

THE GROWTH OF BACTERIA IN A CONTINUOUS FLOW OF BROTH

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While we are in the habit of restricting the conception of a bacterial colony to aggregates of cells on solid media, growth in fluid media follows the same general course and for purposes of comparison may be considered as a colony.

The growth curve for a colony of bacteria in a fluid medium follows very closely that of a colony of multicellular organisms up to the time when the maximum number is approached. When the normal population is reached in the usual colony of multicellular organisms it continues approximately level with only a slow increase or decrease, while the bacterial colony soon passes into a period of rapid decline. The analogy between the two kinds of colonies is not perfect because of the difference in conditions. In a colony of multicellular animals there is a constant supply of food, and natural or artificial provision for the removal of the products of metabolism. In the bacterial colony the quantity of food is limited and the products of growth accumulate. If the bacterial colony should be maintained under conditions which would permit a continuous supply of nutritive material and the removal of the products of growth, would the population be limited to its normal number and would it maintain itself indefinitely?

It is well known that colonies of bacteria do maintain themselves indefinitely, under certain natural conditions. An infected tonsil or a pus sac on a dead tooth harbor colonies of bacteria which may persist for years, and in which it is probable that the bacterial population is fairly constant.

Experiments designed to answer these questions were made in a culture flask (fig. 1) holding about 75 cc., provided with an inlet tube into which broth from a supply flask flowed. The rate of flow was regulated to about one drop per minute. This rate was

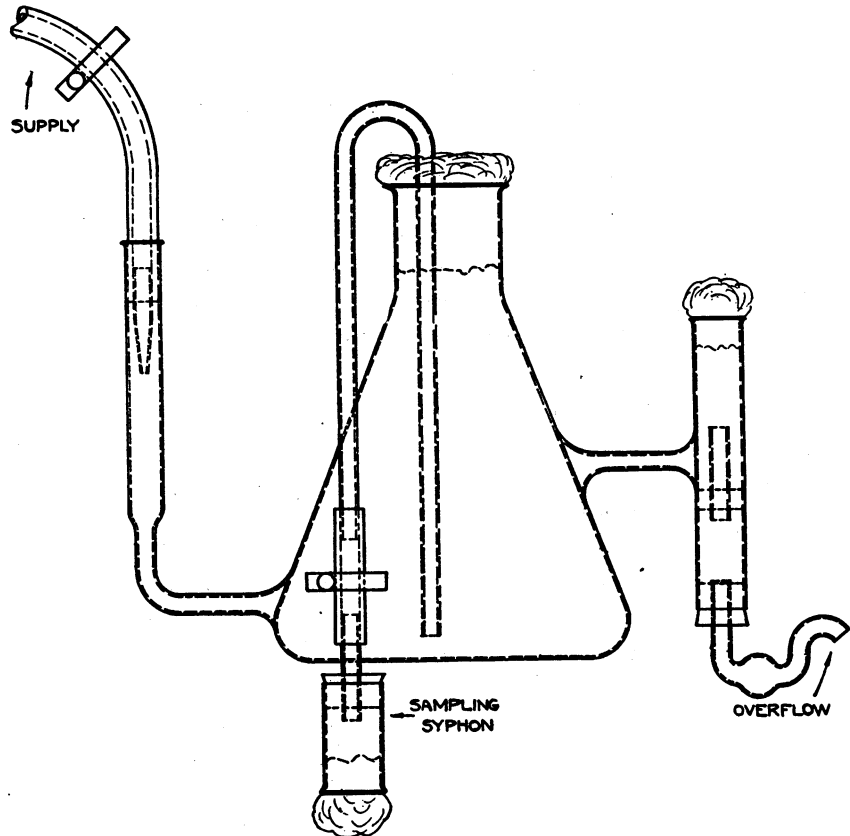


FIG. 1. APPARATUS USED IN STUDYING THE GROWTH OF BACTERIA IN A CONTINUOUS FLOW OF BROTH

sufficient to displace the medium in the flask once in 24 to 30 hours. Since the tube from the supply flask projected into the center of the inlet tube there was no chance for growth to work back into the supply flask. An overflow tube projected into a large vertical tube plugged with cotton at the top and trapped at

the bottom. Samples were taken from the culture flask by a siphon protected at the outlet by a tube plugged with cotton.

The broth contained only a trace of sugar to obviate the inhibiting effect of a high hydrogen-ion concentration.

In table 1 are shown the results obtained when a culture of *Str. lactis* was grown under these conditions at 30°C. In this experiment the population was maintained at a normal level for many days. On the sixteenth day, a failure of the regulating

TABLE 1
Str. lactis in continuous flow of low sugar broth

AGE	FLOW OF BROTH PER CENT OF CAPACITY OF FLASK	BACTERIA PER CUBIC CENTIMETER
<i>days</i>		
1	100.0	1,160,000,000
2	62.2	1,380,000,000
3	71.1	1,250,000,000
4	77.7	
5	57.7	905,000,000
6	80.0	
7	80.0	1,060,000,000
8	71.1	990,000,000
9	60.0	1,165,000,000
10	84.4	1,085,000,000
11	88.8	1,270,000,000
12	26.6	625,000,000
13	82.2	
14	82.2	
15	82.2	760,000,000
16	1,111.1	1,265,000,000

device caused all of the broth in the supply flask to flow through the culture flask in one night so that the medium was changed 11 times in a few hours. The count made the following day showed a normal population but, since some hours had elapsed, it does not necessarily follow that this number had been maintained throughout the rapid flow.

Similar results, shown in table 2, were obtained with *Esch. coli* which maintained a constant population in flowing broth for 30 days.

All of our previous experiments on the effect of *Str. lactis* and

Esch. coli on each other would lead us to expect that these two organisms would not grow equally well in the same flask. Table 3 gives the results obtained when they were inoculated together in a flask through which a low sugar broth was flowed slowly for

TABLE 2
Esch. coli in continuous flow of low sugar broth

AGE	FLOW OF BROTH PER CENT OF CAPACITY OF FLASK	BACTERIA PER CUBIC CENTIMETER
<i>days</i>		
1	78.5	870,000,000
2	89.2	1,255,000,000
3	71.4	1,730,000,000
4	64.2	
5	89.2	3,500,000,000
6	96.4	4,950,000,000
7	92.8	2,300,000,000
8	78.5	3,850,000,000
9	92.8	2,095,000,000
10	89.2	
11	71.4	
12	92.8	3,900,000,000
13	96.4	3,300,000,000
14	64.2	3,200,000,000
15	42.8	3,200,000,000
16	85.7	8,800,000,000
17	85.7	3,600,000,000
18	64.2	
19	107.1	2,730,000,000
20	71.4	2,955,000,000
21	71.4	1,645,000,000
22	78.5	3,000,000,000
24	132.1	1,480,000,000
25	28.5	
26	89.2	1,715,000,000
27	60.7	2,190,000,000
29	60.7	2,785,000,000
30	107.1	2,060,000,000

19 days. The total count was obtained on a medium containing milk powder and tomato juice on which both cultures grew readily and the *Esch. coli* count on an ammonium phosphate agar on which *Str. lactis* did not grow. The lactic colonies were obtained by difference. The synthetic medium did not support

an active growth of the *Esch. coli* culture and it is possible that the count obtained was below the number actually present. The discrepancy between the coli counts in tables 2 and 3 may be due to this factor rather than to the inhibiting effect of the lactics.

There was evident at the beginning an inhibition of the *Str. lactis* but in 8 days a normal population had been reached.

TABLE 3
Esch. coli and *Str. lactis* in continuous flow of low sugar broth

AGE	FLOW OF BROTH PER CENT OF CAPACITY OF FLASK	COLI PER CUBIC CENTI- METER	LACTIC PER CUBIC CENTI- METER
<i>days</i>			
1	42.8	267,000,000	245,000,000
2	71.4	436,000,000	274,000,000
3	71.4	2,330,000,000	195,000,000
4	57.1	3,060,000,000	770,000,000
5	57.1	3,525,000,000	470,000,000
6	50.0	2,550,000,000	725,000,000
7	96.4	2,965,000,000	700,000,000
8	125.0	1,455,000,000	2,000,000,000
9	107.1	1,045,000,000	2,125,000,000
10	128.5	1,750,000,000	1,640,000,000
11	92.8	1,880,000,000	1,555,000,000
12	196.4	1,740,000,000	1,460,000,000
13	125.0	665,000,000	2,020,000,000
14	85.7	850,000,000	2,500,000,000
15	192.8	2,720,000,000	1,270,000,000
16	114.2	1,480,000,000	2,250,000,000
17	135.7	980,000,000	900,000,000
18	121.4	146,000,000	714,000,000
19	117.8	173,000,000	717,000,000

In the latter part of the experiment there was a distinct falling off in the number of *Esch. coli* but, in refilling, the culture flask was contaminated and the experiment terminated before definite results were obtained.

In all of these experiments the population level was maintained in spite of the heavy emigration of cells from the culture. If the cells were evenly distributed throughout the medium it would be necessary for the culture to reproduce itself completely about once every 30 hours, and in some cases even more frequently. In

this respect the conditions were not exactly comparable to those in a colony of multicellular organisms and another experiment was devised which, in some measure at least, overcame this objection.

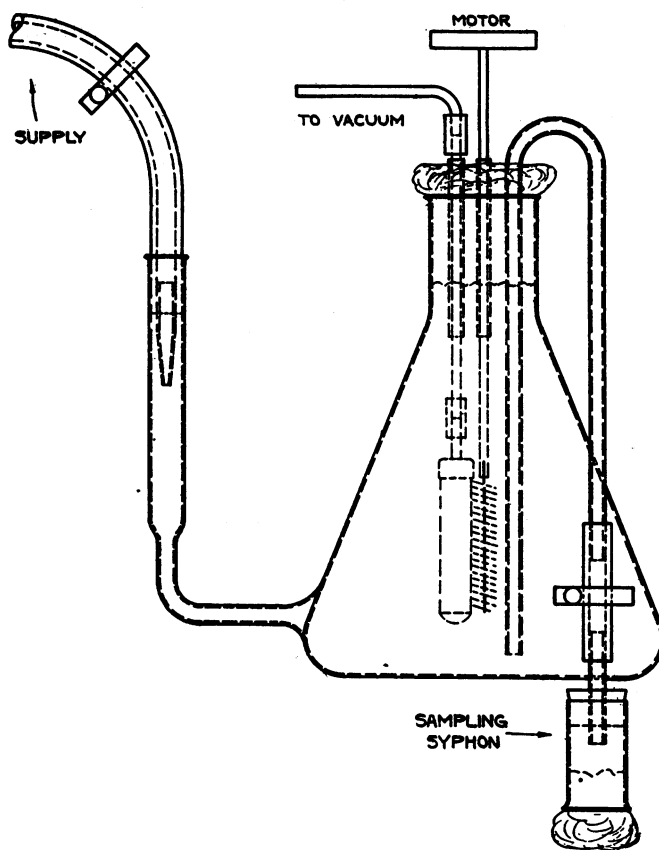


FIG. 2. FLASK ARRANGED TO REMOVE OVERFLOW THROUGH FILTER

In this experiment the broth flowed into the culture flask (fig. 2) as usual but the overflow was carried off through a Berkefeld filter suspended in the culture. The accumulation of cells on the surface of the filter was reduced to a minimum by a revolving brush. The brush rotated constantly but it was neces-

sary to turn the filter manually at frequent intervals to expose the entire surface to the action of the brush. When the filter was removed at the end of the experiment it was free of sediment except on the end where the brush did not reach. The flow of broth into the culture was regulated to balance as closely as possible the outflow through the filter. The greater part of the time about one-third of the filter was submerged and there was no great variation in the volume of the culture.

The rate of flow was sufficient to change the broth in the culture flask from once to over five times every 24 hours. An active culture of *Str. lactis* was used. The results are given in table 4.

TABLE 4
The growth of Str. lactis in a continuous flow with surplus removed by filtration

AGE FROM INOCULATION	OVERFLOW PER CENT OF CAPACITY OF FLASK	BACTERIA PER CUBIC CENTIMETER
<i>hours</i>		
24	185	
41	414	
65	557	243,000,000
89	428	355,800,000
113	300	291,500,000
140	328	291,800,000
159	214	368,600,000
166	100	
183	0	404,200,000
207	0	278,500,000
231	0	48,000,000
255	0	21,250,000

Notwithstanding the abundant supply of nutritive material and the prompt removal of filterable products the population maintained itself at a nearly constant level for 7 days. The comparatively low number maintained in this experiment is probably normal for the culture used, which was not identical with the culture used in the earlier experiments.

Since there was no migration of cells from the culture in this experiment, the only multiplication that could have taken place was that necessary to balance the death rate. If growth continued at the normal rate there must be a correspondingly rapid death rate to hold the population at a nearly constant level.

If the cells did not disintegrate rapidly there would be an accumulation of dead cells and a microscopic count should show a much higher number than the plate count. A microscopic count made at 113 hours was 2,680,000,000 or about ten times the plate count. Since this culture had a decided tendency to form short chains the difference observed was only what would be expected.

It would be difficult to determine positively whether the cells do or do not disintegrate rapidly after death. In an earlier paper¹ it was shown that in a lactic culture in which multiplication had ceased and the number of viable cells was so reduced that 1/10,000 cc. was required to produce growth in milk the direct count was approximately the same as when the culture was at its maximum. This would indicate that the disintegration of the cells was slow.

When the flow of broth was stopped in the flask with the filter, three days elapsed before there was a material decrease in the count. This, we think, indicates that the cells were not dying so long as the flow continued.

It appears from these experiments that, if the conditions are comparable, bacteria will follow the laws governing the population of a colony of multicellular organisms and will maintain a constant population indefinitely. This, however, does not explain why, with an abundant food supply and with filterable products of growth removed, the population should rise rapidly to a fixed number and remain constant at that level.

It is probable that our analogy should be drawn, not between colonies of bacteria and multicellular organisms but between bacteria and an individual multicellular organism.

The normal size of an animal, a tree, or an insect is fixed by heredity. Neither favorable environment nor nutrition will cause an animal or a plant to develop beyond certain limits which are fixed for each species. Even the parts of an individual are constant for the species or strain.

Bacterial colonies on solid media show the effects of a similar law. The typical streptococcus colony on agar is invariably a

¹ Rogers, L. A. and Whittier, E. O. Limiting factors in the lactic fermentation. *Jour. Bact.*, 16, 211-229 (1928).

small sphere, and while the size may be increased somewhat by providing an especially favorable medium, nothing will make it differ materially from its ancestor colonies. In liquid media the maximum population is fairly constant at a number which seems to be fixed for each species or strain.

An analogy of this nature serves a useful purpose as a means of illustrating an obscure phenomenon but should be accepted with caution. The fact that different types of organisms follow the same general course of growth and maintenance does not necessarily prove that they are governed by identical laws. In any event the consideration of heredity as a factor does not really explain why growth ceases when the colony has reached a certain size or the population has attained a certain density.

It is difficult to conceive of the control of a phenomenon of this nature which does not have its basis in some physico-chemical balance. Heredity can only determine the point at which this balance becomes effective in checking growth.

There is a possible application of these results to the fermentation industry. In the usual intermittent method a large part of the time is used in sterilizing equipment and medium, developing the culture to its maximum strength and, when fermentation is completed, emptying the tanks and preparing them for another run. If a fermenting vat could be supplied continually with fresh fermentable material and the surplus removed, it should be possible to maintain the fermentation at its maximum speed for an extended period. We have been able to maintain a lactic fermentation under laboratory conditions for an indefinite time. In these experiments we have used a mixed culture containing a lactobacillus and a mycoderm. This combination grows vigorously and produces lactic acid.

The culture flask, filled to the overflow level, held about 800 cc. and was maintained at 35°C. A heavy suspension of sterilized calcium carbonate was run into the culture at intervals and kept in suspension by a stirrer. The fermenting material was an infusion broth containing tomato juice and 5.92 per cent lactose. The experiment, the results of which are shown in table 5, was continued for 20 days. On the second day after inoculation the

flow of broth was started slowly and 140 cc. overflow obtained on the morning of the third day.

On account of the difficulty in adjusting the flow accurately there was a variation in the overflow from 140 to 900 cc. per day. This caused a variation in the total amount of sugar fermented and the rate per hour. The maximum fermentation was obtained in the period in which 900 cc. flowed through the flask. In this

TABLE 5
The continuous lactic fermentation of broth containing 5.92 per cent lactose

AGE	OVERFLOW	SUGAR FERMENTED			LACTIC ACID PER CENT OF TOTAL SUGAR FERMENTED
		Grams	Per cent of total in overflow	Grams per hour	
<i>days</i>	<i>cc.</i>				
3	140	8.08	97.4	0.33	
4	575	34.04	97.4	1.41	
5	670	39.06	98.4	1.62	
6	900	52.65	98.8	2.19	
7	750	41.85	94.2	1.74	
8	400	18.76	79.2	0.78	57.9
9	560	28.73	86.6	1.19	
10	500	23.50	79.3	0.97	
11	480	23.18	81.5	0.96	
12	500	26.45	89.3	1.10	75.8
13	460	25.30	92.9	1.05	75.3
14	460	26.27	96.4	1.09	
15	340	19.52	96.9	0.81	
16	450	26.24	98.4	1.09	
17	340	19.28	85.7	0.80	
18	230	13.09	96.1	0.54	
19	630	35.15	94.2	1.46	68.8
20	575	27.37	80.4	1.14	89.1

period 52 grams of sugar were fermented at the rate of 2.19 grams per hour. Some undetermined factor affected the efficiency of the fermentation as shown by the percentage of sugar fermented. This varied from 77 to 98.8 per cent. In other words, on one day the broth came through the culture flask with 25 per cent of the sugar unfermented. This variation was independent of the rate of flow. It is possible that it may have been caused by failure to keep the reaction of the culture within the limits of greatest activity.

The efficiency of the fermentation in producing lactic acid is shown by the sixth column of table 5. This shows the percentage of the total sugar fermented which was converted to lactic acid. In other words it is lactic acid expressed as percentage of the theoretical yield.

SUMMARY

Cultures of *Str. lactis* or *Esch. coli*, in flasks through which broth was flowed slowly, maintained a constant population level as long as the experiment was continued.

Under similar conditions *Str. lactis* and *Esch. coli* grew together, each one maintaining a high population. At the beginning, there was an apparent inhibition of *Str. lactis* and, later, a distinct falling off in the number of *Esch. coli*.

In a culture flask with a continuous flow of fresh broth into the flask, and the overflow removed by filtration so that there was no emigration of cells out of the flask, *Str. lactis* maintained a constant population level.

Under laboratory conditions a continuous lactic fermentation could be maintained indefinitely by providing a continuous supply of sterile lactose broth. Under favorable conditions an 800-cc. culture delivered every 24 hours from 500 to 900 cc. of effluent from which practically all of the sugar had been fermented.