TABLE I.
Citric Acid Content of Tissues.*
(Expressed as mg. %.)

	Intact Rats				Nephrectomized Rats			
	Sodium Chloride Injected		Sodium Malate Injected		Sodium Chloride Injected		Sodium Malate Injected	
Tissue	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
Liver	1.6	1.0-2.2	1.4	1.2-1.7	2.5	1.7-3.1	2.3	1.4-2.7
Kidney	5.5	3.0-7.0	11.7	8.0-16.1	—	— —	—	— —
Muscle	2.7	1.7-3.7	2.6	1.5-2.9	4.2	2.9-5.2	3.3	2.2-4.2
Blood	4.0	3.3-4.8	4.0	3.4-4.3	4.6	2.9-7.5	4.7	2.5-6.3

*Averages represent 5 rats in each group except those for kidneys of intact rats, where 8 animals were used.

In order to determine whether the "extra" citric acid present in the kidney after malate injection may have been formed elsewhere and was merely being cleared from the blood by the kidneys, further studies were made on a second series of rats treated in the same manner as those just described, but which were bilaterally nephrectomized immediately preceding the injection. The table shows that again there was little difference between the citric acid content of the blood, liver, and muscle of the control and malate-injected animals.

Thus it is evident that under the conditions employed, the citric acid formed following the injection of sodium malate into rats is produced chiefly, if not entirely, in the kidney.

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On Factors Limiting Bacterial Growth. I.

ALFRED D. HERSHEY AND J. BRONFENBRENNER.
From the Laboratories of the Department of Bacteriology, Washington University School of Medicine.

There is as yet no agreement among bacteriologists as to the specific physical and chemical influences responsible for changes observed in the rate of growth during the life of the bacterial culture.[1] In Fig. 1 typical curves are presented, illustrating these changes as they occurred during the development of a broth culture of *Bacterium coli*. The plotted values were calculated by us from the data of Martin,[2] by means of the formula B-A/T for the rate of population-increase,

[1] Topley and Wilson, *The Principles of Bacteriology and Immunity*, 1929, **1**, 75.
[2] Martin, D., *J. Gen. Physiol.*, 1931, **15**, 697.

and log B-log A/T log 2 for the rate of growth, where A represents the population at the beginning and B at the end of the observed time-interval T. It will be seen that during the period of rapid increase in population, the rate of growth shows a sharp rise, followed by a continuous decrease. It has been the purpose of our experiments to reinvestigate the causes for these changes.

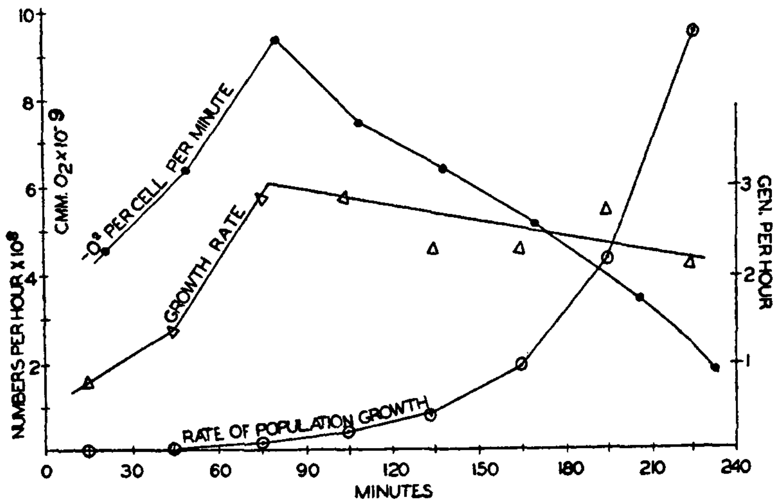

FIG. 1.
Rates of growth and oxygen-uptake per cell during period of increase of a broth culture of *Bact. coli.* (From data of Martin.[2])

The possibility had presented itself that measurements of rate of oxygen-consumption per cell might be used as a convenient index of comparative growth-rates. The observations of Martin on the oxygen-uptake of *Bact. coli* cultures seemed to support this inference. In Fig. 1 we have plotted the rates of oxygen-consumption which he observed, using a single time-scale for these and for the corresponding growth-rates. A comparison of these curves shows that a close relation exists between the rate of growth, and the rate of oxygen-uptake per cell, throughout the duration of the experiment. The present paper deals with the influence of certain factors on the rate of oxygen-consumption per cell. Future publications will present fuller justification for extending conclusions based on these data to the question of actual growth-rates.

The methods we have used were essentially the same in all experiments. Bacterial cells to be examined were centrifuged from the culture fluid, resuspended in sterile salt solution, and standardized both by photoelectric estimation of turbidity using solutions of copper sulfate as standard for comparison, and by the dilution and pour-

plate method to obtain the viable count. The medium used was ordinary 0.5% beef extract—1.0% peptone broth, containing sufficient phosphate buffer of pH 6.6 to give a final concentration of M/20. Whenever the culture-fluids were to be examined, they were sterilized by heat or by filtration, with or without readjustment of pH. The required amount of suspension, buffer, and saline were pipetted onto the floor of the respirometer-vessel, and peptone broth of 5 times the desired concentration was placed in a side arm. When necessary, as in the examination of culture-filtrates, this procedure was reversed, the cells being placed in the side arm and the culture-fluid in the vessel proper. Rolls of starch-free filter paper moistened with 10% KOH served to adsorb the carbon-dioxide liberated. The vessel was then attached to its differential manometer of the Barcroft type, together with a compensator containing the same amounts of nutrient broth, and the apparatus was allowed to reach equilibrium in a waterbath held at 37 ± .001°C., by means of a thyratron-relay. Manometric readings were begun and continued at 5-minute intervals following the introduction of the substrate from the side-arm. With the corrected values of average oxygen-uptake for the successive 5-minute intervals, curves were laid down, and extrapolated graphically to the vertical axis, where the hypothetical quantity of oxygen consumed per 5 minutes at zero time could be read off. In this way it was possible to obtain consistent values for oxygen-uptake per cell to the exclusion of complicating factors of cellular multiplication and changing composition of medium.

It is evident that changes in the rate of growth may be dependent either on the physiology of the cell, on the nature of its environment, or on both. Our first experiments were directed toward separate study of changes in cellular activity, and alterations in the medium of growth in relation to the multiplication-rate observed in broth cultures of *Bact. coli.* The cultures for examination were prepared by inoculation of 100 ml. of infusion-broth in 500 ml. Erlenmeyer flasks with 0.1 ml. of an 18-hour test-tube culture in the same medium and incubated at 37.5°C. without resort to special methods of aëration. The results obtained by the methods outlined above may be summarized very briefly for our present purpose by saying that neither the changes detected in the rate of oxygen-uptake of cells taken from cultures of different age, nor differences in the effect of the corresponding culture-filtrates upon growth and respiration, were sufficiently great to explain the divergence of growth-rates observed at different times in the parent-cultures. Some additional environmental factor apparently was involved.

The remaining factor, which in the above experiments had been

kept constant, was the density of population of the test-suspension. By adaptation of the methods already used, we next measured the oxygen-consumption per cell at zero time in nutrient broth into which a standard amount of bacterial suspensions of varying density were introduced. The results of these tests with 2 and 5 ml. volumes of culture-suspension respectively are shown in Fig. 2.

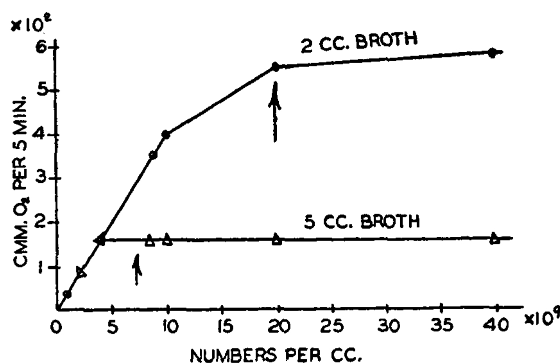

FIG. 2.

Rates of O_2-uptake at zero time per ml. of 2 and 5 ml. broth cultures seeded with different numbers of *Bact. coli.*

Arrows indicate maximal numbers attained in cultures growing under the respective conditions from small inocula.

The first observation of interest is that with equal surface-area and with populations in excess of 10^9 cells per ml., the volume of the culture has a marked influence upon the energy available per unit-volume of the medium. The difference in oxygen-uptake per ml. between the 2 and 5 ml. cultures respectively could be attributed to the limited amount of oxygen which can dissolve in the medium through a given surface, thus giving advantage to cells in the culture where the surface-volume ratio was larger. There is nothing surprising in this finding, which we report only because it seems to imply that nearly all quantitative studies of bacterial metabolism reported in the literature have been carried out under conditions where the available supply of oxygen was limited, even when forced aëration was maintained. It should be noted that the curve showing oxygen-starvation in the 5 ml. culture was obtained under conditions in which the depth of the medium exposed to the air was only 2 mm., and which were shaken in the usual manner 78 times per minute through an amplitude of 5 cm., while with 2 ml. cultures, having a depth of about 0.8 mm., we have not seen any indication of oxygen-insufficiency.

The second fact of interest shown by these curves is that the

limiting density of population in test-suspensions which permitted the rate of oxygen-consumption per cell to remain at its optimum in the 2 cases, was very nearly the same as that reached in normal cultures at the time when active multiplication ceased under the corresponding conditions. These points are indicated in Fig. 2 by arrows. This correlation suggests that analysis of factors limiting oxygen-consumption per cell will, in fact, explain cessation of growth.

The evidence that in cultures having a total volume of 5 ml., under the conditions of our experiments, in which the density of population approaches 10^9 cells per ml., the oxygen-uptake per cell is limited solely by oxygen-supply, may be summarized as follows:

(1) Increasing the surface-volume ratio from 5 to 12.5 by decreasing the volume from 5 to 2 ml., increased the oxygen-uptake per cell; whereas increasing this ratio from 12.5 to 25 by further reducing the volume to 1.0 ml., left oxygen-uptake per cell unaffected. (2) Increasing the rate of shaking increased the oxygen-uptake per cell in 5 ml. cultures, but not in 2 ml. cultures. (3) Increasing the partial pressure of atmospheric oxygen markedly increased this value in 5 ml. cultures, but very little in 2 ml. cultures.

It is clear, therefore, that under ordinary conditions of cultivation in stationary cultures, where the surface-volume ratio varies from about 0.2 in test-tube cultures to somewhat more or less in flasks of different shape containing the same depth of medium, the rates of solution and diffusion of oxygen will be of critical importance in the economy of the culture. We are at present pursuing the question of the extent to which oxygen-deprivation limits the rate of growth and the maximal population attained under various conditions of aëration.

We have indicated that in the 2 ml. cultures used in the respirometer-experiments, oxygen was available in excess of the demand within the range of density of population employed. What is it then that limits the respiration and growth in these cultures? It was noted that shortly after the maximal value was attained under these conditions, the rate of oxygen-uptake of the culture rapidly decreased to values approaching zero, whereas in 5 ml. cultures it remained constant at its maximum during several hours. If cells from the apparently lifeless 2 ml. culture were transferred to fresh medium, they took up oxygen and multiplied normally. The pH of the original culture had remained within physiologic limits. It appeared, therefore, that under these conditions important changes in the composition of the culture-fluid had occurred, although no such changes had been found in earlier experiments employing fil-

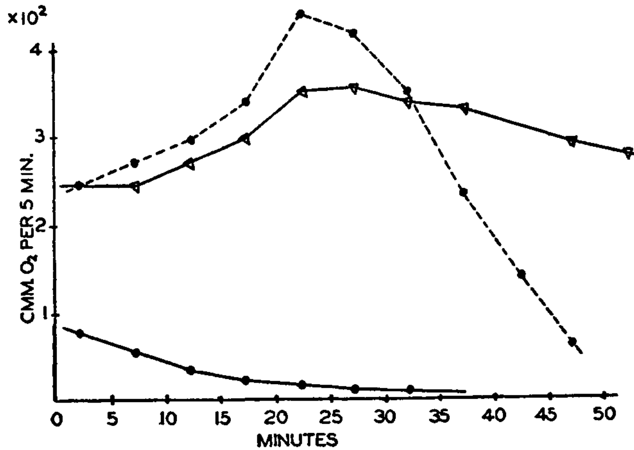

FIG. 3.

Rates of O_2-uptake of 2.2 x 10^9 *Bact. coli* in media containing filtrate of an aërated broth culture.

-●------ 1.5 ml. 1% peptone + 0.5 ml. saline.
-△—△— 1.5 ml. filtrate + 0.5 ml. 3% peptone.
-●——●— 1.5 ml. filtrate + 0.5 ml. saline.

trates of cultures maintained under more usual conditions. In Fig. 3 we have shown data obtained by reseeding the sterile filtrate obtained at the end of the growth-cycle from an intensely aërated culture. It may be seen that this filtrate does not support the respiration of *Bact. coli,* but that its usefulness is largely restored by the addition of 1% peptone. These findings suggest that growth of *Bact. coli* ceases under these conditions as a consequence of oxidation of essential available food materials. So far our experiments have not lent support to the commonly accepted view that accumulation of growth-inhibitory metabolic products is responsible for cessation of growth in bacterial cultures. It appears that under the usual conditions of cultivation, rates of growth and respiration of *Bact. coli* are limited by the rate at which oxygen can reach the cells, and that this limitation prevents rapid exhaustion of the nutrient materials. If, however, oxygen is available in excess, growth soon ceases as a result of oxidative removal of foodstuffs.