109	135	26	19.3
148	163	139	24	17.3

TABLE 20. (Contd)

Sample No.	First 2 Plates Mean Plate Count	20 Plates Mean Plate Count.	Difference	Percentage Difference
165	143	139	4	2.9
63	107	161	54	33.5
220	176	162	14	8.6
10	153	166	13	7.8
59	155	166	11	6.6
62	136	166	30	18.1
157	234	175	59	33.7
143	176	176	0	0
16	137	178	41	23.0
18	175	191	16	8.4
65	205	195	10	5.1
14	16	198	182	91.9
23	131	221	90	40.7
207	161	230	69	30.0
7	232	232	0	0
150	241	238	3	1.3
56	339	240	99	41.3
57	210	246	36	14.6
169	231	260	29	11.2
6	356	299	57	19.1
200	225	312	87	27.9
20	306	314	8	2.6
46	344	314	30	9.6
66	216	326	110	33.7
144	327	326	1	.3
26	377	359	18	5.0
8	402	367	35	9.5
201	391	435	44	10.1
64	527	437	90	20.6
198	278	475	197	41.5
60	449	553	104	18.8
17	561	573	12	2.1
37	537	674	137	20.3
183x	442	698	256	36.7
152	923	729	194	26.6
197x	1780	751	1029	137.0
21	337	772	435	56.4
196x	1069	815	254	31.2
61	1055	866	189	21.8
202	1956	1628	328	20.2
54	1076	1724	648	37.6
205	2127	2242	115	5.1
203y	2992	3045	53	1.7

TABLE 21.

COMPARISON of MEAN COUNTS using 5 and 20 PLATES, in ASCENDING ORDER of MEANS of 20 PLATE SERIES.

Sample No.	First 5 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
204x	3	2	1	50.0
205x	2	3	1	33.3
164	4	4	0	0
170	4	5	1	20.0
204	2	5	3	60.0
80	7	6	1	16.7
163	6	6	0	0
159	10	8	2	25.0
162	11	8	3	37.5
199x	7	8	1	12.5
19	10	10	0	0
146	12	10	2	2.0
196	13	10	3	30.0
202x	11	11	0	0
160	15	12	3	25.0
204y	14	12	2	16.7
142	15	14	1	7.1
81	14	16	2	12.5
200x	16	18	2	11.1
155	20	19	1	5.3
70	17	19	2	10.5
39	22	20	2	10.0
222	22	20	2	10.0
208	20	21	1	4.8
52	23	22	1	4.6
201x	22	22	0	0
71	21	23	2	8.7
41	23	24	1	4.2
221	23	24	1	4.2
45	25	25	0	0
171	29	28	1	3.6
40	33	29	4	13.8
42	30	29	1	3.5
219	30	29	1	3.5
82	30	30	0	0
147	27	30	3	10.0
182	28	30	2	6.7

T A B L E 21. (Contd)

Sample No.	First 5 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
206	22	30	8	26.7
214	31	30	1	3.3
212	26	32	6	18.8
215	33	33	0	0
211	41	36	5	13.9
213	37	36	1	2.8
171x	41	39	2	5.1
210	38	39	1	2.6
55	37	40	3	7.5
72	39	41	2	4.9
48	49	42	7	16.7
197	46	44	2	4.5
217	40	46	6	13.0
75	47	48	1	2.1
203x	54	56	2	3.6
44	63	65	2	3.1
218	105	66	39	59.1
78	61	67	6	9.0
216	72	67	5	7.5
53	58	68	10	14.7
51	79	69	10	14.5
79	73	72	1	1.4
166	65	79	14	17.7
199	76	81	5	6.2
145	60	84	24	28.6
161	71	85	14	16.5
168	91	88	3	3.4
43	81	90	9	10.0
74	88	101	13	12.9
11	125	106	19	17.9
203	108	110	2	1.8
38	106	111	5	4.5
183	111	112	1	.9
49	123	116	7	6.0
209	112	116	4	3.5
47	102	120	18	15.0
149	127	120	7	5.8
15	130	126	4	3.2
151	129	135	6	4.4
148	157	139	18	13.0
165	154	139	15	10.8

TABLE 21. (Contd)

Sample No.	First 5 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
63	110	161	51	31.7
220	167	162	5	3.1
10	150	166	16	9.6
59	158	166	8	4.8
62	149	166	17	10.2
157	233	175	58	33.1
143	204	176	28	15.9
16	140	178	38	21.4
18	197	191	6	3.1
65	172	195	23	11.8
14	59	198	139	70.2
23	120	221	101	45.7
207	203	230	27	11.7
7	245	232	13	5.6
150	210	238	28	11.8
56	275	240	35	14.6
57	224	246	22	8.9
169	289	260	29	11.2
6	308	299	9	3.0
200	273	312	39	12.5
20	306	314	8	2.6
46	315	314	1	.3
66	219	326	107	32.8
144	323	326	3	.9
26	363	359	4	1.1
8	343	367	24	6.5
201	408	435	27	6.2
64	534	437	97	22.2
198	271	475	204	42.9
60	463	553	90	16.3
17	524	573	49	8.6
37	551	674	123	18.3
183x	519	698	179	25.6
152	826	729	97	13.3
197x	1031	751	280	37.3
21	758	772	14	1.8
196x	1120	815	305	37.4
61	1000	866	134	15.5
202	2045	1628	417	25.6
54	1433	1724	291	16.9
205	2189	2242	53	2.4
203y	2906	3045	139	4.6

TABLE 22.

COMPARISON of MEAN COUNTS using 10 and 20 PLATES, in ASCENDING ORDER of 20 PLATE SERIES.

Sample No.	First 10 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
204x	4	2	2	100
205x	3	3	0	0
164	4	4	0	0
170	4	5	1	20.0
204	3	5	2	40.0
80	6	6	0	0
163	6	6	0	0
159	8	8	0	0
162	10	8	2	25.0
199x	6	8	2	25.0
19	10	10	0	0
146	12	10	2	20.0
196	11	10	1	10.0
202x	10	11	1	9.1
160	15	12	3	25.0
204y	14	12	2	16.7
142	14	14	0	0
81	16	16	0	0
200x	19	18	1	5.6
155	19	19	0	0
70	19	19	0	0
39	21	20	1	5.0
222	22	20	2	10.0
208	21	21	0	0
52	21	22	1	4.6
201x	22	22	0	0
71	24	23	1	4.4
41	24	24	0	0
221	24	24	0	0
45	23	25	2	8
171	30	28	2	7.1
40	29	29	0	0
42	29	29	0	0
219	28	29	1	3.5

TABLE 22. (Contd)

Sample No.	First 10 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
82	29	30	1	3.3
147	28	30	2	6.7
182	28	30	2	6.7
206	29	30	1	3.3
214	30	30	0	0
212	28	32	4	12.5
215	33	33	0	0
211	38	36	2	5.6
213	37	36	1	2.8
171x	40	39	1	2.6
210	42	39	3	7.7
55	39	40	1	2.5
72	42	41	1	2.4
48	43	42	1	2.4
197	45	44	1	2.3
217	43	46	3	6.5
75	48	48	0	0
203x	56	56	0	0
44	63	65	2	3.1
218	77	66	11	16.7
78	64	67	3	4.5
216	70	67	3	4.5
53	62	68	6	8.8
51	72	69	3	4.4
79.	73	72	1	1.4
166	81	79	2	2.5
199	81	81	0	0
145	81	84	3	3.6
161	115	85	30	35.3
168	85	88	3	3.4
43.	85	90	5	5.6
74	97	101	4	4.0
11	114	106	8	7.6
203	110	110	0	0
38.	104	111	7	6.3
183	119	112	7	6.3
49	124	116	8	6.9
209	109	116	7	6.0
47	123	120	3	2.5
149	119	120	1	.8

TABLE 22. (Contd)

Sample No.	First 10 Plates Mean Plate Count	20 Plates Mean Plate Count	Difference	Percentage Difference
15	116	126	10	7.9
151	128	135	7	5.2
148	146	139	7	5.0
165	142	139	3	2.2
63	196	161	35	21.7
220	165	162	3	1.9
10	141	166	25	15.1
59	162	166	4	2.4
62	155	166	11	6.6
157	206	175	31	17.7
143	182	176	6	3.4
16	146	178	32	18.0
18	195	191	4	2.1
65	197	195	2	1.0
14	103	198	95	48.0
23	114	221	107	48.4
207	229	230	1	.4
7	239	232	7	3.0
150	241	238	3	1.3
56	251	240	11	4.6
57	267	246	21	8.5
169	260	260	0	0
6	316	299	17	5.7
200	282	312	30	9.6
20	313	314	1	.3
46	313	314	1	.3
66	265	326	61	18.7
144	313	326	13	4.0
26	349	359	10	2.8
8	367	367	0	0
201	414	435	21	4.8
64	481	437	44	10.1
198	495	475	20	4.2
60	622	553	69	12.5
17	545	573	28	4.9
37	721	674	47	7.0
183x	515	698	183	26.2
152	758	729	29	4. 0
197x	774	751	23	3.1
21	884	772	112	14. 5
196x	916	815	101	12.4
61	979	866	113	13.1
202	1812	1628	184	11.3
54	1779	1724	55	3.2
205	2225	2242	17	.8
203y	2871	3045	174	5.7

T A B L E 23.

COMPARISON of the PERCENTAGE DIFFERENCE between the MEAN OBTAINED from 2 with that OBTAINED from 20 PLATES EXPRESSED as a FREQUENCY TABLE.

Percentage Difference between Mean of 2 PLATES & that of 20 PLATES.	Frequency.	Frequency, expressed as a percentage of Number of Samples.
0 - 4.4	28	23.3
4.5 - 9.4	21	17.5
9.5 - 14.4	15	12.5
14.5 - 19.4	12	10.0
19.5 - 24.4	12	10.0
24.5 - 29.4	6	5.0
29.5 - 34.4	9	7.5
34.5 - 39.4	4	3.3
39.5 - 44.4	5	4.2
44.5 - 49.4	1	.8
49.5 - 54.4	1	.8
54.5 - 59.4	1	.8
59.5 - 64.4	-	-
64.5 - 69.4	-	-
69.5 - 74.4	1	.8
74.5 - 79.4	-	-
79.5 - 84.4	-	-
84.5 - 89.4	-	-
89.5 - 94.4	1	.8
94.5 - 99.4	-	-
Over 99.4	3	2.5

The percentage difference is greater than 19.4 in 36.5 per cent of the samples, but if the samples with mean plate counts of less than 50 are excluded, the figure is reduced to 21.5 per cent.

TABLE 24.

COMPARISON of the PERCENTAGE DIFFERENCE between the MEAN OBTAINED from 5 with that OBTAINED from 20 PLATES expressed as a FREQUENCY TABLE

Percentage Difference between MEAN of 5 PLATES and that of 20 PLATES.	Frequency	Frequency, expressed as a percentage of Number of Samples.
0 - 4.4	35	29.3
4.5 - 9.4	24	20.0
9.5 - 14.4	22	18.3
14.5 - 19.4	16	13.3
19.5 - 24.4	3	2.5
24.5 - 29.4	6	5.0
29.5 - 34.4	5	4.3
34.5 - 39.4	3	2.5
39.5 - 44.4	1	.8
44.5 - 49.4	1	.8
49.5 - 54.4	1	.8
54.5 - 59.4	1	.8
59.5 - 64.4	1	.8
64.5 - 69.4	-	-
69.5 - 74.4	1	.8
74.5 - 79.4	-	-
Over 79.4	-	-

The percentage difference is greater than 19.4 in 19.1 per cent of the samples, but if the samples with mean plate counts of less than 50 are excluded, the figure is reduced to 11.7 per cent.

TABLE 25.

COMPARISON of the PERCENTAGE DIFFERENCE between the MEAN obtained from 10 with that obtained from 20 PLATES, expressed as a Frequency Table.

Percentage Difference between Mean of 10 Plates and that of 20 Plates.	Frequency	Frequency, expressed as a percentage of Number of Samples.
0 - 4.4	63	52.5
4.5 - 9.4	29	24.2
9.5 - 14.4	9	7.5
14.5 - 19.4	7	5.8
19.5 - 24.4	3	2.5
24.5 - 29.4	4	3.3
29.5 - 34.4	-	-
34.5 - 39.4	1	.8
39.5 - 44.4	1	.8
44.5 - 49.4	2	1.7
49.5 - 99.4	-	-
Over 99.4	1	.8

The percentage difference is greater than 19.4 per cent in 9.9 per cent of the samples, but if the samples with Mean Plate Counts of less than 50 are excluded, the figure is reduced to 4.1 per cent.

TABLE 26.

COMPARISON of the PERCENTAGE DIFFERENCES between the MEANS obtained from 2, 5 and 10 PLATES respectively with that obtained from 20 PLATES, expressed as a FREQUENCY TABLE.

Percentage Difference between Mean Count of 2, 5 or 10 PLATES and that of 20.	Per Cent Samples		
	2 Plate Series.	5 Plate Series .	10 Plate Series
0 - 4.4	23.3	29.3	52.5
4.5 - 9.4	17.5	20.0	24.2
9.5 - 14.4	12.5	18.3	7.5
14.5 - 19.4	10.0	13.3	5.8
19.5 - 24.4	10.0	2.5	2.5
24.5 - 29.4	5.0	5.0	3.3
29.5 - 34.4	7.5	4.3	-
34.5 - 39.4	3.3	2.5	.8
39.5 - 44.4	4.2	.8	.8
44.5 - 49.4	.8	.8	1.7
49.5 - 54.4	.8	.8	-
54.5 - 59.4	.8	.8	-
59.5 - 64.4	-	.8	-
64.5 - 69.4	-	-	-
69.5 - 74.4	.8	.8	-
74.5 - 79.4	-	-	-
79.5 - 84.4	-	-	-
84.5 - 89.4	-	-	-
89.5 - 94.4	.8	-	-
94.5 - 99.4	-	-	-
Over 99.4	2.5	-	.8

TABLE 27.

COMPARISON of the ACTUAL DIFFERENCE between the MEAN obtained from 2 with that obtained from 20 PLATES, expressed as a FREQUENCY TABLE. SAMPLES giving MEAN COLONY COUNTS of less than 50 on the 1/100 Dilution Plates.

Actual Difference between Mean of 2 PLATE COUNTS & that of 20	Frequency	Frequency, expressed as a percentage of the Number of Samples.
0 - 4.4	38	74.5
4.5 - 9.4	10	19.6
9.5 - 14.4	2	3.9
14.5 - 19.4	1	2.0
Over 19.4	-	-

Total Number of Samples 51.

TABLE 28.

COMPARISON of the ACTUAL DIFFERENCE between the MEAN obtained from 5 with that obtained from 20 PLATES, expressed as a FREQUENCY TABLE. SAMPLES giving MEAN COLONY COUNTS of less than 50 on the 1/100 DILUTION PLATES.

Actual Difference between Mean of 5 Plate Counts and that of 20.	Frequency	Frequency, expressed as a percentage of the Number of Samples.
0 - 4.4	46	90.2
4.5 - 9.4	5	9.8
Over 9.4	-	-

Total Number of Samples - 51.

TABLE 29.

COMPARISON of the ACTUAL DIFFERENCE between the MEAN obtained from 10 with that obtained from 20 PLATES, expressed as a FREQUENCY TABLE. SAMPLES giving MEAN COLONY COUNTS of less than 50 on the 1/100 DILUTION PLATES.

Actual Difference between Mean of 10 Plates Counts and that of 20.	Frequency	Frequency, expressed as a percentage of the Number of Samples.
0 - 4.4	51	100
Over 4.4	-	-

Total Number of Samples - 51.

T A B L E 30.

COMPARISON of the ACTUAL DIFFERENCES between the MEANS obtained from 2, 5 and 10 PLATES respectively with that obtained from 20 PLATES, expressed as a FREQUENCY TABLE.

SAMPLES giving MEAN COLONY COUNTS
of less than 50 on 1/100 DILUTION PLATES.

Actual Difference between Mean Count of 2, 5, or 10 PLATES and that of 20 PLATES.	Per Cent Samples.		
	2 Plate Series	5 Plate Series	10 Plate Series
0 - 4.4	74.5	90.2	100
4.5 - 9.4	19.6	9.8	-
9.5 - 14.4	3.9	-	-
14.5 - 19.4	2.0	-	-
Over 19.4	-	-	-

T A B L E 31.

SAMPLES with MEAN PLATE COUNTS of less than 50, and with a PERCENTAGE DIFFERENCE between MEANS of 5 and 20 PLATES of 19.5 per cent or more.

Sample No.	5 Plates Mean Count	20 Plates Mean Count	Difference between Means	Percentage Difference
204x	3	2	1	50.0
205x	2	3	1	33.3
170	4	5	1	20.0
204	2	5	3	60.0
159	10	8	2	25.0
162	11	8	3	37.5
196	13	10	3	30.0
160	15	12	3	25.0
206	22	30	8	26.7

TABLE 32.

SAMPLES with HIGH MEAN PLATE COUNTS and with a PERCENTAGE DIFFERENCE between MEANS of 5 & 20 PLATES of 19.5 per cent or more.

Sample No.	5 Plates Mean Count	20 Plates Mean Count	Difference between Means	Percentage Difference
183x	519	698	179	25.6
197x	1031	751	280	37.3
196x	1120	815	305	37.4
202	2045	1628	417	25.6

TABLE 33.

SAMPLES with irregular PLATE COUNTS and with a PERCENTAGE DIFFERENCE between MEANS of 5 and 20 PLATES of 19.5 per cent or more.

Sample No.	5 Plates Mean Count	20 Plates Mean Count	Difference between Means	Percentage Difference.
218	105	66	39	59.1
145	60	84	24	28.6
63	110	161	51	31.7
157	233	175	58	33.1
16	140	178	38	21.4
14	59	198	139	70.2
66	219	326	107	32.8
64	534	437	97	22.2
198	271	475	204	42.9
23	120	221	101	45.7

T A B L E 34.

SAMPLES with MEAN PLATE COUNTS of 50 and over with a PERCENTAGE DIFFERENCE between MEANS of 10 and 20 PLATES of 19.5 per cent or more.

Sample No.	10 Plates Mean Count	20 Plates Mean Count	Difference between Means	Percentage Difference
161	115	85	30	35.3
63	196	161	35	21.7
14	103	198	95	48.0
23	114	221	107	48.4
183x	515	698	183	26.2

S E C T I O N II.

THE INFLUENCE of the CREAMING PROPERTIES of MILK, AGITATION and HOMOGENISATION on the BACTERIAL CONTENT and DISTRIBUTION of ORGANISMS in the FLUID.

S E C T I O N II.

THE INFLUENCE of the CREAMING PROPERTIES of MILK, AGITATION and HOMOGENISATION on the BACTERIAL CONTENT and DISTRIBUTION of ORGANISMS in the FLUID.

In estimating the bacterial content of a fluid by the plating method it appears that the difficulties in obtaining uniformity of counts for duplicate tests may be increased in the case of milk, because this liquid is heterogeneous in nature and the bacterial contaminants are liable to occur, not only as groups or individuals freely dispersed in the liquid but also in association with matter in suspension, especially the clusters of fat globules. Consequently the bacterial counts are likely to vary with the extent to which the clusters of fat globules are broken up or aggregated by the manipulations.

An investigation was carried out to determine:-

(1) The influence of the creaming properties of milk on the numbers and distribution of organisms and

(2) The effect of (a) prolonged shaking of the 1/100 dilution and (b) homogenisation of the milk on the mean plate count and uniformity of parallel plate counts.

The Influence of the Creaming Properties of Milk on the Numbers and Distribution of Organisms

Samples of fresh milk a few hours old were tested for bacterial content. Then each was divided into two parts, the one being poured into an ordinary sample bottle and the other into a separating funnel. The sample bottles and separating funnels were deposited in a cool place. After 24 hours the skim milk in each separating funnel was separated from the cream. The whole milk in the sample bottles, the skim milk and the cream were then tested separately for bacterial content, 20 parallel plates being poured. The results are given in Table 35 (page 90).

The mean plate counts of the skim milk samples are very low compared with those of the cream due evidently to the fact that many bacteria have been conveyed to the surface by the rising fat globules. There is, however, no definite relationship between the mean counts of the skim milk and of the cream. The mean plate counts of the cream samples are in most instances higher than those of the corresponding whole milk samples of the same age, but there is no definite correlation.

It is evident from these results that if the cream

is not properly mixed with the rest of the milk, an accurate estimation of the bacterial content cannot be obtained. The ordinary shaking method appears to be satisfactory for the general distribution of the fat clusters throughout the rest of the milk because there is little or no variation in duplicate fat tests in the numerous instances where these have been performed. (An error may occur where a sample bottle contains so much milk that it is impossible to agitate the milk properly by shaking, or where the cream is sticking to the lower part of the stopper and the neck of the bottle). The shaking, however, may not be sufficient to break up the clusters of fat globules. If this is the state of affairs an even distribution of the fat globules throughout the milk and the water employed in preparing the dilutions cannot be obtained. Parallel plate counts of the organisms in such fluids will be irregular. This supposition affords an explanation of the irregularity of parallel plate counts. Nevertheless, it must be remembered that in a large proportion of the samples the parallel plate counts are uniform. It is likely that irregularity of parallel plate counts is due to several factors of which the two most important would seem to be:-

(1) occurrence of the organisms in clusters or groups of varying size.

(2) uneven distribution of the fat globules.

The Influence of prolonged Shaking of the 1/100 dilution on the mean plate Count and on the uniformity of parallel plate Counts.

The 1/100 dilution was prepared by the modified method discussed previously, and then hand shaken by the official method up to 1000 times. In a few instances it was shaken for an hour in an end over end shaker running at 40 revolutions per minute, but this practice was abandoned as signs of churning were seen. Twenty parallel plates were poured after 40, 500, and 1000 shakes respectively, and after one hour in the shaker.

It will be seen from Table 36, (i) & (ii) (page 91 and 92) in which the results are reported that in those samples of which the mean plate counts were less than 50, the prolonged agitation has not altered the value of the mean count appreciably. In general the differences between the mean counts of the plates poured after 40 shakes and after prolonged shaking are of no significance, being less than $\sqrt{S_1^2 + S_2^2}$ where S_1 and S_2 are the standard deviations of the respective means under consideration (24) Further, the prolonged shaking does not appear to have resulted in the distribution of the organisms becoming more uniform throughout the suspension, for in neither the 5 nor

the 20 plate series has the standard deviation been consistently reduced, in a number of instances there being found an increase. The agreement between the counts obtained from the 5 and from the 20 parallel plates appears to be quite satisfactory.

In the samples whose mean plate counts exceeded 50, prolonged shaking has frequently resulted in an increase in the mean plate count. This increase is generally greater in samples with high counts; in most of the samples having counts ranging between 50 and 150, the increase is of little or no significance. The number of samples was insufficient for any conclusions to be drawn as to the existence of any relation between irregularity of distribution of the organisms in the dilution water and an increase in the count on prolonged shaking. But in the case of samples, Number 161, 152 and 169, whose mean counts had very high coefficients of variation, prolonged agitation has resulted in a large increase in the mean plate counts of the first two samples and a slight decrease (after 1000 shakes) in the mean plate count of the last one. There is fairly close agreement between the mean counts obtained from the 5 and from the 20 parallel plates. Prolonged shaking does not appear to have distributed the organisms more uniformly throughout the suspension for, with few exceptions, the coefficients of variation have not

been significantly reduced. In a number of instances, especially where the mean counts are high, there has been an increase.

The results seem to indicate that no advantage is to be gained by increasing the period of shaking. It is possible that a different type of motion in which the suspension is subjected to a vibratory treatment might bring about a more satisfactory dispersion. There is no doubt, in view of the high values of the coefficient of variation, that some method which will give a more uniformly distributed suspension of the organisms is urgently required.

The Influence of Homogenisation of the Milk on the Bacterial Content and Uniformity of Parallel Plate Counts

An experiment was performed to determine whether more uniform plate counts may be obtained when the fat clusters are broken up and the globules composing them are so finely sub-divided that they no longer tend to rise to the surface. Samples were taken from specimens of fresh milk prior to and after homogenisation and estimations were made of their bacterial content. In order to make allowance for the effect of bacterial multiplication in the milk during

homogenisation, corresponding samples of the untreated and homogenised milk were tested simultaneously. The milk was homogenised at a pressure of from 2000 to 3500 pounds per square inch by means of a dairy homogenising plant with a capacity of 10 gallons per hour. Prior to use the machine was scalded by exposure to boiling water for 10 minutes and then allowed to cool. The temperature of the unhomogenised milk was approximately 10°C. and that of the milk passing through the machine was not allowed to exceed 40°C. The time taken for the treatment did not exceed 10 minutes. The results of this experiment are recorded in Table 37 (page 93), the samples being arranged in order of the mean counts of the homogenised milk samples. Twenty parallel plates were employed throughout the experiment. In 5 instances the bacterial content was not estimated prior to homogenisation.

The results are not illuminating probably due to the fact that the samples of milk in most instances were only a few hours old, so that the cream had not 'gathered' nor the bacteria multiplied to any extent. In all samples the mean plate count of the milk prior to homogenisation is much lower than that of the milk after treatment, but there is no definite relationship between them. Further there is no significant difference between the coefficients of variation of the corresponding mean counts; thus the

average coefficient of variation of the mean counts of all the samples prior to homogenisation is 13.5 while that of the mean counts of the corresponding homogenised samples is 12.8. The coefficients of variation of the homogenised milk samples, however, would probably have been lower but for the fact that in many samples the plate counts were over 300 or 400 and consequently there was a greater error in making the actual counts. On the other hand, the coefficients of variation of some of the unhomogenised milk samples are high owing to the effect of the low values of the means. It is probable that in most samples the organisms occurred singly, in pairs or in small clusters, fairly uniformly distributed throughout the milk and only to a limited extent in contact with the clusters of fat globules. If this supposition is correct, and it is supported to some extent by the fact that in none of the unhomogenised milk samples is the coefficient of variation high, the process of homogenisation would have little influence on the uniformity of parallel plate counts. The higher bacterial counts of the homogenised milk samples appear to be due largely to the breaking up of the bacterial clusters by the treatment into smaller cell-groupings and single cells. There would be little contamination of the milk during homogenisation as not only was the machine carefully scalded prior to use but the first portion of the treated milk was discarded.

SUMMARY

(1) The cream has a much higher bacterial content than the underlying milk and therefore if not properly mixed with the rest of the milk an accurate estimate of the bacteri content will not be obtained. While the official shaking method appears to be satisfactory for the distribution of the clusters of fat globules throughout the milk, it may not be sufficient to break up these clusters and disperse the organisms in contact with them.

(2) Prolonged shaking of the 1/100 dilution by hand does not appear to distribute the organisms more uniformly throughout the fluid but it frequently increases appreciably the values of the mean counts of samples which contain large numbers of bacteria. There seems, however, to be little advantage to be gained by prolonging the period of shaking by hand.

(3) Homogenisation increases considerably the values of the mean counts of samples of milk. This is apparently due to the breaking up of clusters of bacteria. It does not, however, bring about a more uniform distribution of the bacteria in comparatively fresh samples probably owing to the fact that in such samples the bacteria most frequently occur singly or in small groups and not in association with the clusters of fat globules.

TABLE 35.

THE INFLUENCE of the CREAMING PROPERTIES of MILK on the TOTAL BACTERIAL CONTENT and UNIFORMITY of PARALLEL PLATE COUNTS.
THE TABLE is based on the RESULTS obtained from the USE of 20 PARALLEL PLATES.

	WHOLE FRESH MILK			WHOLE MILK 24 HOURS old			SKIM MILK 24 HOURS old			CREAM 24 Hours old.		
Sample	Mean Plate Count	Standard Deviation	Coefficient of Variation	Mean Plate Count	Standard Deviation	Coefficient of Variation	Mean Plate Count	Standard Deviation	Coefficient of Variation	Mean Plate Count	Standard Deviation	Coefficie of Variati
204	2	2.3	116.5	5	4.2	83.1	3	1.4	46.7	67	10.2	15.2
205	3	1.2	38.7	2242	207.3	9.3	33	6.7	20.3	3948	886.5	22.5
170	5	2.7	54.0	-	-	-	2	.94	47.2	58	10.0	17.2
199	8	5.2	64.4	81	15.1	18.7	1	1.1	110.0	46	5.8	12.7
196	10	5.2	52.3	815	238.6	29.3	44	7.1	16.1	9545	1505.3	15.8
202	11	3.4	30.5	1628	513.8	31.6	2	1.5	75.0	516	72.5	14.1
200	18	10.2	56.8	312	100.2	32.1	1	1.5	150.0	459	56.5	12.4
201	22	3.3	14.9	435	73.8	17.0	1	1.3	130.0	Uncountable	-	-
171	39	7.2	18.5	28	4.4	15.6	9	16.8	186.4	180	33.4	18.5
182	30	7.8	26.1	2003	50.3	2.5	1	1.1	110.0	2769	1349.0	48.7
197	44	4.8	11.0	751	494.4	65.8	93	30.4	32.7	3861	793.3	20.5
203	56	5.1	9.1	110	6.8	61.7	5	1.9	38.0	1634	193.9	11.9
183	112	12.3	11.0	698	381.3	54.6	157	17.8	11.3	7070	1627.5	23.0
198	475	236.6	49.8	Uncountable	-	-	186	34.9	18.8	Uncountable	-	-

TABLE 36 (1)

THE INFLUENCE of PROLONGED SHAKING of the 1/100 DILUTION on the MEAN PLATE COUNT and on the DISTRIBUTION of ORGANISMS in the SUSPENSION.

FIVE PLATE COUNTS in ASCENDING ORDER of MEANS of 20 PLATE SERIES.

Sample Number	40 Shakes Mean	40 Shakes Standard Deviation	40 Shakes Coefficient of Variation	500 Shakes Mean	500 Shakes Standard Deviation	500 Shakes Coefficient of Variation	1000 Shakes Mean	1000 Shakes Standard Deviation	1000 Shakes Coefficient of Variation	Shaker 1 Hour Mean	Shaker 1 Hour Standard Deviation	Shaker 1 Hour Coefficient of Variation
164	4	1.1	27.7	8	2.4	30.6	8	2.3	28.9	-	-	-
163	6	2.0	33.7	5	1.2	23.1	7	3.9	55.3	-	-	-
162	8	4.8	60.1	10	4.4	44.0	8	2.7	33.9	-	-	-
159	8	2.8	35.6	10	3.9	40.0	11	3.1	27.8	-	-	-
146	12	2.7	22.6	23	3.5	15.4	12	2.8	23.6	10	2.7	27.1
160	12	4.8	40.2	19	3.4	17.7	12	2.6	22.0	-	-	-
142	15	5.5	36.5	7	2.9	42.0	14	1.9	13.7	16	4.1	26.0
155	19	5.8	30.4	25	3.5	14.1	24	3.6	14.9	-	-	-
167	26	22.7	87.2	17	4.8	28.2	20	4.2	21.2	-	-	-
147	27	9.0	33.3	30	5.8	19.4	23	7.2	31.4	28	17.2	61.5
166	79	21.3	26.9	91	44.5	48.9	113	48.8	43.2	-	-	-
145	60	8.7	14.6	186	40.7	21.9	139	14.7	10.6	105	18.8	18.0
161	85	59.8	70.3	433	102.0	23.5	440	97.8	22.2	--	-	-
168	88	15.2	17.3	129	24.3	18.9	128	18.2	14.2	-	-	-
154	107	34.1	33.1	169	51.9	30.7	133	52.8	39.7	-	-	-
149	127	10.8	8.5	131	23.0	17.6	133	8.5	64.1	-	-	-
151	129	23.3	18.1	146	10.8	7.4	186	49.9	26.7	-	-	-
165	139	20.1	14.5	143	21.1	14.8	155	28.8	18.6	-	-	-
148	157	25.0	15.9	147	16.5	11.2	171	10.0	58.3	172	18.1	10.1
157	175	44.0	25.1	264	95.0	36.0	309	141.4	45.8	-	-	-
143	204	54.7	26.8	331	80.0	23.6	276	60.0	21.8	285	44.1	15.5
150	210	52.6	25.0	268	38.8	14.5	267	15.4	57.6	-	-	-
169	260	152.7	58.7	248	36.0	14.5	131	23.7	18.1	-	-	-
144	323	40.6	12.6	531	72.0	13.6	544	63.3	11.6	495	50.1	10.1
156	633	235.9	36.9	1014	331.1	32.7	708	276.0	39.0	-	-	-
152	826	745.4	90.2	889	777.3	8.7	1449	801.7	55.3	-	-	-
153	659	206.9	31.4	1344	339.3	2.5	1267	530.1	41.8	-	-	-
158	2483	969.2	39.0	3088	1116.7	36.2	4091	708.9	17.3	-	-	-
10	150*	69.9	46.6	166	31.1	18.7	280	44.4	15.9	-	-	-
7	245*	29.7	12.1	248	28.5	11.5	-	-	-	-	-	-

* 25 Shakes.

TABLE 36 (11)

THE INFLUENCE of PROLONGED SHAKING of the 1/100 DILUTION on the MEAN PLATE COUNT and on the DISTRIBUTION of ORGANISMS in the SUSPENSION.

TWENTY PLATE COUNTS in ASCENDING ORDER of MEANS.

Sample Number	40 Shakes Mean	40 Shakes Standard Deviation	40 Shakes Coefficient of Variation	500 Shakes Mean	500 Shakes Standard Deviation	500 Shakes Coefficient of Variation	1000 Shakes Mean	1000 Shakes Standard Deviation	1000 Shakes Coefficient of Variation	Shaker 1 Hour Mean	Shaker 1 Hour Standard Deviation	Shaker 1 Hour Coefficient of Variation.
164	4	1.1	27.7	7	1.7	25.0	8	2.4	30.4	-	-	-
163	6	2.0	33.7	4	1.6	40.4	8	2.6	32.6	-	-	-
162	8	4.8	60.1	11	5.5	50.3	10	4.0	40.5	-	-	-
159	8	2.8	35.6	7	3.2	46.2	13	3.7	28.3	-	-	-
146	10	3.1	31.0	16	17.0	106.1	11	3.4	31.1	-	-	-
160	12	4.8	40.2	14	4.9	34.7	17	10.7	63.0	-	-	-
142	14	4.1	29.1	12	4.2	35.3	18	6.8	37.8	18	6.5	36.0
155	19	5.8	30.4	24	2.8	11.7	23	3.5	15.3	-	-	-
167	26	22.7	87.2	18	5.1	28.2	21	5.5	26.4	-	-	-
147	30	5.8	19.3	25	8.6	34.4	20	6.6	33.2	33	8.8	26.6
166	79	21.3	26.9	82	19.6	23.9	120	22.1	18.5	-	-	-
145	84	23.2	27.6	202	31.9	15.8	135	20.9	15.5	102	19.8	19.4
161	85	59.8	70.3	233	192.6	82.6	386	84.0	21.8	-	-	-
168	88	15.2	17.3	130	17.2	13.3	134	22.6	16.9	-	-	-
154	113	32.4	28.6	150	60.5	40.3	143	58.4	40.8	-	-	-
149	120	12.3	10.3	136	22.2	16.3	152	15.4	10.2	-	-	-
151	135	18.5	13.7	159	27.0	17.0	178	44.6	25.1	-	-	-
165	139	20.1	14.5	140	26.1	18.6	152	31.6	20.8	-	-	-
148	139	18.9	13.6	159	16.9	10.6	164	13.2	8.0	166	16.3	98.4
157	175	45.9	25.1	283	59.1	20.9	350	104.7	29.9	-	-	-
145	176	58.0	33.0	314	54.3	17.3	313	55.3	17.7	295	53.1	18.0
150	238	68.1	28.6	278	45.9	16.5	303	70.7	23.3	-	-	-
169	260	152.7	58.7	332	214.0	64.5	203	941.6	463.8	-	-	-
144	326	29.0	8.9	524	58.9	11.2	555	56.9	10.3	538	85.9	16.0
156	638	235.9	37.0	957	271.9	28.7	722	242.1	33.5	-	-	-
152	729	480.5	65.9	992	555.8	56.0	1609	602.9	37.5	-	-	-
153	743	200.3	27.0	1227	252.3	20.6	1493	335.0	22.4	-	-	-
158	2843	969.2	39.0	2938	565.4	19.3	3536	658.8	18.6	-	-	-
10	166[M]	50.7	30.6	203	54.3	26.7	232	57.9	25.0	-	-	-
7	232[M]	56.0	25.5	259	54.2	20.9	-	-	-	-	-	-

M 25 Shakes.

TABLE 37.

The Influence of Homogenisation of Milk on the Bacterial Content and Uniformity of Parallel Plate Counts. The Table is based on the Results obtained from the use of 20 Parallel Plates, the Results being arranged in Ascending Order of Means of the Homogenised Samples.

	BEFORE HOMOGENISATION			AFTER HOMOGENISATION		
Sample	Mean Plate Count	Standard Deviation	Coefficient of Variation	Mean Plate Count	Standard Deviation	Coefficient of Variation
			per cent			per cent
249	42	5.1	12.0	62	8.7	14.0
253	53	5.7	10.7	112	9.9	8.9
266	-	-	-	125	8.8	7.0
212	32	6.8	21.3	158	14.5	9.0
260	-	-	-	199	32.3	16.2
265	-	-	-	221	13.1	5.9
267	-	-	-	351	23.2	6.6
250	76	16.8	22.1	385	66.4	17.2
264	-	-	-	417	39.9	9.6
254	126	14.0	11.0	457	64.9	14.2
219	29	2.2	7.7	668	59.7	8.9
261	69	5.7	8.3	685	58.0	8.5
252	126	7.7	6.1	701	40.6	5.8
220	162	14.4	8.9	772	100.2	13.0
255	276	31.4	11.3	967	104.4	10.8
251	305	34.2	11.2	1073	94.8	8.8
262	-	-	-	1132	157.4	13.9
258	999	188.6	18.9	1926	508.7	26.4
257	553	75.3	13.6	2954	476.1	16.1
259	1266	238.9	18.9	4752	818.1	17.2
256	1011	205.9	20.4	4802	631.7	13.2

S E C T I O N III.

THE OCCURRENCE of COLIFORM BACTERIA in MILK.

S E C T I O N III.

THE OCCURRENCE of COLIFORM BACTERIA in MILK.

Of all the common non-pathogenic bacterial contaminants of milk the coliform organisms are generally recognised to be the most important. Many of them occur commonly in intestinal matter and are characteristic of animal excrements, while others occur less frequently in faeces but are fairly common in the soil, and even on cereal grains and other foods (27 -60) Their presence in milk is considered generally to be due to its contamination, directly or indirectly, with animal excrements. Therefore the Coliform Test is widely used in determining the purity of milk.

The test as carried out by the Bacteriology Department of The West of Scotland Agricultural College by the Durham's fermentation tube method, involves the use of three test tubes of bile-salt lactose bouillon with Andrade's indicator. One tenth c.c. of the milk is added to the first test tube; 1 c.c. of the 1/100 dilution (equivalent to 1/100 c.c. of the original sample) is added to the second test tube and 1/10 c.c. of the 1/100 dilution (equivalent to 1/1000 c.c. of the original sample) is added to the third test tube.

The inoculated medium is incubated at 37°C. for 48 hours and then examined, the presence of both acid and gas being regarded as a "positive" reaction.

An investigation was carried out to determine:-

(1) the prevalence of coliform bacteria in milk;

(2) the influence of season upon their prevalence;

(3) the correlation, if any, between the presence of coliform bacteria in milk and the total bacterial content, and

(4) the types of coliform bacteria commonly present in milk.

The Prevalence of Coliform Bacteria in Milk.

Table 38 (pages 152 & 153) gives the prevalence of coliform bacteria in 21,569 samples of market milk. The samples were generally bulk samples, taken without warning from the milk of individual farmers on arrival at a creamery or dairy. They were therefore typical of the general supplies. The tests were carried out at intervals of a month and in most instances extended over a period of several years. In recording the results, the farmers have been placed in groups according to the district, creamery or dairy to which they belong, and the results of each year's tests are given separately.

The following is a summary of the results given in Table 38:-

Percentage of Samples	Results of Coliform Test		
	1/10 c.c.milk	1/100 c.c.milk	1/1000 c.c.mil
48.3	-	-	-
21.4	+	-	-
14.0	+	+	-
16.3	+	+	+

It is probable that these results are much better than those generally obtained from samples of milk for the following reasons:-

(1) Arrangements were made in the despatch of the samples so that they could be tested on the same day. Consequently the samples of morning milk were tested at an age of from 8 to 10 hours and those of evening milk at an age of from 20 to 22 hours.

(2) Many of the samples were from creameries and dairies whose supplies have been submitted to such tests for a number of years. In these instances there is frequently after a time a distinct improvement in the purity of the milk due to the greater care taken in its production and treatment. This improvement is evident when a comparison is made of the results of successive years tests of samples from the same group of farms. (See Table 38).

The Influence of Season on the Prevalence of Coliform Bacteria in Milk

A comparison of the results of the tests of samples from the same group of farms reveals the fact that the proportion of samples which contain coliform bacteria in 1/10 c.c. (hereafter referred to as coliform positive samples), varies widely during the year. The proportion of these samples is generally much higher during the summer and early autumn than during the rest of the year. (See Tables 39 to 44 (pages 154 to 159), and Figures 4 to 9). Each table and figure gives the results of the monthly tests of samples from the one group of farms. These monthly tests in the case of each group of farms were generally performed on the same day, and the mean of the minimum and maximum atmospheric temperatures for that day is given in the table and corresponding figure. It is therefore possible to determine whether or not this seasonal variation in the proportion of coliform positive samples is due largely to atmospheric temperature. It is evident that there is a distinct correlation between the proportion of coliform positive samples and the mean of the minimum and maximum atmospheric temperatures, the proportion of coliform positive samples being generally larger the higher the atmospheric temperature. The fact that the correla-

lation is not perfect indicates that there may be other seasonal factors.

The effect of atmospheric temperature on the proportion of coliform positive samples is probably due largely to its influence on the rate of multiplication of the bacterial contaminants both in milk which has not been cooled and on utensils which have not been properly sterilised.

The Relation of Total Bacterial Content to the Presence of Coliform Bacteria

To determine whether there is any relationship between the total bacterial content of milk and the presence of coliform bacteria, the results of the tests of 21,857 samples were examined. Estimations were made of the average bacterial content of the coliform positive samples, i.e., the samples containing coliform bacteria in 1/10 c.c., and of the coliform negative samples, i.e., the samples containing no coliform organisms in 1/10 c.c. The results are given in Table 45 (pages 160-161), the samples being arranged in groups as in Table 38. A few additional samples (288) have been included. These samples were omitted from Table 38 owing to the fact that no coliform test had been made for 1/1000 c.c. amounts. It will be seen that in all the series of tests the average bacterial

content of the coliform negative samples is less than that of the coliform positive. The ratio of the former to the latter varies from 1 : 1. 6 to 1 : 11.6. The average bacterial content of all the coliform negative samples (10,458 in number) is 25,294 and of all the coliform positive samples (11,399 in number) is 160,577. The ratio of the former to the latter is 1 : 6.3.

The higher total bacterial content of the coliform positive samples may be due to several factors of which the following appear to be the most important:-

(1) Higher initial contamination of such milk, not only with coliform bacteria but also with others, as a result of faulty methods and especially in warm weather, contaminated utensils.

(2) The multiplication of coliform and other bacteria in uncooled milk. Samples of uncooled milk not only give in many instances coliform positive tests but they generally contain large numbers of bacteria.

If the results of the monthly tests of samples from the same group of farms are examined, it will be seen that there is a well marked correlation not only between the average bacterial content and the proportion of coliform positive samples, but also between the average bacterial

content and the mean of the minimum and maximum atmospheric temperatures. (See Tables 39 to 44 (pages 154 to 159) and Figures 4 to 9). The reasons for this correlation become evident from a consideration of the factors just mentioned.

The Types of Coliform Bacteria which occur in Cows' Milk.

Three hundred and fifty-nine cultures of coliform bacteria were isolated from samples of market milk during a period extending from the middle of November to the middle of April (hereafter referred to as the "Winter period") when the cows were confined to the byres and not at pasture. Three or four cultures as a rule were isolated from each sample of milk. Then an additional 438 cultures were isolated during a period extending from the middle of June to the beginning of November (hereafter referred to as the "Summer period"), when the cows were at pasture for the whole or part of the day. In this case only one culture was isolated from each sample of milk, as it had previously been found that in most instances cultures, which had been isolated from the same sample, were identical in type. The total number of cultures isolated was 797.

Method of Isolation.

The cultures were isolated from Durham's fermentation

tubes containing MacConkey's bile-salt lactose peptone water, which had been employed in the routine examination of milk for purity and showed acid and gas production. Three loopfuls of the culture were transferred from the fermentation tube to a tube of sterile 0.85 per cent. salt solution or peptone water. Two tubes ("A" and "B") of melted MacConkey's neutral-red bile-salt lactose agar at 43°C. were then inoculated, three loopfuls of the saline or peptone water suspension being transferred to tube "A" and three or four loopfuls of the inoculated agar in tube "A" to tube "B". The agar was poured into plates and these were incubated at 37°C. for twenty-four hours or until the colonies had developed sufficiently to be picked off. Some types of coliform bacteria when living under aerobic conditions, do not produce sufficient acid in this medium for their colonies to have a rose-red colour. Therefore pure cultures were prepared not only from rose-red surface and deep colonies but also in many instances from pale surface colonies.

In order to confirm the fact that the colonies selected were those of coliform bacteria, cultures were prepared from them in Durham's fermentation tubes containing MacConkey's bile-salt lactose peptone water and Andrade's indicator. The cultures were incubated at 37°C. and all those which showed acid and gas production were stroked on agar slopes. Cultures

which did not produce acid and gas in the Durham's tubes within fourteen days were discarded. The agar slope cultures were incubated at 37°C. for twenty-four hours and then placed until required in a cool dark cupboard. They were transferred to fresh agar slopes at intervals of four weeks.

Method of Identification.

For the identification of these cultures the following tests were employed, an agar slope culture twenty-four hours old being used as the inoculum in each instance.

A. Fermentation of Glucose , Saccharose, Dulcitol, Adonitol, Inulin, Mannitol, Raffinose and Salicin.

The fermentation was carried out by means of Durham's fermentation tubes, the medium consisting of peptone water with Andrade's indicator and 0.5 per cent of the sugar or glucoside. The tubes after inoculation were incubated at 37°C. They were examined daily. If there was no evidence of acid and gas production within fourteen days the test was recorded as negative. If acid was produced but only a trace of gas, as sometimes occurred in the case of saccharose and inulin fermentation, the test was repeated. If the results of repeat tests were still indefinite the culture was replated in MacConkey's bile-salt lactose agar by the method already described. The plates were incubated and a representative colony was picked off and transferred to

MacConkey's bile-salt lactose peptone water. If the organisms produced acid and gas in this medium, an agar slope sub-culture was prepared and then the identification tests were repeated.

All the cultures isolated during the first period fermented mannitol with production of acid and gas and therefore none of the cultures isolated during the second period were tested in this sugar.

B. Action on Milk.

Sub-cultures in litmus milk were prepared from the agar slope cultures and incubated at 37°C. for seven days to determine whether the organisms produced a permanent acid reaction and curdling.

C. The Methyl-red and Voges-Proskauer Reactions.

Sub-cultures were prepared in a peptone water medium containing 0.5 per cent peptone, 0.5 per cent glucose and 0.5 per cent dipotassium hydrogen phosphate. After three days incubation at 37°C. each culture was divided into two portions. The reaction of the first portion was tested by adding two drops of a solution of methyl-red. (A solution of 0.1 gram. methyl-red in 300 c.c. alcohol, made up to 500 c.c. with distilled water). The other portion was tested for acetyl-methyl-carbinol by shaking it up with a strong solution of caustic potash and keeping it at room temperature

for a few hours, the Voges-Proskauer test. In some instances where the Methyl-red and Voges-Proskauer reactions did not correlate, repeat Voges-Proskauer tests were made using sub-cultures 24 and 48 hours old.

D. Production of Indole.

Cultures in peptone water medium were incubated at 37°C. for ten days and then tested for indole by means of Ehrlich's rosindole reagent and a saturated solution of potassium persulphate. For this test Witte's and Fair-child's peptone powders were employed.

E. Liquefaction of Gelatin.

Two tubes of gelatin medium were heavily inoculated from each agar slope culture and the mouths of the tubes covered with rubber caps. One tube was kept at 37°C. and the other at room temperature. The former was examined at intervals of a few days, the tube being placed in cold water for a few hours to permit the gelatin if undigested by the bacteria, to solidify. If the gelatin failed to solidify, the tube was kept for a further twenty-four hours at room temperature before the reaction was recorded. In some in-stances there was a delay in the solidification of the gelatin. This was generally observed in cultures which digested the gelatin after a further period of incubation. Cultures which did not digest the gelatin after twelve weeks

incubation at 37°C. were recorded as non-liquefiers. The culture kept at room temperature was examined weekly. If there was no evidence of liquefaction after eight weeks, it was incubated at 37°C. and examined at intervals in the same manner as the other tube. If there was no evidence of liquefaction after four weeks' incubation, it was recorded as a non-liquefier.

F. Ability to grow in Koser's Citrate Medium.

The medium employed consisted of 1.5 grams. sodium ammonium hydrogen phosphate (microcosmic salt), 1 gram. potassium dihydrogen phosphate, 0.2 gram. magnesium sulphate and 2 grams. sodium citrate in 1000 c.c. distilled water. It was tubed in 10 c.c. amounts in test tubes which had been specially cleaned by boiling in a solution of sodium hypochlorite and rinsing in distilled water. Sterilisation was carried out in the autoclave.

The tubes were inoculated from agar slope cultures by means of a platinum needle, a very small quantity of inoculum being used. They were then incubated at 37°C. and examined daily. Cultures which rendered the medium turbid within ten days were termed positive, those which failed to do this were termed negative. Where the reaction was negative or where only a faint turbidity was produced, the test was repeated in duplicate. It was considered necessary in view

of the small quantity of inoculum employed to confirm in this way the fact that the inoculation of the medium had been properly carried out in the first instance.

G. Motility.

The organisms were grown in peptone water medium. The method at first adopted was to incubate the cultures at 37°C. and examine them for motility at the end of 6, 8 and 24 hours. Organisms which appeared to be non-motile were re-examined, fresh cultures in peptone water being prepared for this purpose. In the case of non-motile bacteria at least two, and in many instances, four or five re-examinations were made. The other method employed was to keep the peptone water cultures at room temperature and test them for motility at the end of twenty-four and forty-eight hours. This gave much better results than the first method as the organisms were generally more actively motile at room temperature. Further, there was a great reduction in the proportion of cultures which were apparently non-motile in the first tests but proved in the later tests to be motile.

TYPES of COLIFORM BACTERIA found in MILK

The general characters of the cultures isolated were as follows:- small Gram-negative, non-sporing bacilli; capable of growing at 37°C.; fermenting glucose and lactose with formation of acid and gas; producing coagulation and permanent acidity of milk within seven days at 37°C.; as a rule not liquefying gelatin but occasionally as in the case of B.
as far as possible according to MacConkey's method (29)
clo
and arranged in order of frequency. See Table 46 (pages 162-163)
as
and also the following:-
and
B. "MacConkey's No.71" (B.communior)
and also the following:-

B. "MacConkey's No.71" (B.communior)

Distinguishing characters:- acid and gas produced in mannitol, saccharose and dulcitol but not in adonitol and inulin; indole reaction positive; Voges-Proskauer reaction negative; gelatin not liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser reaction negative; methyl-red reaction positive.

Number of cultures isolated during the winter period (when cows were not at pasture), 127; during the summer period (when the cows were at pasture), 66. Total number isolated 193.

One hundred and eighty-one cultures gave positive

reactions (i.e. produced acid and gas) in salicin; 12 gave negative reactions.

B."MacConkey's No.34" (B. coli communis)

Distinguishing characters:- acid and gas produced in mannitol and dulcitol, but not in saccharose, adonitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter period, 55; during the summer period 40. Total number isolated 95. Four cultures were atypical; of these 3 produced acid and gas in raffinose and 1 gave a positive Koser reaction.

Sixty-nine cultures gave positive reactions in salicin and 26 gave negative reactions.

B."MacConkey's No.108" (B.cloacae)

Distinguishing characters:- acid and gas produced in mannitol and saccharose, but not in adonitol, dulcitol and inulin; indole negative; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 32, during the summer, 39. Total number isolated 71. Eighteen

cultures were atypical. Sixteen did not liquefy gelatin; of these 3 gave negative Koser reactions and 2 gave neutral methyl-red reactions. Two cultures which liquefied gelatin, were atypical in that they gave negative Koser reactions.

Sixty-four cultures gave positive reactions in salicin, 7 gave negative reactions.

B. "MacConkey's No.103" (B.lactis aerogenes)

Distinguishing characters:- acid and gas produced in mannitol, saccharose and adonitol, but not in dulcitol and inulin; indole negative; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 10; during the summer, 51. Total number isolated 61. Nine cultures were atypical, 5 gave neutral methyl-red reactions and 4 gave positive.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.1"

Distinguishing characters:- acid and gas produced in mannitol and adonitol, but not in saccharose, dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 28; during the summer, 17. Total number of cultures isolated 45. Three cultures were atypical; of these 2 produced acid and gas in raffinose and 1 gave a positive Koser reaction.

Forty-two cultures gave positive reactions in salicin; 3 gave negative reactions.

B. "MacConkey's No.7"

Distinguishing characters:- acid and gas produced in mannitol, but not in saccharose, dulcitol, adonitol and inulin; indole negative; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser positive; Methyl-red positive.

Number of cultures isolated during the winter, 17; during the summer 26. Total number isolated 43. One culture was atypical in that it gave a Koser negative reaction.

Thirty cultures gave positive reactions in salicin and 13 gave negative reactions.

B. "MacConkey's No.4"

Distinguishing characters:- acid and gas produced in mannitol, but not in saccharose, dulcitol, adonitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas not produced

in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 10; during the summer, 16. Total number isolated, 26. One culture was atypical in that it fermented raffinose with production of acid and gas.

Thirteen cultures gave positive reactions in salicin and 13 gave negative reactions.

B. "MacConkey's No.106"

Distinguishing characters:- acid and gas produced in mannitol and saccharose; but not in adonitol, dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 14, during the summer 8. Total number isolated 22.

Sixteen cultures gave positive reactions in salicin, 6 gave negative reactions.

B. "MacConkey's No.98"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, adonitol and inulin, but not in dulcitol; indole negative; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 2; during the summer, 19. Total number isolated 21. Two cultures were atypical in that they gave negative Koser reactions. All the cultures gave positive reactions in salicin.

B. "MacConkey's No.73"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and dulcitol but not in adonitol and inulin; indole negative; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 4; during the summer, 15. Total number isolated 19. Thirteen cultures were atypical. Ten did not liquefy gelatin. Of these 1 was Koser negative and 2 were methyl-red neutral. Three cultures which liquefied gelatin were atypical in that 2 were methyl-red neutral and 1 methyl-red positive.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.65"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, dulcitol, adonitol and inulin; indole positive; Voges-Proskauer positive; gelatin liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 3; during the summer, 14. Total number isolated 17. One of these cultures did not liquefy gelatin. B. "MacConkey's No.65" is peculiar in that there is a direct correlation between the indole and Koser reactions.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.5"

Distinguishing characters:- acid and gas produced in mannitol, but not in saccharose, dulcitol, adonitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 5; during the summer 10. Total number isolated 15.

Three cultures gave positive reactions in salicin and 12 gave negative reactions.

B. "MacConkey's No.67"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, dulcitol and adonitol but not in inulin; indole negative; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter 1; during the summer, 14. Total number isolated, 15.

Four cultures were atypical; three of these had neutral methyl-red reactions and one had a negative Koser reaction.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.74"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and dulcitol, but not in adonitol and inulin; indole negative; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas produced in

raffinose; Koser positive; methyl-red positive.

Number of cultures isolated during the winter, 4; during the summer, 9. Total number isolated 13.

Five cultures were atypical, 4 of these gave negative Koser reactions and 1 gave a negative methyl-red reaction.

Eleven of the cultures gave positive reactions in salicin and 2 gave negative reactions.

B. "MacConkey's No.36"

Distinguishing characters:- acid and gas produced in mannitol and dulcitol, but not in saccharose, adonitol and inulin; indole negative; Voges-Proskauer negative; gelatin liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser positive; methyl-red positive.

Number of cultures isolated during the winter, 4; during the summer, 8. Total number isolated 12. Nine of these cultures did not liquefy gelatin. Eleven cultures gave positive reactions in salicin and 1 a negative reaction.

B. "MacConkey's No.101"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and adonitol, but not in dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin

not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red positive.

Number of cultures isolated during the winter, 5; during the summer 7. Total number isolated 12. In 10 cultures there was a direct correlation between the indole and Koser reactions. Two cultures gave negative Koser reactions. All the cultures gave positive reactions in salicin.

B. "MacConkey's No.35" (B. Schafferi)

Distinguishing characters:- acid and gas produced in mannitol and dulcitol, but not in saccharose, adonitol and imulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 6; during the summer, 5. Total number isolated 11.

Seven cultures gave positive reactions in salicin and 4 gave negative reactions.

B. "MacConkey's No.109"

Distinguishing characters:- acid and gas produced

in mannitol and saccharose, but not in adonitol, dulcitol and inulin; indole negative; Voges-Proskauer negative; gelatin not liquefied, motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red positive.

Number of cultures isolated during the winter, 4; during the summer, 7. Total number isolated 11. Two cultures were atypical, giving negative Koser reactions. Of these one gave a neutral methyl-red reaction.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.102"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and adonitol but not in dulcitol and inulin; indole negative; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 3; during the summer, 7. Total number isolated 10. Two cultures were atypical in that they did not liquefy gelatin.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.68"

Distinguishing characters:- acid and gas produced in

mannitol, saccharose, dulcitol, and adonitol but not in inulin; indole negative; Voges-Proskauer negative; gelatin not liquefied; non motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red positive.

Number of cultures isolated during the winter, 2; during the summer, 6. Total number, 8.

All the cultures gave positive salicin reactions.

B. "MacConkey's No.72"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and dulcitol but not in adonitol and inulin; indole positive; Voges-Proskauer negative;gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 4; during the summer, 4. Total number isolated 8.

All the cultures gave positive reactions in Salicin.

B. "MacConkey's No.104"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and adonitol, but not in dulcitol and inulin; indole negative; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer, 6.

All the cultures gave positive reactions in salicin.

Anomalous Type "A".

Distinguishing characters:- acid and gas produced in mannitol, saccharose, adonitol, dulcitol, but not in inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 5; during the summer, 1. Total number isolated 6.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.2" or B. acidi lactici (Hüppe).

Distinguishing characters:- acid and gas produced in mannitol and adonitol, but not in saccharose, dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 0; during the summer, 5.

All the cultures gave positive reactions in salicin.

Anomalous Type "H".

Distinguishing characters:- acid and gas produced in mannitol and saccharose, but not in adonitol, dulcitol and inulin; indole positive; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer, 5. Four of the cultures were atypical. One of these was Koser positive and methyl-red positive. Three did not liquefy gelatin. One of the latter gave a negative reaction in raffinose.

Four of the cultures gave positive reactions in salicin, one gave a negative reaction.

B. "MacConkey's No.99"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, adonitol and inulin; but not in dulcitol; indole negative; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in

raffinose; Koser positive; methyl-red positive.

Number of cultures isolated during the winter, 0; during the summer, 4. Two cultures were atypical in that they gave neutral methyl-red reactions.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.105"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and inulin, but not in adonitol and dulcitol; indole negative; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 3; during the summer, 1. Total number isolated 4. Three cultures failed to liquefy gelatin and one gave a positive methyl-red reaction.

Three cultures gave positive reactions in salicin; one gave a negative reaction.

Anomalous Type "E"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, adonitol, but not in dulcitol and inulin; indole positive; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 1; during the summer, 3. Total number isolated 4. One culture gave a negative Koser reaction. In the other cultures there was a direct correlation between the indole and Koser reactions. All the cultures gave positive reactions in salicin.

B. "MacConkey's No.69"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, dulcitol and imulin but not in adonitol; indole negative; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 3; during the summer, 0.

One culture failed to liquefy gelatin. All the cultures gave positive salicin reactions.

B. "MacConkey's No.75"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and dulcitol but not in adonitol and imulin; indole negative; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in

raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer, 3. One culture was atypical in that it gave a negative Koser reaction.

All the cultures gave positive reactions in salicin.

B. "MacConkey's No.100"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and adonitol, but not in dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 1; during the summer, 2. Total number isolated 3. One culture was atypical in that it gave a positive Koser reaction.

All the cultures gave positive reactions in salicin.

B."MacConkey's No.107"

Distinguishing characters:- acid and gas produced in mannitol and saccharose, but not in adonitol, dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in

raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 0; during the summer, 3.

Two cultures gave positive reactions in salicin, one gave a negative reaction.

Anomalous Type "B".

Distinguishing characters:- acid and gas produced in mannitol, saccharose and inulin, but not in adonitol and dulcitol; indole positive; Voges-Proskauer positive; gelatin not liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red negative.

Number of cultures isolated during the winter, 3; during the summer, 0.

One culture gave a positive reaction in salicin, 2 gave negative reactions.

B. "MacConkey's No.6"

Distinguishing characters:- acid and gas produced in mannitol, but not in saccharose, dulcitol, adonitol and inulin; indole positive; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas not produced

in raffinose; Koser negative; methyl-red reaction negative.

Number of cultures isolated during the winter, 0; during the summer, 2.

One culture gave a positive reaction in salicin and 1 a negative reaction.

Anomalous Type "C"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and adonitol, but not in dulcitol and imulin; indole positive; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red negative.

Number of cultures isolated during the winter, 1; during the summer 1. Total number isolated 2. One culture was gelatin negative.

Both cultures gave positive reactions in salicin.

Anomalous Type "L"

Distinguishing characters:- acid and gas produced in mannitol and saccharose, but not in adonitol, dulcitol and imulin; indole positive; Voges-Proskauer negative; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 0; during the summer, 2.

Both cultures gave negative reactions in salicin.

Anomalous Type "Q"

Distinguishing characters:- acid and gas produced in mannitol and saccharose, but not in adonitol, dulcitol or inulin; indole negative; Voges-Proskauer negative; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; Methyl-red positive.

The number of cultures isolated during the winter, 0; during the summer, 2.

Both cultures gave positive reactions in salicin.

Anomalous Type "R"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, and adonitol, but not in dulcitol and inulin; indole negative; Voges-Proskauer negative; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methly-red positive.

The number of cultures isolated during the winter, 0; during the summer, 2. One culture was atypical in that it

gave a negative methyl-red reaction.

Both cultures gave positive reactions in salicin.

B. "MacConkey's No.33"

Distinguishing characters:- acid and gas produced in mannitol, dulcitol and adonitol, but not in saccharose and imulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

Number of cultures isolated during the winter, 1; during the summer 0.

The culture gave a positive reaction in salicin.

B. "MacConkey's No.66"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, dulcitol, and adonitol but not in imulin; indole positive; Voges-Proskauer negative; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red reaction positive.

Number of cultures isolated during the winter, 0; during the summer 1.

The type is peculiar in that there is a direct

correlation between the indole and Koser reactions. It gave a positive reaction in salicin.

B. "MacConkey's No.97"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, adonitol and inulin, but not in dulcitol; indole positive; Voges-Proskauer positive; gelatin liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative. Number of cultures isolated during the winter, 0; during the summer 1. There was a direct correlation between the indole and Koser reactions. The culture was atypical in that it did not liquefy gelatin. It gave a positive reaction in salicin.

Anomalous Type "D"

Distinguishing characters:- acid and gas produced in mannitol, but not in saccharose, adonitol, dulcitol and inulin; indole positive; Voges-Proskauer positive; gelatin not liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red negative.

Number of cultures isolated during the winter, 1; during the summer 0.

The culture gave a positive reaction in salicin.

Anomalous Type "J"

Distinguishing characters:- acid and gas produced in mannitol, and saccharose, but not in adonitol, dulcitol and inulin; indole positive; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer, 1.

The culture gave a positive reaction in salicin.

Anomalous Type "K"

Distinguishing characters:- acid and gas produced in mannitol, saccharose and dulcitol, but not in adonitol and inulin; indole positive; Voges-Proskauer positive; gelati not liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser negative; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer 1.

The culture gave a positive reaction in salicin.

Anomalous Type "M"

Distinguishing characters:- acid and gas produced

in mannitol, sacchrose, adonitol and inulin, but not in dulcitol; indole negative; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer, 1.

The culture gave a positive reaction in salicin.

Anomalous Type "N".

Distinguishing characters:- acid and gas produced in mannitol and saccharose, but not in adonitol, dulcitol and inulin; indole negative; Voges-Proskauer positive; gelatin not liquefied; non-motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; methyl-red negative.

Number of cultures isolated during the winter, 0; during the summer 1.

The culture gave a positive reaction in salicin.

Anomalous Type "P"

Distinguishing characters:- acid and gas produced in mannitol, but not in saccharose, adonitol, dulcitol and inulin; indole positive; Voges-Proskauer negative; gelatin liquefied; motile.

Additional characters:- acid and gas not produced in raffinose; Koser negative; methyl-red positive.

The number of cultures isolated during the winter, 0; during the summer, 1.

The culture gave a positive reaction in salicin.

Anomalous Type "X"

Distinguishing characters:- acid and gas produced in mannitol, saccharose, adonitol, dulcitol and inulin; indole positive; Voges-Proskauer positive; gelatin liquefied; motile.

Additional characters:- acid and gas produced in raffinose; Koser positive; Methyl-red negative.

The number of cultures isolated during the winter, 0; during the summer, 1. The type is peculiar in that there is a direct correlation between the indole and Koser reactions.

The culture gave a positive reaction in salicin.

THE CORRELATION between CERTAIN REACTIONS

The Salicin Reaction

The salicin reaction as proposed by Kligler (61) and Levine (42 and 50), has not been employed in distinguish-

ing the type of bacterium because it was found that there was no clear correlation between this reaction and the distinguishing characters used by MacConkey (29) in his classification, even in the case of species which are generally accepted, e.g. B. coli communis (B. "MacConkey's No.34"), B. communior (B. MacConkey's No.71") and B. cloacae (B.MacConkey's No.108"). It will be seen from Table 47 (pages 164-165) that 69 of the B. coli communis cultures gave positive reactions in salicin and 26 gave negative; 181 of the B. communior cultures gave positive reactions and 12 gave negative; 64 of the B. cloacae cultures gave positive reactions and 7 gave negative. Roger, Clark and Evans (35) attached no importance to the salicin test for purposes of classification as they found that 94.6 per cent. of their cultures gave positive reactions. Thomas and Sandman (62) noted that salicin was very frequently fermented by lactose positive organisms. Winslow, Kligler and Rothberg(54) found that salicin was fermented by most but not all of the colon group and by all of the B. aerogenes group and that there was no clear correlation between the saccharose, salicin and dulcitol reactions. Further, Bardsley (59) considered Kligler's method of classification based on the saccharose and salicin reactions of little significance. In the present investigation it was found that of the 797 cultures isolated 86.6 per cent gave positive reactions in salicin.

Levine (43 and 50) noted that cultures which gave

positive Voges-Proskauer reactions frequently gave positive salicin reactions and (64) that there was also a correlation between the indole and salicin reactions. In the present investigation 235 or 95.1 per cent of the cultures which gave positive Voges-Proskauer reactions gave positive salicin reactions, 404 or 83.0 per cent of the cultures which produced indole gave positive salicin reactions. The salicin reaction therefore correlated much better with the Voges-Proskauer reaction than with the indole reaction.

The Raffinose Reaction.

It has been shown by numerous workers (44, 49, 50, 51, 54, 57 and 61) that there is an almost perfect direct correlation between the saccharose and raffinose reactions, i.e., organisms which ferment saccharose with production of acid and gas in most instances ferment raffinose with production of acid and gas and conversely. In the present investigation 567 cultures were tested in raffinose in addition to saccharose and there was a direct correlation between the two reactions in the case of 560 cultures, i.e. 98.8 per cent. Of the 7 cultures which failed to give this correlation 2 belonged to the type B. "MacConkey's No.1"; 1 to type "No.4"; 3 to type "No.34" and 1 to the anomalous type "H". It is worthy of note that with the exception of type "H", these types give negative reactions (i.e., do not produce

acid and gas) in saccharose.

In a number of instances where cultures produced only slight acidity and traces of gas in saccharose, the reactions in raffinose were of value for confirmatory purposes.

The Methyl-red Reaction.

There is an almost perfect inverse correlation between the methyl-red and the Voges-Proskauer reactions. Organisms which give positive Voges-Proskauer reactions as a rule give negative methyl-red reactions and conversely (37, 38, 40, 41, 43, 49, 50, 51, 53-56, 59). In the present investigation this inverse correlation occurred in 771 cultures out of a total of 797, i.e., in 96.7 per cent. The correlation was therefore almost perfect. Of the 26 atypical cultures, 17 gave neutral methyl-red reactions (See Table 48, page 166). It is worthy of note that with one exception, a culture belonging to the anomalous type "H", all the atypical cultures gave negative indole reactions and 21 of them gave positive Voges-Proskauer reactions. Burton and Rettger (47), Chen and Rettger (55) and other observers found that most, if not all, of their atypical strains gave positive Voges-Proskauer reactions. Ruchhoft, Kallas, Chinn and Coulter (63) found that there was a variability in methyl-red reactions in aerogenes strains

that were regular in their indole and Koser reactions (indole negative, Koser positive) and considered that such strains were"normal members of an aerogenes section that has considerable variation in the organic acid utilizing velocity of the various strains".

The Koser Citrate Reaction.

It has been shown by Koser (65 and 58) that the aerogenes - cloacae types of coliform bacteria (generally methyl-red negative and Voges-Proskauer positive) are capable of utilizing the citrate radicle as the sole source of carbon, while the B. coli communis and allied types (generally methyl-red positive and Voges-Proskauer negative) of faecal origin are unable to attack this radicle. Consequently the former grow readily in a synthetic citrate medium while the latter do not develop. Koser found that in some instances organisms resembling faecal types (methyl-red positive and Voges-Proskauer negative) but of soil origin, can attack the citrate and develop in the synthetic citrate medium.

There was a direct correlation between the Voges-Proskauer and Koser reactions in the case of 664 cultures, i.e., 83.2 per cent. There were 133 exceptions. (See Table 49, page 167). Ninety-four of the latter were peculiar in that they gave negative indole and negative Voges-Proskauer reactions. These 94 cultures were indole

negative, methyl-red positive, Voges-Proskauer negative and Koser positive. Cultures of this type were frequently obtained by Koser (58 and 66) from the soil. They have also been isolated from water by Bardsley (59), Raghavachari (67) Lewis and Pittman (68) and Ruchhoft, Kallas, Chinn and Coulter (63) They are rarely found in human and animal faeces. In the present investigation it was found that a positive Koser reaction was not characteristic of all the cultures which were indole negative, methyl-red positive and Voges-Proskauer negative as 7 of such cultures were Koser negative.

Fifteen of the cultures which did not give correlating Voges-Proskauer and Koser reactions were peculiar in that they were indole positive and Voges-Proskauer positive. These cultures were indole positive, methyl-red negative, Voges-Proskauer positive and Koser negative. There were, however, 23 other indole positive Voges-Proskauer positive cultures and these gave positive Koser reactions (most of the latter belonged to types not included in Table 49).

A perfect correlation therefore was not obtained between the Voges-Proskauer and the Koser citrate reactions. Exceptions occurred most frequently in cultures which were indole negative and Voges-Proskauer negative or indole

positive and Voges-Proskauer positive.

Raghavachari (67) found that there was a well marked inverse correlation between the indole and Koser citrate reactions. Similar results have been obtained in the present investigation. Of the 797 cultures isolated there was an inverse correlation between these two reactions in 743, i.e. 93.2 per cent of the cultures. The Koser reaction therefore correlated to a very much higher degree with the indole reaction than with the Voges-Proskauer. The inverse correlation between the indole and Koser reactions did not occur in the case of 54 cultures. (See Table 50, page 168). Twenty-three of these exceptional cultures belonged to types which gave positive indole and positive Voges-Proskauer reactions, e.g. B. "MacConkey's Nos.65 and 97" and "Anomalous Types E, H and X". Such cultures were indole positive, Voges-Proskauer positive and Koser positive. A positive Koser reaction was not characteristic of all the cultures which gave positive indole and positive Voges-Proskauer reactions as in 15 instances such cultures were Koser negative. It is worthy of note, however, that all the cultures, 17, of B. "MacConkey's No.65" were Koser positive and therefore exceptional. Of the 97 cultures which gave negative indole and negative Voges-Proskauer reactions, only 7 gave Koser reactions which did not correlate inversely with the indole

reactions. It is interesting to note that most of the cultures (10 out of 12) of the type B. "MacConkey's No.101" were exceptional. Organisms belonging to this type are indole positive and Voges-Proskauer negative.

There is therefore a well marked inverse correlation between the indole and Koser reactions of coliform bacteria, the exceptions occurring most frequently in the case of types giving positive indole and positive Voges-Proskauer reactions (especially B."MacConkey's No.65") and in the case of type B. "MacConkey's No.101".

THE OCCURRENCE of FAECAL and NON-FAECAL TYPES of COLIFORM BACTERIA in MILK during the WINTER and SUMMER PERIODS

It is generally accepted that there are two types of coliform bacteria (a) faecal types and (b) non-faecal types.

The Faecal Types are comparatively rare in natural waters and soil and on grains provided there has been no contamination with faeces and sewage, but they occur abundantly in human and animal faeces. They are characterised generally by producing approximately equal volumes of carbon dioxide and hydrogen in the anaerobic fermentation of glucose, i.e.

they have a "low gas ratio". In most cases they are indole positive, methyl-red positive, Voges-Proskauer negative and Koser citrate negative. B. coli communis (B. "MacConkey's No.34") and B. communior (B. "MacConkey's No.71") are faecal types.

The Non-faecal (soil and grain) types are comparatively rare in human and animal faeces but occur commonly in the soil and surface waters and on grains. The most prevalent of these are the aerogenes - cloacae types. They are characterised generally by producing over 1.5 times as much carbon dioxide as hydrogen in the anaerobic fermentation of glucose, i.e. they have a "high gas ratio". In most instances, but not all, they are indole negative. They are generally methyl-red negative, Voges-Proskauer positive and Koser citrate positive. B.lactis aerogenes (B. "MacConkey's No.103") and B. cloacae (b. "MacConkey's No.108) are examples of such types.

Other types (58 and 66) which occur commonly in soils but are less prevalent than the aerogenes-cloacae types, resemble faecal types in that they are methyl-red positive and Voges-Proskauer negative. In some instances they are indole positive, in others indole negative. They are most frequently Koser citrate positive. Koser found that such organisms occurred only in rare instances in the cultures he isolated from faeces and concluded that they were non-faecal

types and that the citrate reaction therefore correlated more closely with the source of coliform bacteria than the methyl-red, Voges-Proskauer and indole reactions. His results have been confirmed by Rauchhoft, Kallas, Chinn, and Coulter (63) and other observers. Consequently the Koser citrate reaction is frequently employed for the differentiation of faecal from non-faecal types of coliform bacteria.

It will be seen from Table 46 (page 162) and pages 108 - 132 that the types most frequently isolated from samples of milk were as follows:-

WINTER PERIOD

Type	Number of Cultures Isolated	Percentage
B. "MacConkey's No.71"	127	35.4
B. "MacConkey's No.34"	55	15.3
B. "MacConkey's No.108"	32	8.9
B. "MacConkey's No.1"	28	7.8
B. "MacConkey's No.7"	17	4.7
B. "MacConkey's No.106"	14	3.9
B. "MacConkey's No.4"	10	2.8
B. "MacConkey's No.103"	10	2.8

SUMMER PERIOD

Type	Number of Cultures Isolated	Percentage
B. "MacConkey's No.71"	66	15.1
B. "MacConkey's No.103"	51	11.6
B. "MacConkey's No.34"	40	9.1
B. "MacConkey's No.108"	39	8.9
B. "MacConkey's No.7"	26	5.9
B. "MacConkey's No.98"	19	4.3
B. "MacConkey's No.1"	17	3.9
B. "MacConkey's No.4"	16	3.6
B. "MacConkey's No.73"	15	3.4
B. "MacConkey's No.65"	14	3.2
B. "MacConkey's No.67"	14	3.2
B. "MacConkey's No.5"	10	2.3

BOTH PERIODS

Type	Number of Cultures Isolated	Percentage
B. "MacConkey's No.71"	193	24.2
B. "MacConkey's No.34"	95	11.9
B. "MacConkey's No.108"	71	8.9
B. "MacConkey's No.103"	61	7.7
B. "MacConkey's No.1"	45	5.6
B. "MacConkey's No.7"	43	5.4
B. "MacConkey's No.4"	26	3.3
B. "MacConkey's No.106"	22	2.8
B. "MacConkey's No.98"	21	2.6
B. "MacConkey's No.73	19	2.4
B. "MacConkey's No.65"	17	2.1

B. "MacConkey's Nos.1, 4, 5, 34, 71 and 106" are indole positive, methyl-red positive, Voges-Proskauer negative and Koser negative and are therefore faecal in type. "Nos. 67, 73, 98, 103 and 108" are indole negative, Methyl-red negative, Voges-Proskauer positive and Koser positive and are therefore non-faecal in type. "No.7" is indole negative, methyl-red positive, Voges-Proskauer negative and Koser positive. "No.65" is indole positive, methyl-red negative, Voges-Proskauer positive and Koser positive. These two organisms having regard to their indole and Voges-Proskauer reactions are indeterminate in type, but having regard to the Koser reaction are non-faecal.

Of all the cultures isolated, 36.1 per cent (i.e. over one third) belonged to the faecal types, B. "MacConkey's Nos.34 and 71". The percentage of these types, however, varied greatly according to period. Thus 50.7 per cent of the winter cultures were of these two types but only 24.2 per cent of the summer cultures. The percentage of the soil type, "No.108", was the same (8.9) for both periods. While that of the soil type, "No.103", was only 2.8 in the case of the winter cultures and 11.6 in the case of the summer.

The numbers and percentages of indole positive cultures, Voges-Proskauer negative cultures and Koser negative cultures, which were isolated from milk during

the winter and summer periods and during the combined periods, are given in Table 51 (page 169). As previously mentioned, during the winter period the practice was to isolate several cultures from the one sample of milk, but these most frequently proved to be of the same type, consequently during the summer only one culture was isolated from each sample. Owing to the fact that the Koser reaction correlates inversely with the indole reaction and directly with the Voges-Proskauer the values in the table for the different reactions correspond to a great extent. Further, these reactions (indole positive, Voges-Proskauer negative and Koser negative) are more characteristic of the faecal types of coliform bacteria than of the non-faecal types. It will be seen from the table that of the cultures isolated from samples of milk during the winter period, 75.2 per cent were indole positive, 81.3 per cent. were Voges-Proskauer negative and 73.3 per cent. were Koser negative. Of the cultures isolated during the summer period, 49.5 per cent. were indole positive, 58.9 per cent. were Voges-Proskauer negative and 46.8 per cent. were Koser negative. Of the total number of cultures isolated, 61.1 per cent. were indole positive, 69.0 per cent. were Voges-Proskauer negative and 58.7 per cent. were Koser negative. It is evident from these figures that there is a much closer correlation between the

indole and Koser reactions than between the Voges-Proskauer and Koser. Accordingly if the Koser reaction is accepted as being the most reliable for the determination of the source of coliform organisms, the indole reaction will be more reliable than the Voges-Proskauer for the same purpose.

These results show that the proportion of faecal to non-faecal types of coliform bacteria in the samples of the milk of the winter period was much higher than it was in the samples of the summer period. If the differentiation of faecal from non-faecal types is based on the Koser reaction then in the winter period 73.3 per cent of the coliform cultures isolated were of faecal origin (Koser negative) and 26.7 per cent were of non-faecal origin (Koser positive), i.e. the ratio of faecal to non-faecal types was approximately 3:1. In the summer period only 46.8 per cent of the cultures were of faecal origin and 53.2 per cent were of non-faecal origin, i.e. the ratio of faecal to non-faecal types was approximately 1:1.

Conclusions.

Coliform bacteria are of frequent occurrence in milk. Even where fresh samples less than 24 hours old are tested, over 50 per cent may give positive coliform tests with 1/10 c.c. amounts. The proportion of coliform positive

samples, however, varies greatly according to season, being much higher in summer than in winter. This variation is accounted for largely by atmospheric temperature, as there is a well marked correlation between the proportion of coliform positive samples and the mean of the minimum and maximum atmospheric temperatures. The effect of the higher temperature in summer appears to be due to its influence both on the uncooled milk and on incompletely sterilised utensils so that more rapid multiplication of contaminating organisms occurs. Milk which has been contaminated with coliform bacteria has generally a much higher total bacterial content than specimens free from coliform bacteria in 1/10 c.c. samples, and it appears that in certain cases at least this excess is partly contributed to by organisms other than coliform bacteria.

During the colder part of the year when the cows are confined to cattle sheds and not at pasture, the faecal types of coliform bacteria, e.g. B. "MacConkey's Nos.1, 4, 34, 71 and 106" occur much more frequently in milk than the non-faecal types, e.g. B. "MacConkey's Nos. 67, 73, 98, 103 and 108". During the warmer part of the year when the cows are at pasture, the faecal and non-faecal types occur with almost equal frequency. In the present investigation 73.3 per cent of the cultures isolated during the winter period were faecal in type. The ratio of faecal to non-faecal cultures was

therefore approximately 3:1. On the other hand, only 46.8 per cent of the cultures isolated during the summer period were faecal in type, the ratio of the faecal to non-faecal types being approximately 1:1. The importance of these results lies in the fact that the presence of coliform organisms in milk is generally accepted as an indication that it has been contaminated directly or indirectly with faeces. Non-faecal types, however, are comparatively rare in bovine faeces according to various observers; therefore in all probability the milk is contaminated with such types from other and less harmful sources, e.g., hay, straw, silage, grain and particles of soil or dust derived from the animals skins or from the atmosphere of the milking sheds.

The greater preponderance of faecal types during the winter period is in all probability to be explained by the greater exposure of the milk to contamination with dung, when the cows are confined to the sheds and not at pasture.

Summary.

(1) Seven hundred and ninety-seven cultures of coliform bacteria were isolated from samples of milk and the degrees of correlation between certain characters examined with the following results:-

(a) There was no clear correlation between the salicin

reaction and the distinguishing characters used by MacConkey in his classification. 95.1 per cent of the cultures which were Voges-Proskauer positive and 83.0 per cent of the cultures which were indole positive gave positive salicin reactions.

(b) In 98.8 per cent of the cultures examined there was a direct correlation between the saccharose and raffinose reactions. The correlation was therefore almost perfect.

(c) In 96.7 per cent of the total number of cultures isolated there was an inverse correlation between the methyl-red and Voges-Proskauer reactions. The exceptions in most instances were cultures of the aerogenes-cloacae type.

(d) There was a direct correlation between the Koser citrate and the Voges-Proskauer reactions in 83.2 per cent of the cultures. Exceptions occurred most frequently in cultures which were indole negative and Voges-Proskauer negative or indole positive and Voges-Proskauer positive. There was an inverse correlation between the Koser citrate and indole reactions in 93.2 per cent of the cultures, the exceptions occurring most frequently in type B. "MacConkey's No.101" and in types giving indole positive and Voges-Proskauer positive reactions, especially B. "MacConkey's No.65". There was therefore a much closer correlation

between the Koser and indole reactions than between the Koser and Voges-Proskauer. Consequently if the Koser reaction is accepted as being the most reliable to differentiate the faecal from the non-faecal types of coliform bacteria, the indole reaction is more reliable than the Voges-Proskauer for the same purpose.

(2) Of the cultures isolated from samples of milk during the colder part of the year (winter and spring) when the cows were confined to the cattle sheds and not at pasture, 75.2 per cent were indole positive, 81.3 per cent were Voges-Proskauer negative and 73.3 per cent were Koser citrate negative. Of the cultures isolated from milk during the warmer part of the year (summer and autumn) when the cows were at pasture, 49.5 per cent were indole positive, 58.9 per cent were Voges-Proskauer negative and 46.8 per cent were Koser negative.

(3) The types of coliform bacteria most frequently isolated were B. "MacConkey's No.71, B.communior (24.2 per cent); "No.34", B. coli communis (11.9 per cent); "No.108", B.cloacae (8.9 per cent); "No.103", B. lactis aerogenes (7.7 per cent); "No.1" (5.6 per cent); "No.7 (5.4 per cent); "No.4" (3.3 per cent); "No.106" (2.8 per cent); "No.98"

(2.6 per cent); "No.73" (2.4 per cent);"No.65" (2.1 per cent).

(4). The ratio of faecal to non-faecal types of coliform bacteria was approximately 3:1 when the cows were confined to the byres or cattle sheds and it was approximately 1:1 when they were at pasture.

(5). 21,569 samples of milk were examined by means of the coliform test. In 48.3 per cent of the samples the test gave negative results for 1/10, 1/100 and 1/1000 c.c.; in 21.4 per cent it gave positive results for 1/10 c.c. and negative results for 1/100 and 1/1000 c.c.; in 14.0 per cent it gave positive results for 1/10 and 1/100 c.c. and negative for 1/1000 c.c.; and in 16.3 per cent it gave positive results for 1/10, 1/100 and 1/1000 c.c. It is probable that these results are better than those generally obtained for milk.

(6). The proportion of coliform positive samples is generally much higher in summer and early autumn than during the winter and spring. This seasonal variation is largely due to atmospheric temperature, there being a well marked direct correlation between the proportion of coliform

positive samples and the mean of the minimum and maximum atmospheric temperatures.

(7). Coliform negative samples of milk generally contain fewer bacteria than coliform positive. Of 21,857 samples examined, 10,458 were coliform negative and had an average bacterial content of 25,294; 11,399 were coliform positive and had an average bacterial content of 160,577. The ratio of the average bacterial content of the coliform negative samples to that of the coliform positive was 1:6.3, i.e. the coliform positive samples contained on an average 6.3 times as many bacteria as the coliform negative.

(8). There is a well marked direct correlation between the average bacterial content of a series of samples, the proportion of coliform positive samples and the mean of the minimum and maximum atmospheric temperatures.

TABLE 38.

THE PREVALENCE of COLIFORM BACTERIA in MILK from GROUPS of FARMS in the SOUTH WEST of SCOTLAND.

Group of Farms	Year	Number of Samples in each Year	Samples Containing No Coliform bacteria in 1/10 1/100 & 1/1000 c.c.		Samples Containing Coliform bacteria in 1/10 but Not in 1/100 and 1/1000 c.c.		Samples Containing Coliform bacteria in 1/10 and 1/100 but Not in 1/1000 c.c.		Samples Containing Coliform bacteria in 1/10, 1/100 and 1/1000 c.c.	
			Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
A	1st	72	35	48.6	12	16.7	8	11.1	17	24.1
B	1st	250	136	54.4	46	18.4	33	13.2	35	14.0
C	1st	175	132	75.4	21	12.0	14	8.0	8	4.6
D	1st	140	47	34.0	44	31.0	18	13.0	31	22.0
	2nd	207	108	52.0	39	19.0	24	12.0	36	17.0
	3rd	240	117	48.8	42	17.5	32	13.3	49	20.4
	4th	248	111	44.7	64	25.8	41	16.5	32	13.0
E	1st	156	65	42.0	37	24.0	32	20.5	22	14.0
	2nd	354	190	54.0	72	20.0	53	15.0	39	11.0
	3rd	300	201	67.0	65	21.7	22	7.3	12	4.0
	4th	279	164	58.8	59	21.1	36	12.9	20	7.9
F	1st	570	320	56.0	130	23.0	62	11.0	58	10.0
	2nd	528	372	70.5	94	17.8	36	6.8	26	4.9
	3rd	516	349	68.0	105	20. 0	35	7.0	27	5.0
G	1st	528	278	53.0	116	22.0	70	13.0	64	12.0
	2nd	556	323	58.0	137	25.0	58	10.0	38	7.0
	3rd	537	366	68.2	119	22.2	39	7.3	13	2.4
H	1st	603	377	62.5	117	19.4	63	10.5	46	7.6
	2nd	615	347	56.4	131	21.3	76	12.4	61	9.9
I	1st	240	95	39.6	46	19.2	41	17.1	58	24.1
	2nd	377	151	40.0	65	17.0	67	18.0	94	25.0
	3rd	443	236	53.3	100	22.6	67	15.1	40	9.0
J	1st	966	248	25.7	197	20.4	254	26.3	267	27.6

TABLE 38. (Contd)

THE PREVALENCE of COLIFORM BACTERIA in MILK from GROUPS of FARMS in the SOUTH WEST of SCOTLAND.

Group of Farms	Year	Number of Samples in each Year	Samples Containing No Coliform bacteria in 1/10 1/100 & 1/1000 c.c.		Samples Containing Coliform bacteria in 1/10 but Not in 1/100 and 1/1000 c.c.		Samples Containing Coliform bacteria in 1/10 and 1/100 but Not in 1/1000 c.c.		Samples Containing Coliform bacteria in 1/10, 1/100 & 1/1000 c.c.	
			Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
K	1st	765	275	36.0	176	23.0	124	16.2	190	24.8
	2nd	766	343	44.8	191	24.9	135	17.6	97	12.7
	3rd	669	315	47.0	145	22.0	103	15.0	106	16.0
L	1st	72	27	38.0	14	19.0	15	21.0	16	22.0
	2nd	78	43	55.1	9	11.5	8	10.3	18	23.1
	3rd	72	36	50.0	15	20.8	9	12.5	12	6.7
M	1st	792	212	26.8	127	16.0	143	18.2	310	39.0
	2nd	1143	332	29.0	209	18.0	215	19.0	387	34.0
	3rd	1319	226	17.1	274	20.8	284	21.5	535	40.6
N	1st	686	418	60.9	145	21.1	55	8.0	68	9.9
	2nd	635	409	64.4	132	20.8	65	10.2	29	4.6
	3rd	618	420	68.0	124	20.0	43	7.0	31	5.0
O	1st	694	351	50.6	141	20.3	96	13.8	106	15.3
	2nd	589	315	53.5	132	22.4	76	12.9	66	11.2
P	1st	364	210	58.0	91	25.0	41	11.0	22	6.0
	2nd	848	542	63.9	198	23.3	77	9.1	31	3.7
Q	1st	463	196	42.0	98	21.0	64	14.0	105	23.0
	2nd	520	221	43.0	157	30.0	79	15.0	63	12.0
R	1st	457	142	31.1	118	25.8	84	18.4	113	24.7
	2nd	478	238	49.8	130	27.2	59	12.3	51	10.7
S	1st	641	385	60.0	126	19.7	62	9.7	68	10.6
		21,569	10,424	48.3	4,610	21.4	3,018	14.0	3,517	16.3

T A B L E 39.

The Influence of Season and Atmpspheric Temperature on the proportion of Coliform Positive Samples+ and on the Average Bacterial Content of Samples of Milk from the same Group of Farms ("F,1").

Average Number of Samples per Month - 44.

Month in which Samples Received	Average Bacterial Content per c.c. (Coliform Positive & Coliform Negative)	Proportion of Coliform Positive Samples, expressed as a Percentage.	Mean of the Minimum and Maximum atmospheric temperatures for the Day on which Samples Received.
January	16,798	20.4	36.0
February	11,373	11.4	35.0
March	17,664	29.5	40.5
April	25,702	22.7	45.5
May	74,057	56.8	48.5
June	216,145	81.8	55.5
July	182,936	84.0	57.5
August	35,627	43.0	53.5
September	57,525	75.0	55.5
October	9,377	25.0	45.5
November	10,995	32.0	47.5

+ Samples containing Coliform Organisms in 1/10 c.c.

FIGURE 4.

GRAPHIC REPRESENTATION OF TABLE 39.

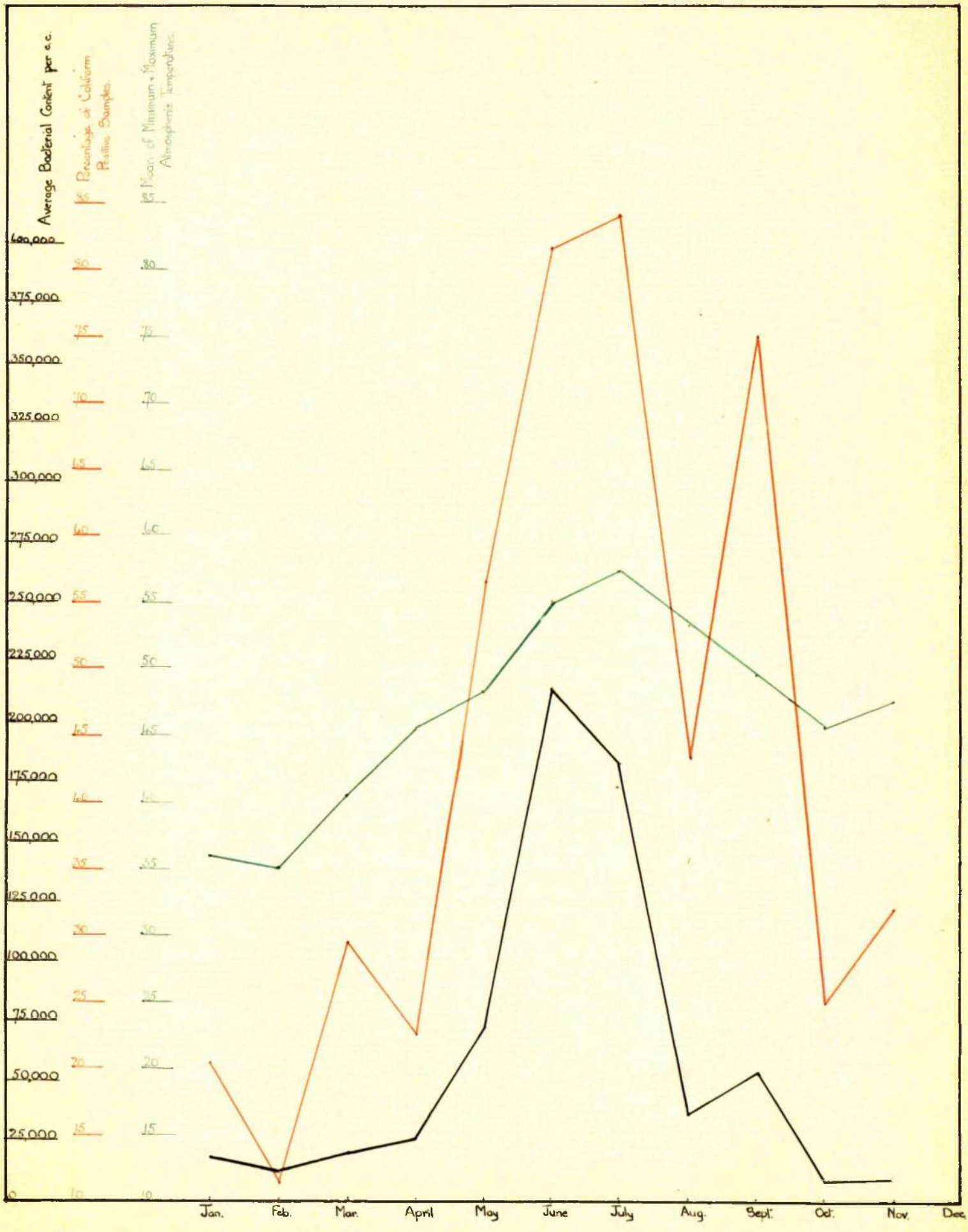


TABLE 40.

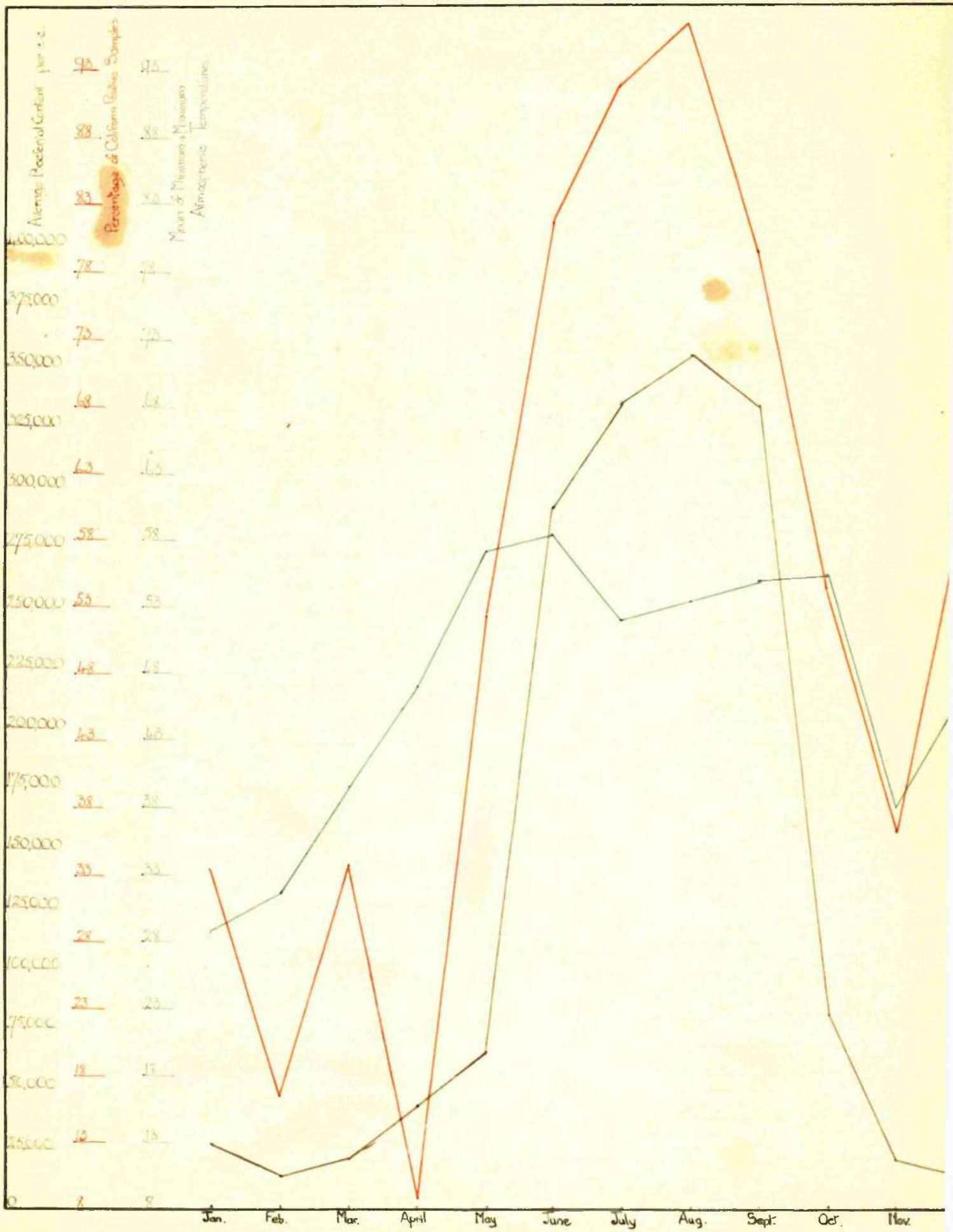
The Influence of Season and Atmospheric Temperature on the Proportion of Coliform Positive Samples+ and on the Average Bacterial Content of Samples of Milk from the same Group of Farms ("D")

Average Number of Samples per Month - 24.

Month in which Samples Received	Average Bacterial content per c.c. (Coliform Positive & Coliform Negative)	Proportion of Coliform Positive Samples, expressed as a Percentage	Mean of the Minimum and Maximum atmospheric temperatures for the Day on which Samples Received.
January	26,417	33.3	28.5
February	13,179	16.7	31.5
March	20,713	33.7	39.5
April	42,217	8.7	47.0
May	65,970	52.2	57.0
June	288,200	81.8	58.5
July	334,279	91.7	52.0
August	355,217	96.5	53.5
September	333,931	79.7	55.0
October	77,332	54.5	55.5
November	18,927	36.4	38.0
December	15,940	60.0	47.0

+ Samples containing Coliform Organisms in 1/10 c.c.

FIGURE 5
GRAPHIC REPRESENTATION OF TABLE 40



T A B L E 41.

The Influence of Season and Temperature on the Proportion of Coliform Positive Samples[+] and on the Average Bacterial Content of Samples of Milk from the same Group of Farms ("E")

Average Number of Samples per Month - 30.

Month in which Samples Received	Average Bacterial content per c.c. (Coliform Positive & Coliform Negative)	Proportion of Coliform Positive Samples, expressed as a Percentage	Mean of the Minimum and Maximum atmospheric temperatures for the Day on which Samples Received.
January	21,304	24.0	31.5
February	33,179	35.3	41.0
March	29,407	15.0	42.0
April	33,263	41.0	42.5
May	46,126	39.0	48.5
June	112,444	63.0	55.5
July	277,859	96.0	57.5
August	252,431	91.0	57.0
September	90,488	62.0	55.0
October	26,131	47.0	45.5
November	9,950	25.0	47.5
December	11,764	24.0	39.5

[+] Samples containing Coliform Organisms in 1/10 c.c.

Figure 6
Graphic Representation of Table 41.

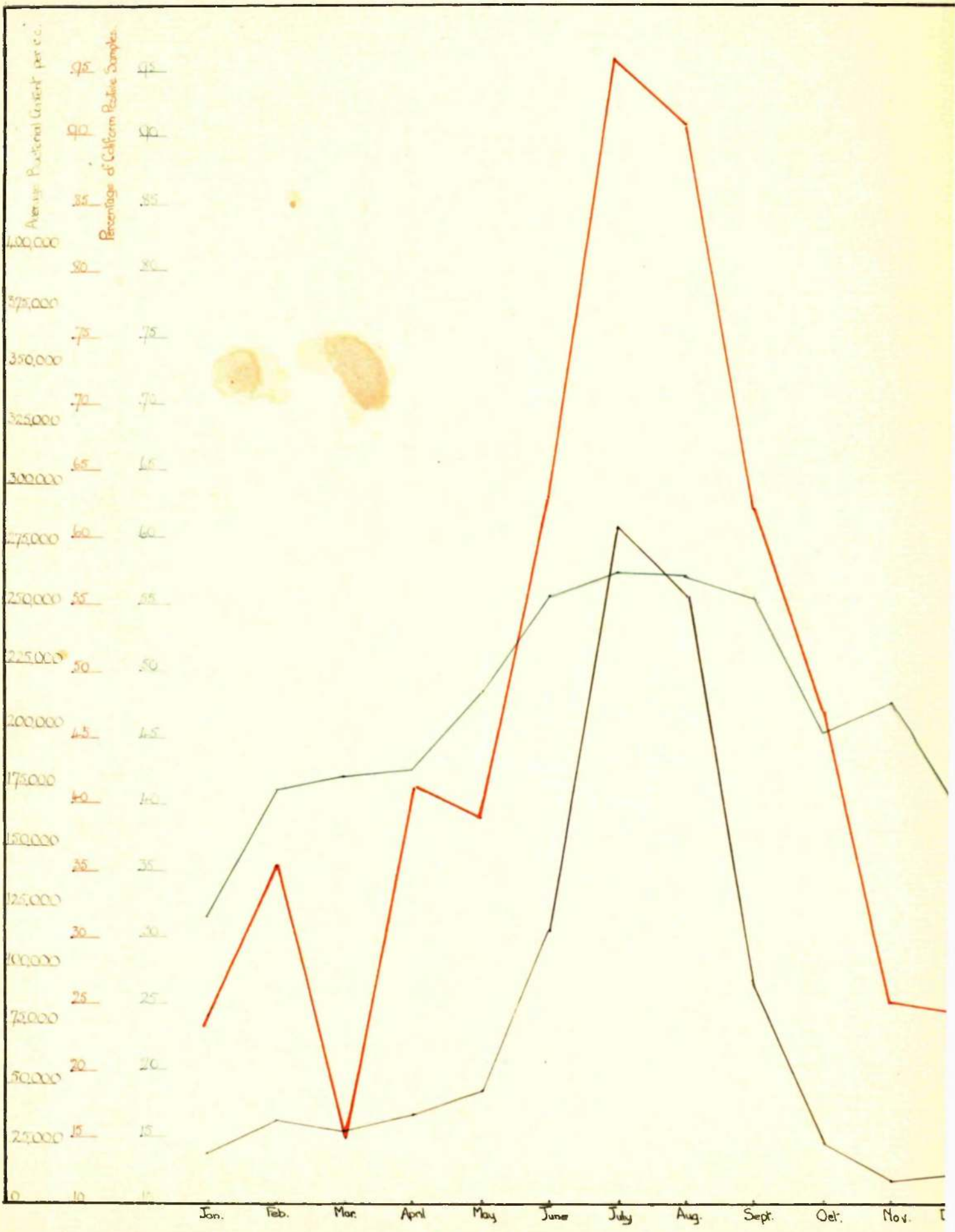


TABLE 42.

The Influence of Season and Atmospheric Temperature on the Proportion of Coliform-Positive Samples+ and on the Average Bacterial Content of Samples of Milk from the same Group of Farms ("K")

Average Number of Samples per Month - 65.

Month in which Samples Received	Average Bacterial Content per c.c. of all the Samples (Coliform Positive & Coliform Negative)	Proportion of Coliform Positive Samples, expressed as a Percentage	Mean of the Minimum and Maximum atmospheric temperatures for the Day on which Samples Received
January	32,997	29.0	34.3
February	52,538	54.0	42.0
March	33,977	36.0	36.8
April	75,209	57.0	46.3
May	47,676	33.0	41.3
June	374,274	86.0	53,5
July	654,738	82.0	53.8
August	82,185	91.0	52.3
September	421,986	94.0	53.0
October	78,259	71.0	48.5
November	57,347	45.3	42.0
December	44,030	70.0	45.5

+ Samples containing Coliform Organisms in 1/10 c.c.

FIGURE 7.
GRAPHIC REPRESENTATION OF TABLE 42.

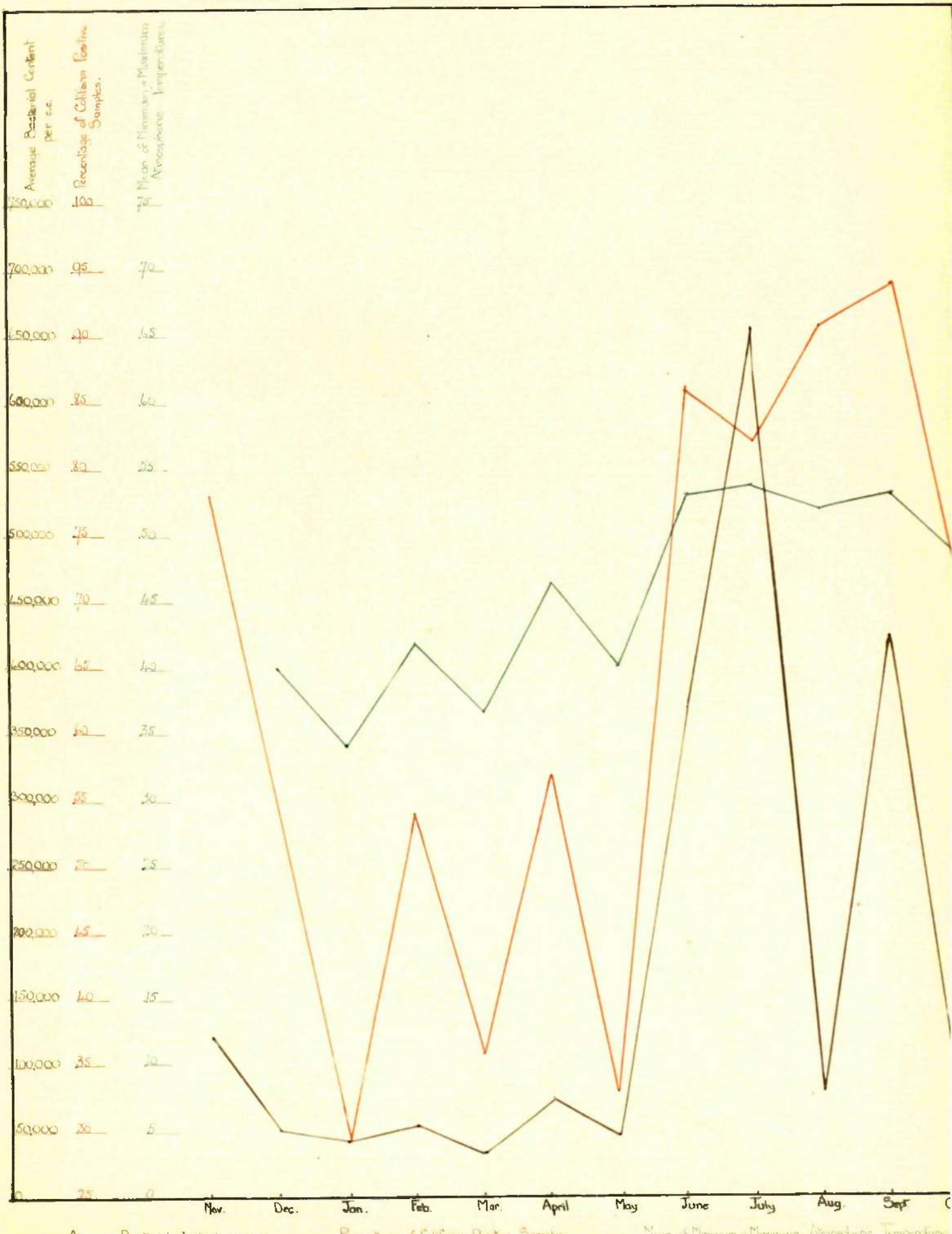


TABLE 43.

The Influence of Season and Atmospheric Temperature on the Proportion of Coliform Positive Samples +and on the Average Bacterial Content of Samples of Milk from the same Group of Farms ("X")

Average Number of Samples per Month - 46.

Month in which Samples Received	Average Bacterial Content per c.c. of all the Samples (Coliform Positive & Coliform Negative)	Proportion of Coliform Positive Samples, expressed as a Percentage	Mean of the Minimum and Maximum atmospheric temperatures for the Day on which Samples Received
January	13,444	12.0	35.5
February	17,210	7.7	35.0
March	19,297	20.5	47.0
April	32,757	19.6	47.5
May	71,076	36.0	54.5
June	59,143	33.0	49.5
July	190,241	59.0	59.5
August	226,179	72.0	60.0
September	158,231	56.0	60.0
October	17,159	29.0	38.5
November	10,375	21.0	47.0
December	10,806	16.0	37.5

+Samples containing Coliform Organisms in 1/10 c.c.

FIGURE 8
GRAPHIC REPRESENTATION OF TABLE 4.3

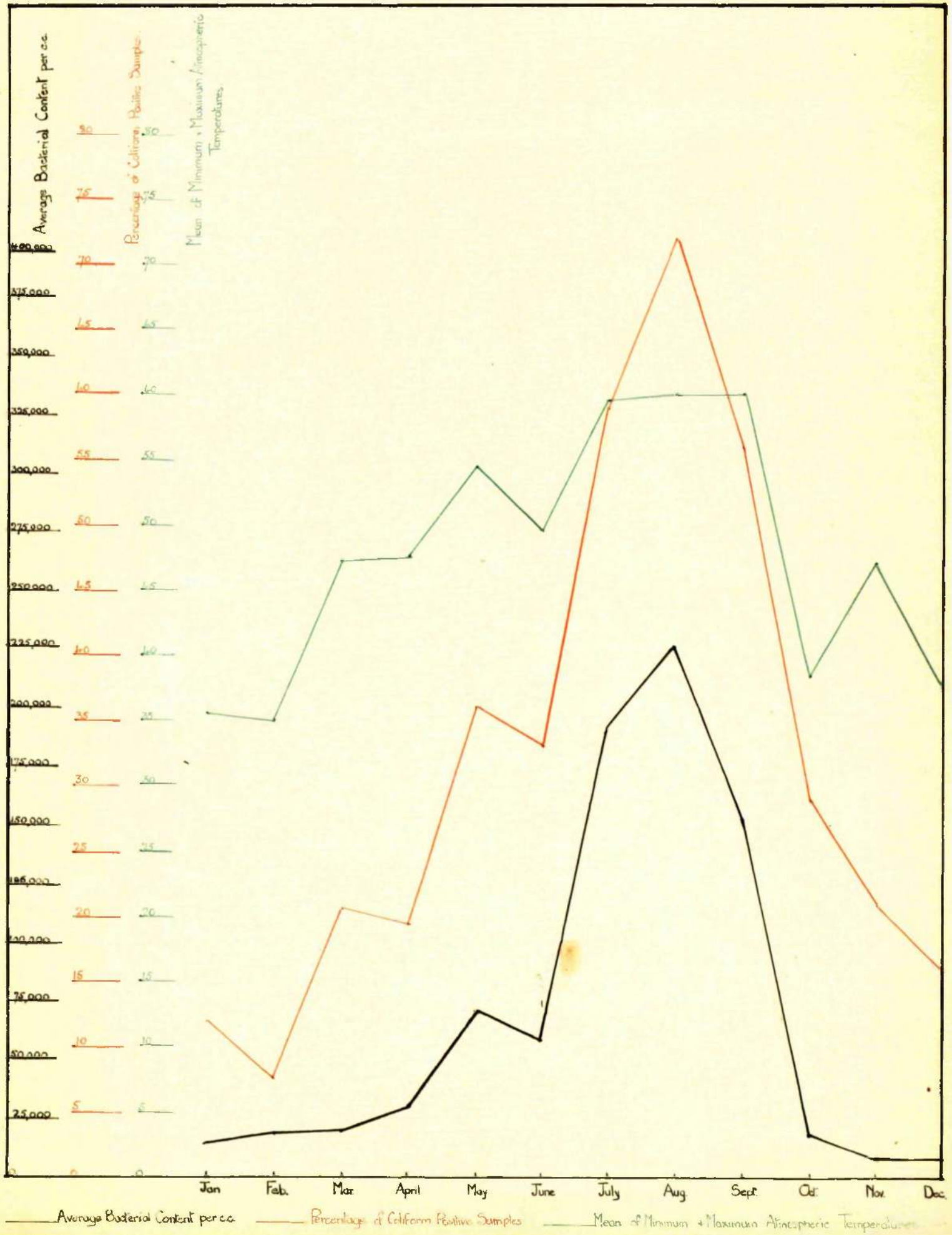


TABLE 44.

The Influence of Season and Atmospheric Temperature on the Proportion of Coliform Positive Samples[†] and on the Average Bacterial Content of Samples of Milk from the same Group of Farms ("F,2")

Average Number of Samples per Month - 44.

Month in which Samples Received	Average Bacterial Content per c.c. of all the Samples (Coliform Positive & Coliform Negative)	Proportion of Coliform Positive Samples, expressed as a Percentage	Mean of the Minimum and Maximum atmospheric temperatures for the Day on which Samples Received
January	12,480	20.0	38
February	21,853	11.11	32.5
March	15,011	11.11	39.5
April	9,550	25.0	46
May	23,195	25.6	49.5
June	18,920	22.7	55.0
July	21,934	36.4	59.0
August	108,845	61.4	60.0
September	183,944	83.7	52.5
October	18,937	30.2	50.5
November	18,673	15.9	45.5
December	11,209	15.9	41.0

[†]Samples containing Coliform Organisms in 1/10 c.c.

T A B L E 45.

A COMPARISON between the AVERAGE BACTERIAL CONTENT of COLIFORM NEGATIVE and COLIFORM POSITIVE SAMPLES of MILK from GROUPS of FARMS in the SOUTH WEST of SCOTLAND.

Group of Farms	Year	Number of Samples in each Year	COLIFORM NEGATIVE SAMPLES (Samples containing no Coliform Organisms in 1/10 c.c.) Number of Samples	Average Bacterial Content per c.c. A.	COLIFORM POSITIVE SAMPLES (Samples containing Coliform Organisms in 1/10 c.c.) Number of Samples	Average Bacterial Content per c.c. B.	Ratio of Average Bacterial Content of Coliform Negative Samples to Average Bacterial Content of Coliform Positive Samples. (A: B).
A.	1st	72	33	13,021	39	150,551	1: 11.6
B.	1st	250	136	27,133	114	95,235	1: 3.5
C.	1st	396	168	43,026	228	242,623	1: 5.6
	2nd	175	132	31,688	43	53,395	1: 1.7
D.	1st	140	47	42,049	93	74,381	1: 1.8
	2nd	207	108	24,874	99	250,732	1: 10.1
	3rd	274	125	44,118	149	165,497	1: 3.8
	4th	248	111	16,100	137	70,577	1: 4.4
E.	1st	156	65	25,195	91	94,946	1: 3.8
	2nd	354	190	24,684	164	144,554	1: 5.9
	3rd	300	201	24,381	99	55,505	1: 2.3
	4th	279	164	31,205	115	109,063	1: 3.5
F.	1st	570	320	19,430	250	115,857	1: 6.0
	2nd	528	372	14,830	156	23,567	1: 1.6
	3rd	516	349	14,599	167	79,288	1: 5.4
G.	1st	782	364	31,275	418	165,848	1: 5.3
	2nd	556	323	20,839	233	100,653	1: 4.9
	3rd	537	366	16,071	171	46,954	1: 2.9
H.	1st	603	377	25,006	226	145,366	1: 5.8
	2nd	615	347	23.944	268	98,047	1: 4.1
I.	1st	359	133	30,757	226	211,969	1: 6.9
	2nd	377	151	29,293	226	150,338	1: 5.1
	3rd	443	236	21,152	207	99,398	1: 4.7
J.	1st	966	248	31,436	718	140,132	1: 4.5

TABLE 45. (Contd.)

Group of Farms	Year	Number of Samples in each year	COLIFORM NEGATIVE SAMPLES (Samples containing no Coliform Organisms in 1/10 c.c.) Number of Samples	Average Bacterial Content per c. c. A.	COLIFORM POSITIVE SAMPLES (Samples containing Coliform Organisms in 1/10 c.c.) Number of Samples	Average Bacterial Content per c.c. B.	Ratio of Average Bacterial Content of Coliform Negative Samples to Average Bacterial Content of Coliform Positive Samples. (A: B)
K.	1st	765	275	18,009	490	157,855	1: 8.8
	2nd	766	343	36,512	423	115,411	1: 3.2
	3rd	669	315	28,860	354	169,742	1: 5.9
L.	1st	72	27	75,100	45	141,222	1: 1.9
	2nd	78	40	17.728	38	88,841	1: 5.0
	3rd	72	36	55,103	36	135.153	1: 2.5
M.	1st	843	229	40,458	614	368,231	1: 9.1
	2nd	1143	332	44,552	811	237,465	1: 5.3
	3rd	1319	226	62,281	1093	294,375	1: 4.7
N.	1st	686	418	23,224	268	74,430	1: 3.2
	2nd	635	409	17,746	226	47,217	1: 2.7
	3rd	618	420	14,241	198	61,707	1: 4. 3
O.	1st	694	351	39,008	343	192,758	1: 4.9
	2nd	589	315	28,941	274	100,129	1: 3.5
P.	1st	439	259	22,787	180	77,571	1: 3.5
	2nd	848	542	15,958	306	53,958	1: 3.4
Q.	1st	463	196	37,064	267	103,569	1: 2.8
	2nd	520	221	24,802	299	123,315	1: 5.0
R.	1st	457	200	47,998	257	155,352	1: 3.2
	2nd	478	238	32,805	240	111,103	1: 3.4
TOTAL		21,857	10,458		11,399		

The Average Bacterial Content of all the Coliform Negative Samples is - 25.294, and that of all the Coliform Positive Samples is - 160,577. The Ratio of the former to the latter is - 1: 6.3.

TABLE 46.

TYPES of COLIFORM BACTERIA found in MILK DURING the WINTER PERIOD (when the COWS were not at Pasture) and DURING the SUMMER PERIOD (when the Cows were at Pasture).

GENERAL CHARACTERS:- Small Gram - Negative, Non-Sporing Bacilli: Fermenting Glucose and Lactose with formation of acid and Gas: Producing coagulation and permanent acidity of Milk within Seven days at 37°C: as a rule not liquefying gelatin but occasionally liquefying it slowly.

Type of Bacterium	Distinguishing Characters									Additional Characters			Number of Cultures Isolated						Number of Cultures with Exceptional Characters (G. = Gelatin; R. = Raffinose; K = Koser; M.R. = Methyl-red; n. = neutral Reaction.)
	Saccharose	Dulcitol	Adonitol	Inulin	Mannitol	Indole	Voges-Proskauer Reaction.	Gelatin	Motility	Raffinose	Koser	Methyl-Red Reaction	WINTER PERIOD		SUMMER PERIOD		COMBINED PERIODS		
													Total	per cent	Total	per cent	Total	per cent	
1. B. "MacConkey's No.71"	+	+	-	-	+	+	-	-	+	+	-	+	127	35.4	66	15.1	193	24.2	
2. B. "MacConkey's No.34"	-	+	-	-	+	+	-	-	+	-	-	+	55	15.3	40	9.1	95	11.9	R + (3); K + (1).
3. B. "MacConkey's No.108"	+	-	-	-	+	-	+	+	+	+	+	-	32	8.9	39	8.9	71	8.9	G - (11): G-K-(3): G - M.R.n.(2); K-(2).
4. B. "MacConkey's No.103"	+	-	+	-	+	-	+	-	-	+	+	-	10	2.8	51	11.6	61	7.7	M.R.+(4); M.R.n.(5).
5. B. "MacConkey's No. 1"	-	-	+	-	+	+	-	-	+	-	-	+	28	7.8	17	3.9	45	5.6	R + (2); K + (1)
6. B. "MacConkey's No. 7"	-	-	-	-	+	-	-	-	+	-	+	+	17	4.7	26	5.9	43	5.4	K - (1).
7. B. "MacConkey's No. 4"	-	-	-	-	+	+	-	-	+	-	-	+	10	2.8	16	3.6	26	3.3	R + (1).
8. B. "MacConkey's No.106"	+	-	-	-	+	+	-	-	+	+	-	+	14	3.9	8	1.8	22	2.8	
9. B. "MacConkey's No.98"	+	-	+	+	+	-	+	-	-	+	+	-	2	.6	19	4.3	21	2.6	K - (2)
10. B. "MacConkey's No.73"	+	+	-	-	+	-	+	+	+	+	+	-	4	1.1	15	3.4	19	2.4	G-(7); G-K-(1); G-M.R.n.(2); M.R.+(1); M.R.n.(2).
11. B. "MacConkey's No.65"	+	+	+	+	+	+	+	+	-	+	+	-	3	.8	14	3.2	17	2.1	G - (1).
12. B. "MacConkey's No. 5"	-	-	-	-	+	+	-	-	-	-	-	+	5	1.4	10	2.3	15	1.9	
13. B. "MacConkey's No.67"	+	+	+	-	+	-	+	-	-	+	+	-	1	.3	14	3.2	15	1.9	K-(1); M.R.n.(3).
14. B. "MacConkey's No.74"	+	+	-	-	+	-	-	-	+	+	+	+	4	1.1	9	2.1	13	1.6	K-(4); M.R. -(1).
15. B. "MacConkey's No.36"	-	+	-	-	+	-	-	+	+	-	+	+	4	1.1	8	1.8	12	1.5	G - (9).
16. B. "MacConkey's No.101"	+	-	+	-	+	+	-	-	-	+	+	+	5	1.4	7	1.6	12	1.5	K - (2).
17. B. "MacConkey's No.35"	-	+	-	-	+	+	-	-	-	-	-	+	6	1.7	5	1.1	11	1.4	
18. B. "MacConkey's Mal09"	+	-	-	-	+	-	-	-	+	+	+	+	4	1.1	7	1.6	11	1.4	K-(1); K-M.R.n.(1).
19. B. "MacConkey's Mal02"	+	-	+	-	+	-	+	+	+	+	+	-	3	3.8	7	1.6	10	1.3	G-(2);
20. B. "MacConkey's No.68"	+	+	+	-	+	-	-	-	-	+	+	+	2	.6	6	1.4	8	1.0	
21. B. "MacConkey's No.72"	+	+	-	-	+	+	-	-	-	+	-	+	4	1.1	4	.9	8	1.0	
22. B. "MacConkey's No104"	+	-	+	-	+	-	-	-	-	+	+	-	0		6	1.4	6	.8	
23. Anomalous Type "A"...	+	+	+	-		+	-	-	+	+	-	+	5	1.4	1	.2	6	.8	
24. B. "MacConkey's No. 2"	-	-	+	-	+	+	-	-	-	-	-	+	0		5	1.1	5	.6	
25. Anomalous Type "H"...	+	-	-	-		+	+	+	+	+	-	-	0		5	1.1	5	.6	G - (2); R-G-(1); K+M.R.+(1)

TABLE 46. (Contd)

Type of Bacterium	Distinguishing Characters									Additional Characters			Number of Cultures Isolated						Cultures with Exceptional Characters.
	Saccharose	Dulcitol	Adonitol	Inulin	Mannitol	Indole	Voges-Proskauer Reaction.	Gelatin	Motility	Raffinose	Koser	Methyl-red Reaction	WINTER PERIOD Total	WINTER PERIOD Per cent	SUMMER PERIOD Total	SUMMER PERIOD Per cent	COMBINED PERIODS Total	COMBINED PERIODS per cent	
26. B. "MacConkey's No. 99"	+	-	+	+	+	-	-	-	-	+	+	+	0		4	.9	4	.5	M.R.n.(2).
27. B. "MacConkey's No.105"	+	-	-	+	+	-	+	+	+	+	+	-	3	.8	1	.2	4	.5	G-(3); M.R.+(1).
28. Anomalous Type "E"....	+	-	+	-		+	+	-	-	+	+*	-	1	.3	3	.7	4	.5	K-(1).
29. B. "MacConkey's No. 69"	+	+	-	+	+	-	+	+	+	+	+	-	3	.8	0		3	.4	G-(1).
30. B. "MacConkey's No. 75"	+	+	-	-	+	-	+	-	-	+	+	-	0		3	.7	3	.4	K-(1).
31. B. "MacConkey's No.100"	+	-	+	-	+	+	-	-	+	+	-	+	1	.3	2	.5	3	.4	K+(1).
32. B. "MacConkey's No.107"	+	-	-	-	+	+	-	-	-	+	-	+	0		3	.7	3	.4	
33. Anomalous Type "B"....	+	-	-	+		+	+	-	+	+	-	-	3	.8	0		3	.4	
34. B. "MacConkey's No. 6"	-	-	-	-	+	+	+	-	-	-	-	-	0		2	.5	2	.3	
35. Anomalous Type "C"....	+	-	+	-		+	+	+	+	+	-	-	1	.3	1	.2	2	.3	G-(1).
36. Anomalous Type "L"....	+	-	-	-		+	-	+	+	+	-	+	0		2	.5	2	.3	
37. Anomalous Type "Q"....	+	-	-	-		-	-	+	+	+	+	+	0		2	.5	2	.3	
38. Anomalous Type "R"....	+	-	+	-		-	-	+	+	+	+	+	0		2	.5	2	.3	M.R.-(1).
39. B. "MacConkey's No. 33"	-	+	+	-	+	+	-	-	+	-	-	+	1	.3	0		1	.1	
40. B. "MacConkey's No. 66"	+	+	+	-	+	+	-	-	-	+	+*	+	0		1	.2	1	.1	
41. B. "MacConkey's No. 97"	+	-	+	+	+	+	+	+	-	+	+*	-	0		1	.2	1	.1	G-(1).
42. Anomalous Type "D"....	-	-	-	-		+	+	-	+	-	-	-	1	.3	0		1	.1	
43. Anomalous Type "J"....	+	-	-	-		+	+	-	-	+	-	-	0		1	.2	1	.1	
44. Anomalous Type "K"....	+	+	-	-		+	+	-	+	+	-	-	0		1	.2	1	.1	
45. Anomalous Type "M"....	+	-	+	+		-	+	+	+	+	+	-	0		1	.2	1	.1	
46. Anomalous Type "N"....	+	-	-	-		-	+	-	-	+	+	-	0		1	.2	1	.1	
47. Anomalous Type "P"....	-	-	-	-		+	-	+	+	-	-	+	0		1	.2	1	.1	
48. Anomalous Type "X"....	+	+	+	+		+	+	+	+	+	+*	-	0		1	.2	1	.1	
49. B."MacConkey's No.3"...	-	-	-	+	+	-	+	+	+	-	+	-	0		0		0		
50. B."MacConkey's No.8"..	-	-	-	-	+	-	-	-	-	-	+	+	0		0		0		
51. B."MacConkey's No.70".	+	+	-	+	+	-	-	+	+	+	+	+	0		0		0		

\+ in Saccharose, Dulcitol, Adonitol, Inulin, Mannitol, Raffinose or Salicin Column = Acid & Gas produced.
\- in " " " " " " " " = Acid & Gas not produced.

\+ in Indole, Voges-Proskauer, Koser or Methyl-red Column = presence of reaction.
\- in " " " " " = absence of reaction.

\+ in Gelatin column = gelatin liquefied.
\- in " " = gelatin not liquefied.

\+ in Motility column = presence of motility.
\- in " " = absence of motility.

* Direct correlation between Indole and Koser reactions.

TABLE 47.

The Fermentation of Salicin by Coliform Bacteria Isolated from Milk during the Winter and Summer periods.

Type	Total Number of Cultures	Acid and Gas produced in Salicin Number of Cultures	Acid and Gas Not produced in Salicin Number of Cultures.
B "MacConkey's No. 1"	45	42	3
B "MacConkey's No. 2"	5	5	-
B "MacConkey's No. 4"	26	13	13
B "MacConkey's No. 5"	15	3	12
B "MacConkey's No. 6"	2	1	1
B "MacConkey's No. 7"	43	30	13
B "MacConkey's No. 33"	1	1	-
B "MacConkey's No. 34" (B coli communis)	95	69	26
B "MacConkey's No. 35"	11	7	4
B "MacConkey's No. 36"	12	11	1
B "MacConkey's No. 65"	17	17	-
B "MacConkey's No. 66"	1	1	-
B "MacConkey's No.67"	15	15	-
B "MacConkey's No. 68"	8	8	-
B "MacConkey's No. 69"	3	3	-
B "MacConkey's No. 71" (B. communior)	193	181	12
B "MacConkey's No. 72"	8	8	-
B "MacConkey's No. 73"	19	19	-
B "MacConkey's No. 74"	13	11	2
B "MacConkey's No. 75"	3	3	-
B "MacConkey's No. 97"	1	1	-
B "MacConkey's No. 98"	21	21	-

TABLE 47 (Contd)

Type	Total Number of Cultures	Acid and Gas produced in Salicin Number of Cultures	Acid and Gas not produced in Salicin Number of Cultures
B "MacConkey's No. 99"	4	4	-
B "MacConkey's No.100"	3	3	-
B "MacConkey's No.101"	12	12	-
B "MacConkey's No.102"	10	10	-
B "MacConkey's No.103" (B.lactis aerogenes)	61	61	-
B "MacConkey's No.104"	6	6	-
B "MacConkey's No.105"	4	3	1
B "MacConkey's No.106"	22	16	6
B "MacConkey's No.107"	3	2	1
B "MacConkey's No.108" (B.cloacae)	71	64	7
B "MacConkey's No.109"	11	11	-
Anomalous Type "A"	6	6	-
Anomalous Type "B"	3	1	2
Anomalous Type "C"	2	2	-
Anomalous Type "D"	1	1	-
Anomalous Type "E"	4	4	-
Anomalous Type "H"	5	4	1
Anomalous Type "J"	1	1	-
Anomalous Type "K"	1	1	-
Anomalous Type "L"	2	-	-
Anomalous Type "M"	1	1	-
Anomalous Type "N"	1	1	-
Anomalous Type "P"	1	1	-
Anomalous Type "Q"	2	2	-
Anomalous Type "R"	2	2	-
Anomalous Type "X"	1	1	-

Of 487 indole positive Cultures, 404, i.e., 83.0 per cent, are salicin positive.

Of 247 Voges-Proskauer positive Cultures, 235, i.e., 95.1 per cent are salicin positive.

T A B L E 48.

Cultures which did not give an inverse correlation between the Voges-Proskauer and the Methyl-Red Reactions.

Type	Indole Reaction	Voges-Proskauer Reaction	Total Number of Cultures Isolated	NUMBER OF EXCEPTIONAL CULTURES: Direct Correlation between Voges-Proskauer and Methyl Red-Reactions	NUMBER OF EXCEPTIONAL CULTURES: Neutral Methyl-red Reaction
B "MacConkey's No. 67"	-	+	15		3
B "MacConkey's No. 73"	-	+	19	1	4
B "MacConkey's No. 74"	-	-	13	1	
B "MacConkey's No. 99"	-	-	4		2
B "MacConkey's No.103"	-	+	61	4	5
B "MacConkey's No.105"	-	+	4	1	
B "MacConkey's No.108"	-	+	71		2
B "MacConkey's No.109"	-	-	11		1
Anomalous Type "H"	+	+	5	1	
Anomalous Type "R"	-	-	2	1	

TABLE 49.

Cultures which failed to give a Direct Correlation between the Voges-Proskauer and Koser Reactions

Type	Indole Reaction	Voges-Proskauer Reaction	Total Number of Cultures Isolated	Number of Cultures which failed to give a Direct Correlation
B "MacConkey's No.1"	+	-	45	1
B "MacConkey's No.6"	+	+	2	2
B "MacConkey's No.7"	-	-	43	42
B "MacConkey's No.34"	+	-	95	1
B "MacConkey's No.36"	-	-	12	12
B "MacConkey's No.66"	+	-	1	1
B "MacConkey's No.67"	-	+	15	1
B "MacConkey's No.68"	-	-	8	8
B "MacConkey's No.73"	-	+	19	1
B "MacConkey's No.74"	-	-	13	9
B "MacConkey's No.75"	-	+	3	1
B "MacConkey's No.98"	-	+	21	2
B "MacConkey's No.99"	-	-	4	4
B "MacConkey's No.100"	+	-	3	1
B "MacConkey's No.101"	+	-	12	10
B "MacConkey's No.104"	-	-	6	6
B "MacConkey's No.108"	-	+	71	5
B "MacConkey's No.109"	-	-	11	9
Anomalous Type "B"	+	+	3	3
Anomalous Type "C"	+	+	2	2
Anomalous Type "D"	+	+	1	1
Anomalous Type "E"	+	+	4	1
Anomalous Type "H"	+	+	5	4
Anomalous Type "J"	+	+	1	1
Anomalous Type "K"	+	+	1	1
Anomalous Type "Q"	-	-	2	2
Anomalous Type "R"	-	-	2	2

TABLE 50.

Cultures which failed to give an Inverse Correlation between the Indole and Koser Reactions

Type	Indole Reaction	Voges-Proskauer Reaction	Total Number of Cultures Isolated	Number of Cultures which failed to give inverse Correlation.
B "MacConkey's No. 1"	+	-	45	1
B "MacConkey's No. 7"	-	-	43	1
B "MacConkey's No.34"	+	-	95	1
B "MacConkey's No.65"	+	+	17	17
B "MacConkey's No.66"	+	-	1	1
B "MacConkey's No.67"	-	+	15	1
B "MacConkey's No.73"	-	+	19	1
B "MacConkey's No.74"	-	-	13	4
B "MacConkey's No.75"	-	+	3	1
B "MacConkey's No.97"	+	+	1	1
B "MacConkey's No.98"	-	+	21	2
B "MacConkey's No.100"	+	-	3	1
B "MacConkey's No.101"	+	-	12	10
B "MacConkey's No.108"	-	+	71	5
B "MacConkey's No.109"	-	-	11	2
Anomalous Type "E"	+	+	4	3
Anomalous Type "H"	+	+	5	1
Anomalous Type "X"	+	+	1	1

TABLE 51.

The Numbers and Percentages of Indole Positive Cultures, Voges-Proskauer Negative Cultures and Koser Negative Cultures isolated from Samples of Milk during the Winter and Summer periods and during the Combined Periods.

	WINTER PERIOD		SUMMER PERIOD		COMBINED PERIODS	
Reaction	Number of Cultures	Percentage	Number of Cultures	Percentage	Number of Cultures	Percentage
Indole Positive	270	75.2	217	49.5	487	61.1
Voges-Proskauer Negative	292	81.3	258	58.9	550	69.0
Koser Negative	263	73.3	205	46.8	468	58.7

REFERENCES

REFERENCES

1.+ Koch, Robert. Zur Untersuchung von pathogenen Organismen. Mittheill Kaiserl Gesundheitsanite, 1, 1-48, 1881.

2. Conn, H.W. Standards for determining the purity of milk. The limit of error in bacteriological milk analyses. U.S. Pub.Health Service. Pub.Health Reports, 30. 1915, 2349-2395.

3. Sherman, J.M. The advantage of carbohydrate medium in routine bacteriological examination of milk. J.Bact. 1, 1916, 481-488.

4.+ Slack, Francis, H. Some observations on the bacterial examination of milk. Am. J. Pub. Health. 7,1917, 690-697.

5. Sears, H.J. and Case Lulu L. Some observations on the efficiency of the present standard agar for the estimation of bacteria in milk. J.Bact. 3, 1918, 531-536.

6. Simmons, J.E. A comparison with the standard plate method, of some rapid methods for bacteriologic analyses of milk. J.Infect. Dis., 24,1919, 324-336.

7.+ Supplee, G.C. and Flanigan G.E. Bacterial counts of milk as obtained by various media. Internat. Assoc. Dairy and Milk Inspectors 11th Ann. Report. 1922, 251-259.

8. Zoller, H.F. The value of milk powder agar in the bacteriological laboratory. Am.J.Pub.Health, 13, 1923, 384-387.

9. Frobisher, Martin A. A comparative study of media for the quantitative estimation of bacteria in milk. Am. J.Pub. Health, 13, 1923. 474-478.

10. Ayers, S.H. and Mudge C.S. Milk powder agar for the determination of bacteria in milk. J.Bact. 5,1920. 565-588.

11.+ Bolling, Geo. E. Report of Committee on Methods of Bacterial Analysis of Milk and Milk Products. Internat.Assoc. Dairy and Milk Inspectors 10th Annual Report, 1921, 260-263. See also 11th Annual Report 1922; 12th Annual Report 1923.

12. Norton, John F. and Seymour Margaret. Study of media for the estimation of bacteria in milk. Am.J. Pub. Health, 16, 1926. 35-39.

13. Robertson, A.H. The relation between bacterial counts from milk as obtained by microscopic and plate methods. N.Y. Agr. Exp, Sta. Tech. Bull. 86, 1921.

14. Amer. Pub. Health Assoc.(1928). Standard Methods of Milk Analysis. 5th Edition.

15. Brew, J.D. and Dotterrer, W.D. The number of bacteria in milk. N.Y. Agr. Exp. Sta. Bull. 439. 1917.

16. Breed, R.S. and Stocking, W.A. The accuracy of bacterial counts from milk samples. N.Y. Agr. Exp. Sta. Tech. Bull. 75, 1920.

17. Mattick, A.T.R. Methods of determining the numbers of bacteria in milk. Jour. of Dairy Research, Vol.1. No.2., 1930.

18. Breed, R.S. and Dotterrer, W.D. The number of colonies allowable on satisfactory agar plates. N.Y. Agr.Exp. Sta. Tech. Bull. 53, 1916.

19.+ Kelbourne, Chas. H. Varying Bacteriological Results obtained by different Laboratories. Creamery and Milk Plant Monthly, Chicago. 1918, 7, Oct. pp.54-56; Nov. pp.40-44.

20. Albus, W.R. Some Observations on the Plate Count Method of Enumerating Bacteria in Milk. Jour.Bacteriology. 16, 269.

21. Schacht, F.L. and Robertson, A.H. Laboratory Accuracy of Counting an Agar Plate. Amer.Jour.Pub.Health. Vol.22. No.1. Jan.1932.

22. Ministry of Agriculture (1929) Bacteriological Tests for Graded Milk. Memo. 139/Foods. H.M.Stationery Office, London.

23.+ Prescott, S.C. and Parker, M.E. 1927. Use of blood agar in control of milk supplies. 16th Ann. Rept. Int. Assoc. Dairy and Milk Inspectors.

24. Yule, G.M. "An Introduction to the Theory of Statistics", London 1919.

25. Mahalanobis, P.C. (1925). Agricultural Journal of India. Vol.20. p.100.

26.+ Pearson, Karl (1896) "Regression Heredity and Panmixia". Phil.Trans.Royal Soc. Series A.Vol.CLXXXVII. p.253.

27. MacConkey, A. Lactose-fermenting bacteria in faeces. J.Hyg., 1905, 5, 333-379.

28. MacConkey, A. Bacteriology of milk. J.Hyg., 1906. 6.

29. MacConkey, A. 1909. Further observations on the differentiation of lactose-fermenting bacilli with special reference to those of intestinal origin. J.Hyg., 1909. 9. 86-103.

30. Clemesha, W.W. The Bacteriology of Surface Waters in the Tropics. J.Hyg. 1912. 12, 463-478.

31. Browne, W.W. The production of acid by the Bacillus coli group. J.Inf. Dis., 1914. 15, 580-604.

32. Rogers, L.A., Clark, W.M., and Evans, A.C. The characteristics of bacteria of the colon type found in bovine faeces. J.Inf. Dis., 1914. 15. 100-123.

33. Rogers, L.A., Clark, W.M., and Davies, B.J. The colon group of bacteria. J.Inf. Dis., 1914. 14, 411-475.

34. Browne, W.W. Predominance among the members of the Bacillus coli group in artificially stored waters. J. Inf. Dis., 1915. 17. 72

35. Rogers, L.A., Clark, W.M., and Evans, A.C. The characteristics of bacteria of the colon type occurring on grains. J. Inf. Dis., 1915. 17. 137-159.

36. Greenfield, Myrtle. The presence of soil and faecal strains of organisms of the colon-aerogenes group in waters of Kansas, J.Inf. Dis., 1916. 19. 647-651.

37. Hulton, F. The correlation of certain reactions of colon bacilli and like organisms with source. J. Inf. Dis., 1916. 19. 606.

38. Johnson, B.R. Coli-like organisms of the soil. J.Bact. 1916. 1. 96.

39. Levine, Max. Acid production and other characters of Bacillus coli-like bacteria from faeces and sewage. J. Inf. Dis. 1916. 19. 773-805.

40. Levine, M. On the correlation of the Voges-Proskauer and the methyl-red Reactions. J.Bact.1916. 1. 87.

41. Levine, M. On the significance of the Voges-Proskauer reaction. J.Bact. 1916, 1. 153-164.

42. Levine, M. Preliminary note on the classification of some Lactose fermenting Bacteria. J.Bact.1916. 1. 619-621.

43. Levine, M. The correlation of the Voges-Proskauer and the methyl-red reactions in the colon-aerogenes group of bacteria. J., Inf. Dis., 1916. 18. 358-367.

44. Murray, T.J. A comparative study of colon bacilli isolated from horse, cow and man. J., Inf. Dis., 1916. 19. 161-174.

45. Rogers, L.A., Clark, W.M. and Evans, A.C. Colon Bacteria and Streptococci and their significance in milk. Amer. J. Pub. Health, 1916. 6. 374-380.

46. Rogers, L.A. The Type of Colon Bacillus occurring in surface waters. J. Bact. 1916. 1. 82.

47. Burton, L.V. and Rettger, L.F. Correlations in the colon-aerogenes group. J.Inf. Dis., 1917. 21. 162-195.

48. Hunter, O.W. The colon-aerogenes group from silage. J. Bact. 1917. 2. 635.

49. Johnson, B.R. Levine, M. Characteristics of coli-like micro-organisms from the soil. J.Bact. 1917, 2. 379-401.

50. Levine, M. A statistical classification of the colon-cloacae group. J.Bact.1918. 3. 253-276.

51. Rogers, L.A. Clark, W.M., Lubs., H.A. The characteristics of bacteria of the colon-type occurring in human faeces. J. Bact. 1918. 3, 231-252.

52. Winslow, C. E.A., and Cohen, B. The distribution of Bacillus coli and Bacillus aerogenes in polluted and unpolluted waters. J., Inf. Dis., 1918, 23. 90-101.

53. Wood, D.R. Recent advances in the differentiation of lactose-fermenting (gas producing) bacteria with special reference to the examination of water and food products. J. Hyg., 1918. 18. 46.

54. Winslow, C.-E.A. Kligler, I.J. and Rothberg W. Studies on the classification of the colon-typhoid group of bacteria with special reference to their fermentation reactions. J. Bact. 1919. 4. 429-503.

55. Chen., C.C. and Rettger, Leo F. A correlation study of the colon-aerogenes group of bacteria with special reference to the organisms occurring in the soil. J.Bact., 1920. 5. 253-298.

56. Perry, M.C., and Monfort, W.F. Some atypical colon-aerogenes forms isolated from natural waters. J.Bact. 1921, 6. 53-68.

57. Redman, T. The classification of some lactose fermenting organisms isolated from cheeses, waters and milk. J.Bact. and Path. 1922. 25. 63-76.

58. Koser, Stewart A. Correlation of citrate utilisation by members of the colon-aerogenes group with other differential characteristics and with habitat. J. Bact. 1924. 9. 59-77.

59. Bardsley, Doris A. B.coli an Index of Faecal Pollution of Water Supplies. J. Hyg. 1926, 25. 11-25.

60. Ruchhoft, C.C., Kallas, J.G. Chinn Ben and Coulter E.W. Coli-aerogenes Differentiation in Water Analysis. Part I. J.Bact. 1931. 21. 407-440.

61.+ Kligler, I.J. Studies on the classification of the Colon group. J.Inf. Dis., 1914. 15. 187-204.

62. Thomas and Sandman. Studies on the classification of the colon-typhoid group. Amer. J.Pub. Health, 1916. 6. 579-584.

63. Ruchhoft, C.C., Kallas, J.G., Chinn Ben and Coulter E.W. Coli-aerogenes Differentiation in Water Analysis. Part II. J.Bact. 1931. 22. 125-181.

64.+ Levine, M. Bacteria fermenting lactose and their significance in water analysis. 1921. Bull.62, Eng. Exp. Station, Ames, Iowa.

65. Koser, Stewart A., Utilisation of the Salts of organic acids by the colon-aerogenes group. J.Bact. 1923. 8. 493-520.

66.+ Koser, S.A. 1926. Jour. Inf. Dis., 38, 506-10.

67.+ Raghavachari, T.N.S. 1926. Ind. Jour. Med. Res. 14, 47.

68.+ Lewis, I.M., and Pittman, E.E. 1928. Jour.Amer.Water Works Assoc. 19. 78-92.

+ Not read in original.