RELATIONS BETWEEN PLATE COUNTS AND DIRECT MICROSCOPIC COUNTS OF ESCHERICHIA COLI DURING THE LOGARITHMIC GROWTH PERIOD[1]

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INTRODUCTION

The relation between the plate count and the direct microscopic count of bacteria is of practical as well as theoretical importance. Particularly during the logarithmic growth period, where the constant rates are used for comparative purposes, it is desirable to know whether the number of cells as determined by plating always bear a constant relation to those enumerated microscopically, that is, whether the plate count is a reliable, although relative, measure of the total number of cells present.

That the plate count is usually lower than the total count was noted years ago (Winterberg, 1898; Klein, 1900; Hehewerth, 1901), and this discrepancy has been the subject of a relatively few papers since that time. In general, are the differences between such counts, even during the logarithmic growth period with pure cultures, due entirely to clumping, or to the death of a certain proportion of bacteria, or to a combination of these factors? The more recent studies have brought various facts to light in respect to some of the relationships and have offered explanations in certain cases, without having clearly answered these questions. It is recognized, for example, that in old cultures many cells are not viable, but it is uncertain whether or not this is the case during the period of logarithmic reproduction in a suitable medium.

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Some confusion exists in the literature in regard to the use of the term "total count." Some authors use it literally as meaning that each and every individual cell is counted as one, which is the terminology employed in this paper, while others use it to include both single cells and clumps of bacteria which theoretically give rise to one colony upon plating. In some cases the terms "direct count" and "microscopic count" have been used without further specification. Moreover, there have been apparently unsupported statements that in rapidly-reproducing cultures there is no clumping, and data have sometimes been compared on this assumption. That there is actually a good deal of clumping in young cultures, even with the reputedly "individualistic" organism *Escherichia coli*, has been shown definitely by Glynn, Powell, Rees and Cox (1913), Knaysi (1935), Ziegler and Halvorson (1935), and by our own observations. The clumps, which are not easily broken up by shaking, are most commonly of two organisms, and probably result from incomplete separation after fission. These clumps, alone, if not considered, are sufficient to cause marked discrepancies between plate and total count data. Unless all clumps can be completely broken up, the only fair basis for comparison of the relation between the plate and direct counts—at least so far as viability is concerned—is to use for the direct enumeration a microscopic "group count," in which each clump of two or more cells, as well as single organisms, is counted as one unit.

The explanations of the discrepancies noted by various investigators between plate and microscopic counts may be considered to fall into three classes: (1) counting errors (due to chance) involved in the two methods; (2) clumping; (3) variations in resistance of cells, in which case some cells die either in culture, during the process of plating, or on an unsatisfactory plating medium. A uniform technique, to minimize errors other than those due to chance, is assumed. As regards counting errors, the reliability of results in a given case can be determined statistically, although such data have usually been lacking in published reports. Under the proper conditions the accuracy of bacterial counts obtained either from plates or by direct microscopic enu-

meration, particularly with a Helber type counting chamber, is about the same (Wilson, 1922; Wilson and Kullmann, 1931), and this may be assumed here for purposes of discussion. In respect to clumping, it must be assumed that the number of clumps when the bacterial suspension is examined microscopically is the same as when plated, taking into account the dilution factor.

Various aspects of the effect on the plate count of the diluent used in plating have been discussed by Wilson (1922), Cohen (1922), Falk (1923), Winslow and Falk (1918, 1923), Shaughnessy and Criswell (1925), Winslow and Brooke (1927), and Butterfield (1932). It is clear that there are marked differences in viability between various species exposed to different diluents, often during a period of only a few minutes.

Brew (1914), using the microscopic method of Breed (1911), with milk of various ages, found the microscopic (individual) cell count to be ten or more times greater than the plate count, due largely to clumping. Microscopic group counts were about twice the plate counts, which he considers as probably due to the fact that the agar was not optimum for the development of all types of milk bacteria. Conn (1915), and Baker, Brew and Conn (1920), using the Breed method, showed a reasonably good agreement between the group count and the plate count in milk.

Glynn, Powell, Rees and Cox (1913), with a Helber cell for direct counts, found the group count of staphylococci and *E. coli* somewhat larger than the plate count, the difference becoming progressively greater with increasing age of culture, presumably as a result of more organisms becoming moribund. During the logarithmic growth phase, at optimum temperature, the plate count was from 5 to 30 per cent less than the group count.

Reichenbach (1911), in order to explain the logarithmic order of death, assumed that a certain proportion of the multiplying bacteria of each new generation ceased reproduction and became dormant. Wilson (1922, 1926) determined with pure cultures of *Salmonella suipestifer* and *Salmonella aertrycke*, that the viable count averaged 80 per cent of the total count (with a Helber cell) during the logarithmic period at optimum temperature, although in a few instances results were identical. His explana-

tion of the discrepancy also is that some young cells are actually dying out *in the original culture.*

That some bacteria may be killed during plating, may be inferred from the work of Sherman and Albus (1923, 1924), in which young cultures showed less resistance to temperature and osmotic pressure; young cells are also more easily killed by abrupt environmental changes (Sherman and Cameron, 1934). On the other hand, Barber (1908) found that, with proper care in transferring, single cells of *E. coli* from young cultures were nearly 100 per cent viable. The work of Chesney (1916) on the growth of pneumococcus tends to show that these cells do not die during the logarithmic phase either in culture or upon transplanting, if a favorable environment is present.

Jensen (1928) determined the plate count to be lower than the total count, even during the logarithmic phase. Using Ørskov's (1922) method of direct observation of cells on agar blocks, he followed the growth of cells of *E. coli* from an 18-hour broth culture, and found the original cells and their progeny to have 100 per cent germination until about the end of the logarithmic growth period, after which time there was a decreased reproductivity due to the production of "shadow forms." If cells were transferred during the phase of maximum rate of reproduction to a new medium, some were killed, indicating susceptibility to changes in environment. Kelly and Rahn (1932) by direct observation on agar blocks of single cells of *Aerobacter aerogenes*, *Bacillus cereus* and *Saccharomyces ellipsoideus* from young cultures, found practically 100 per cent germination, infant mortality being nil.

Anderson, Fred and Peterson (1920), in comparing the direct group count with plate counts of *Lactobacillus pentoaceticus*, found the two essentially the same during the logarithmic phase. With increasing age, the plate count became progressively lower than the group count. This progressively greater difference with increased age of culture was also shown for *E. coli* by Henrici (1928), and for *Pseudomonas aeruginosa* by Régnier, David and Kaplan (1932). Ziegler and Halvorson (1935), using for the direct

count the smear method of Henrici (1923, 1928), compared the plate count of *E. coli* with the total count after shaking thoroughly to break up clumps, and concluded that the two are practically identical before the death phase.

The reliability of staining methods used to differentiate between dead and living bacteria has been seriously questioned by Bickert (1930) and others. The data on viability, obtained by tinctorial reactions, are contradictory, but for the sake of completeness, a few recent papers may be mentioned. Gay and Clark (1934), using the Proca-Kayser technique to stain living and dead bacteria, differentially, studied the viability of several forms, and found that even in cultures five hours old there were frequently many cells which showed the color typical of dead organisms. On the other hand, Henrici (1928, Ch. 9), found that cells of the colon bacillus did not stain with Congo red until more than twenty-four hours old. Knaysi (1935) criticizes the Proca-Kayser technique, and recommends neutral red for the same purpose. Comparing the group count of viable cells, as shown by his technique, with the plate count of *E. coli*, he finds that during the logarithmic growth phase there is very close agreement between the two; furthermore, as shown by staining, there are not more than one or two per cent of dead cells present during this period, the percentage increasing with age (personal communication).

PURPOSE

The present study was undertaken to determine the relations between the plate count, the group count, and the total count of *Escherichia coli* during the period of maximum rate of reproduction (logarithmic growth phase), under the best practical conditions. A statistical analysis of the data is included, to indicate the reliability of the results. The organism used was chosen partly because the cells show less tendency than in many species to remain attached together after fission, and partly because its resistance to slight changes in environment and its good growth on artificial media were desirable characteristics. For the purpose of determining whether the temperature of growth has any

influence on viability in culture, several incubation temperatures were employed. The effect on the plate count of the dilution fluid was first investigated.

EFFECT OF THE DILUENT ON THE PLATE COUNT

Preliminary experiments were made on the viability in various dilution fluids of the strain of *E. coli* used, in order to rule out as far as possible the death of cells during plating. Distilled water (pH 6.4) at 22°C. was found to be reasonably satisfactory for this organism, as follows.

Shaken (1 minute), pooled, duplicate broth cultures of known age were serially diluted to a predetermined number of cells (200 to 300 per ml.) convenient for plating and counting. The dilution

TABLE 1

Effect of time of exposure on survival of Escherichia coli in distilled water at 22°C., as determined by plate count

AGE OF CULTURE	TIME OF EXPOSURE, MINUTES					
	0 (5)	10	15	20	25	30
	Per cent survival					
hours						
3	100	99	98	97	97	98
6	100	95	96	94	94	89

was made and plated within five minutes, and the mean count of triplicate plates taken as the initial (0(5) minute) reading. This same dilution was allowed to stand at 22°C., and plated (within three minutes) after various periods of time up to thirty minutes, the same pipette being used at each time interval. Pyrex test tubes and pipettes were employed throughout. Tubes of broth, dilution water, and pipettes had an error of ± 1.0 per cent by volume. Typical data are given in table 1.

This table shows the survival in distilled water at 22°C., for various periods of time, of three-hour and six-hour cultures of *E. coli*. Here the initial plating is taken as 100 per cent. Calculation of the standard deviation of these mean counts, and using plus or minus twice the standard deviation as the criterion of the prob-

able limits of the means, showed that the apparent decreases were within the experimental error. These decreases therefore are without statistical significance, but the data at least indicate a tendency, since in all of many similar experiments the later counts were invariably lower than the initial.

There is also a possible indication from this and other data not presented, that older (six-hour) cultures are apparently more susceptible to the diluent than younger (three-hour) cultures. It should be noted, however, that the three-hour culture was only diluted 1:1000 for plating, while the six-hour culture required a dilution of 1:100,000. It is generally recognized that young cultures are *more* susceptible to environmental changes than old cultures, and in our case the explanation of this apparent paradox may be that the "colloid protective substance" (Winslow and Brooke, 1927) of the original broth is more highly diluted in the old cultures and thus has less protective action over a period of time.

These experiments indicate that even under practical conditions the time of exposure to the diluent may be an important factor in plating, and should be kept low.

RELATIONS BETWEEN PLATE AND DIRECT COUNTS

Methods

A stock culture of *Escherichia coli* (C_1), which was reproducing at a maximum rate in broth at 22°C., was used throughout the work. Two series of plate and direct count data were obtained, the platings at the four temperatures in each series being done on the same day under the same conditions.

Series of culture tubes containing 9 ml. of nutrient broth were inoculated with the same known number of cells (about 2000 per milliliter) from a twelve-hour, 22°C. broth culture. The tubes were incubated at 22°, 27°, 32° and 37°C. At exactly two-hour intervals, two (new) tubes were removed at each temperature, stoppered with rubber stoppers and shaken by hand (without beads) for one minute, and the contents pooled before sampling. A portion was immediately preserved with formalin for the direct count, and another portion serially diluted in 9 ml. distilled water

blanks (pH 6.4) at 22°C., and plated within five to ten minutes, five plates being made from the proper dilution. Plates were incubated for 48 hours at 37°C. before counting, and the dilution giving 100 to 400 colonies per plate—within which range Wilson (1922) has shown the balance between errors of sampling and of over-crowding to be optimum—was used for enumeration. Most of the mean counts reported are averages of five plates, which number is adequate for precise work (Wilson and Kullmann, 1931).

Conditions of incubation, dilution, etc., were controlled so as to minimize experimental errors. Temperatures did not vary more than ± 0.15°C. from those specified, except that of the dilution water which had a variation of ± 0.5°C. All water blanks and tubes of broth contained 9.0 ml. ± 0.1 ml. of liquid when used. The same lot of Difco dehydrated broth and agar was used throughout, agar being poured at 42°C. The average variation in 1 ml. dilution pipettes was ± 1 per cent by volume.

For the direct counts, a Helber counting cell 0.02 mm. deep was used, the organisms being examined in a light field after staining by the addition of methylene blue to the formalinized suspension. For most sampling periods the organisms in 400 small squares of the chamber were counted; this was varied in a few cases when the organisms were too few or too numerous. The number of cells or groups enumerated in each sample varied, except in a few early counts of about 200, from 500 to 3600, which latter are adequate for a precise mean (Wilson and Kullmann, 1931). Two sets of direct counts were made at the same time on each sample—a *total count*, in which each individual cell, whether in a clump or not, was counted as one, and a *group count*, in which each single isolated cell and each clump of two or more cells was also counted as one. It must be assumed that the number of clumps were relatively the same in these suspensions as in the diluted portions which were plated.

Statistical treatment

With a standardized technique, under conditions giving small and constant experimental errors, the chief errors of the estima-

tion of bacterial populations are those due to chance, and the accuracy depends upon the number of cells or colonies counted (Fisher, Thornton and MacKenzie, 1922; Fisher, 1930; Wilson and Kullmann, 1931).

From the five replicate plate counts at each sampling interval, the standard deviation of the mean (standard error) was calculated by the usual statistical formula. These standard errors (coefficients of variation) varied from ± 0.5 to ± 8.3 per cent, and averaged ± 4.0 per cent of the means for the whole series.

The standard error of direct counts is given by $\pm \sqrt{m}$, where m is the number of cells or groups enumerated (Fisher, 1930, p. 57). The standard errors of the *total* counts were between ± 1.7 and ± 7.7 per cent of the counts, with an average value of ± 3.6 per cent. The *group* count error varied from ± 1.9 to ± 8.2 per cent, averaging ± 4.2 per cent of the counts. These direct count deviations are, on the average, of about the same order of magnitude as those of the plate counts, and the accuracy of counts by either method is therefore about the same.

Relations between different kinds of counts were expressed as percentage ratios. The standard error of each ratio was given by the square root of the sum of the squares of the standard errors of numerator and denominator, and plus or minus twice this standard error taken as the reasonably certain maximum variation of the ratio. The probable limits of the ratios, using this criterion, are shown in tables 2 and 3.

Series I

The bacterial counts, when plotted against time on semi-log paper, gave typical sigmoid growth curves (Jennison, 1935). Because of the fact that during the earliest part of the logarithmic growth period the density of cells was too low to make accurate direct counts, the data for comparison here include the latter half of this phase and one subsequent two-hour growth interval at each temperature in tables 2 and 3.

Table 2 gives the total counts, microscopic group (single cells and clumps) counts, and plate counts for the first series of experiments, together with an analysis in the form of ratios to bring

out the relations involved. The data are comparable, since the same number of cells was used for the original inoculation at each temperature, and the same lot of broth for all cultures.

As is to be expected, the plate count (actually a viable group count) is invariably lower than the total, as shown by the plate/total ratios. That these ratios are statistically significant is seen from their probable limits of variation, the ratios not reaching

TABLE 2

Total counts, group counts, and plate counts of Escherichia coli in broth at various temperatures, during approximate logarithmic growth period (Series I)

TEMPERATURE	AGE OF CULTURE	BACTERIA PER MILLILITER, THOUSANDS			RATIOS AND THEIR REASONABLY CERTAIN (TWICE STANDARD ERROR) LIMITS		
		Total count	Group count	Plate count	$\frac{\text{Plate}}{\text{Total}} \times 100$	$\frac{\text{Group}}{\text{Total}} \times 100$	$\frac{\text{Plate}}{\text{Group}} \times 100$
°C.	*hours*						
37°	4	3,880	2,980	2,350	61 (52–70)	77 (61–93)	79 (66–92)
	6	101,000	74,200	60,000	59 (57–61)	73 (69–77)	81 (76–86)
	8	166,000	127,000	126,000	76 (68–84)	77 (72–82)	99 (89–109)
32°	6	10,000	6,620	5,870	59 (50–68)	66 (53–79)	89 (78–100)
	8	88,300	72,000	72,000	82 (77–87)	82 (79–85)	100 (93–107)
	10	121,000	109,000	95,000	79 (66–92)	90 (85–95)	88 (74–102)
27°	8	5,220	4,630	3,500	67 (53–81)	89 (70–108)	76 (59–93)
	10	32,000	27,200	24,400	76 (68–84)	85 (75–95)	90 (80–100)
	12	107,000	95,600	90,000	84 (76–92)	89 (83–95)	94 (84–104)
	14	144,000	130,000	117,000	81 (76–86)	90 (85–95)	90 (84–96)
22°	14	9,000	6,770	6,900	77 (60–94)	75 (60–90)	102 (82–122)
	16	33,600	19,500	20,100	60 (53–67)	58 (50–66)	103 (89–117)
	18	57,700	36,400	43,000	75 (63–87)	63 (57–69)	118 (98–138)
	20	142,000	99,300	100,000	70 (64–76)	70 (66–74)	100 (92–108)
	22	171,000	132,000	129,000	75 (66–84)	77 (72–82)	98 (86–110)

100 per cent in any case. There is no evidence that temperature has any marked effect on the ratios. The plate counts as determined vary from 59 to 84 per cent of the total at various ages and temperatures, giving a mean of 72 per cent (with average probable limits, using the criterion of twice the standard error, of 63 to 81 per cent). Since the viable group count accounts for only 72 per cent of the organisms, the remaining 28 (19 to 37)

per cent of the bacteria must be *either members of clumps* (these clumps being already enumerated in the viable group counts), *or non-viable cells.* Now, the microscopic group count averages 77 (average limits, 68 to 86) per cent of the total, the remaining 23 (14 to 32) per cent of the bacteria being members of clumps enumerated in this count. Since there is no significant difference between the figure of 28 per cent (cells either in clumps or

TABLE 3

Total counts, group counts, and plate counts of Escherichia coli in broth at various temperatures, during approximate logarithmic growth period (Series II)

TEMPERATURE	AGE OF CULTURE	BACTERIA PER MILLILITER, THOUSANDS			RATIOS AND THEIR REASONABLY CERTAIN (TWICE STANDARD ERROR) LIMITS		
		Total count	Group count	Plate count	$\frac{\text{Plate}}{\text{Total}} \times 100$	$\frac{\text{Group}}{\text{Total}} \times 100$	$\frac{\text{Plate}}{\text{Group}} \times 100$
°C.	*hours*						
37°	6	16,200	7,300	7,100	44 (39–49)	45 (37–53)	97 (83–111)
	8	146,000	90,000	90,000	62 (57–67)	62 (57–67)	100 (90–110)
	10	193,000	134,000	134,000	70 (65–75)	70 (65–75)	100 (92–108)
32°	8	15,500	11,300	8,500	55 (48–62)	73 (60–86)	75 (64–86)
	10	145,000	77,000	73,000	50 (47–53)	53 (48–58)	95 (91–99)
	12	171,000	148,000	146,000	85 (78–92)	87 (81–93)	99 (90–108)
27°	10	4,600	2,600	2,200	48 (42–54)	57 (45–69)	85 (71–99)
	12	28,600	15,500	16,000	56 (48–64)	54 (46–62)	103 (86–120)
	14	104,000	73,700	75,000	72 (62–82)	71 (67–75)	102 (88–116)
	16	172,000	116,000	119,000	69 (62–76)	67 (62–72)	103 (97–109)
22°	14	43,500	31,400	10,100	*23 (21–25)	72 (64–80)	*32 (29–35)
	16	59,000	46,000	39,000	66 (55–77)	78 (68–88)	85 (71–99)
	18	141,000	111,000	98,000	70 (70–75)	79 (73–85)	88 (81–95)
	20	220,000	161,000	143,000	65 (60–70)	73 (67–79)	89 (81–97)

* Omitted from averages.

dead) and the value of 23 per cent (cells in clumps), the differences between plate and total counts may be explained by *clumping* rather than by the *death* of some cells.

The group/total ratios, which would approximate unity with less clumping, demonstrate also the very considerable number of groups of cells, which were not even broken up by shaking, present during the logarithmic phase. The great majority of these

clumps, as noted microscopically, were due to so-called biological clumping (incomplete separation of cells after fission), as contrasted with a few larger masses of entangled cells.

The plate/group ratios, in themselves, show only that all of the *groups* (single cells and clumps) present in the original culture will grow on plates, but considered in conjunction with the other ratios, indicate viability of *individual* organisms. Considering the possible errors involved, and the fact that the groups determined microscopically may not be exactly the same in number as those plated, the plate and microscopic group counts show very good agreement, not only on the average, but individually as well. At 37°C., the plate count averages 86 (limits, 76 to 96) per cent of the group; at 32°, 92 (81 to 103) per cent; at 27°, 88 (76 to 100) per cent; and at 22°, 106 (91 to 121) per cent of the group. The average indicated viability in the four experiments is about 94 (83 to 105) per cent, which, within the limits of error of the experiments, is equivalent to practically 100 per cent viability for individual organisms.

Series II

The data and ratios for the second series of experiments—all the culturing and plating for which was done on the same day with identical materials—are shown in table 3. In this case, as in the previous series, the plate count is always significantly lower than the total, varying (omitting the 22°, 14-hour ratio, which is so far off as to indicate a probable technical error) from 44 to 85 per cent, and averaging 62 (average limits, 55 to 69) per cent, of the total under various conditions. In the same manner as before, the other 38 (31 to 45) per cent of the organisms must be either members of clumps or non-viable. The group count averages 67 (limits, 60 to 74) per cent of the total, leaving 33 (26 to 40) per cent of the cells as members of these microscopic groups. In this case also, the difference between 38 and 33 per cent is not significant, and the discrepancy between plate and total count can be wholly explained by clumping of cells.

The plate count at 37° in this case averages 99 (limits, 88 to 110) per cent of the group; at 32°, 90 (82 to 98) per cent; at 27°,

98 (84 to 112) per cent; and at 22°, 87 (77 to 97) per cent (omitting the 14-hour ratio) of the group. In series II also, the evidence is reasonably clear that practically all of the bacteria in the culture will grow on plates, the average viability in the four experiments being 92 (82 to 102) per cent.

DISCUSSION

The question as to whether an appreciable proportion of bacteria are dying out *in culture*, at various temperatures, during the logarithmic period seems to be answered conclusively in the negative. The discrepancies between plate counts and total counts can be wholly explained by clumping of organisms, as shown by the group/total ratios which are essentially the same as the plate/total figures, in most individual cases as well as on the average. The plate/group ratios then, can be taken as a measure of the viability of individual cells. With one or two possible exceptions they indicate, within the limits of error of the methods, a viability of 100 per cent for *E. coli* during the logarithmic growth phase.

As regards the few exceptions, all of which are very nearly within the limits of experimental error, we believe them to be more probably due to technique, where some organisms die during plating, rather than to the fact that some cells are dying out *in culture*. The following indicates the basis for this interpretation.

In the first place, plate counts are more likely to be too low rather than high, due to the death of a certain number of cells especially susceptible to environmental changes, as evidenced by the work of Sherman and Albus (1923, 1924), Jensen (1928), Sherman and Cameron (1934), and others previously cited. Most studies, including our own, on survival of bacteria in diluents also indicate a tendency in the direction of a decrease in numbers of cells, if there is any change at all, rather than the reverse. Secondly, the investigations of Barber (1908), Jensen (1928), and Kelly and Rahn (1932), in which single cells from young cultures were transferred to favorable environments and observed, point to practically 100 per cent viability *in culture*. The evidence on viability, obtained from differential staining of dead and living cells, is considered unreliable for accurate work.

Several investigators state that the ability of organisms to take up stains merely shows different degrees of destruction of cells, although loss of reproductive power usually occurs before staining (Fraser, 1920; Henrici, 1928; Rahn and Barnes, 1933; Knaysi, 1935). Certainly staining is a less direct method than plating to determine viability, and it is felt that at present more weight should be given to plating and direct counting in interpreting viability data.

It appears to us that the most direct evidence indicates that during the logarithmic growth phase, in a suitable medium, all of the cells of *E. coli* are viable. There may be a tendency for some to die during plating if conditions are not optimum. With bacteria which are more susceptible to environmental changes, it is quite possible that a certain proportion may die in culture. It is perhaps more likely, however, that such organisms would die during plating, in which case the evidence for mortality in culture would be indirect, being dependent upon the limitations of experimental technique.

With as "individualistic" an organism as the colon bacillus there was a considerable amount of clumping in broth cultures during the logarithmic phase of growth, as indicated by the average group/total ratio (either microscopic group or viable group), after shaking, of about 70 per cent. The clumps—which were mostly of two cells, at least after shaking—were broken up to a varying extent by the shaking. Largely, at least, because of different amounts of clumping, the ratio of plate count to total (individual) cell count varied to the extent of about 100 per cent with different sampling intervals. Since the plate/total ratio was not constant—with clumps present—throughout the period of logarithmic growth, the plate count under these conditions cannot be considered to be a very reliable measure of the number of organisms relative to the total. Having established that practically all of the individual bacteria are viable, it appears that if clumps could all be broken up the plate counts would be identical with the total counts, within the limits of experimental error. This conclusion was also reached by Ziegler and Halvorson (1935), for *E. coli* cultures before the death phase.

It should be so obvious as not to require mention, that in viability studies the question of clumping must be taken into account in one way or another, although strangely enough many investigators apparently have not considered it of quantitative significance.

CONCLUSIONS

For *Escherichia coli*, in broth culture at different temperatures from 22° to 37°C., the following conclusions may be drawn relative to its behavior during the logarithmic growth period.

1. Even in young cultures there is considerable clumping, the group counts having averaged 70 per cent of the total counts, after shaking.

2. The discrepancies between plate and total counts can be wholly explained, within the limits of experimental error, by clumping of cells, other conditions being optimum.

3. The plate count—due to the varying amounts of clumping at different times—does not bear a constant relation to the total (individual) cell count throughout the logarithmic period. When there is clumping, therefore, a plate count *may* not be a reliable measure of the number of cells present relative to the total.

4. If groups of bacteria can be completely broken up, the plate and total counts will be practically identical during the period of maximum rate of reproduction.

5. Different temperatures of growth seem to have no significant effect upon the viability of these bacteria in culture.

6. The results indicate practically a 100 per cent viability *in culture* for this organism. There may be a tendency for some cells to die during the plating-out process.

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