THE EXTRA OXYGEN CONSUMED DURING GROWTH OF SERRATIA MARCESCENS AS A FUNCTION OF THE CARBON AND NITROGEN SOURCES AND OF TEMPERATURE[1]

DOROTHY J. McLEAN AND KENNETH C. FISHER

Department of Zoology, University of Toronto, Toronto 5, Canada

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It has recently been shown that *Escherichia coli* (Armstrong and Fisher, 1947) and *Serratia marcescens* (McLean and Fisher, 1947) consume oxygen more rapidly during a period of active growth than when the cells are in a resting condition. For these experiments the bacteria were maintained and examined in a medium containing only salts with citrate or glycerol as the carbon source and with ammonia as the sole source of nitrogen. It was observed that the rate of oxygen consumption of cells which had been growing in a respirometer vessel fell, following the exhaustion of the ammonia, to a level that was approximately 60 per cent of that in existence at the moment the uptake of ammonia was completed. The implication is that the growth process requires oxidative energy at a rate higher than that at which oxidative energy is liberated in the resting cell. Before drawing this general conclusion, however, it is necessary to know that the result observed is independent of the nitrogen and carbon sources employed, and that it is not a peculiarity seen only in the particular medium used heretofore. We have therefore repeated this type of experiment with several other nitrogen sources and with several carbon sources; and as a further test of the variability possible in the result, we have in certain instances made observations at four different temperatures. These new data are recorded below along with a few observations on the effect of sulfathiazole on the extra oxygen consumption associated with growth.

MATERIALS AND METHODS

The strain of *Serratia marcescens* used, the culture medium, and the methods of measuring the rate of oxygen consumption and of making the various calculations were all identical with those described previously (McLean and Fisher, 1947). The amount of nitrogen remaining in the medium after it had been separated from the bacteria by filtration was determined as before by digestion, aeration, and nesslerization.

It was desired in the present experiments to examine the metabolism of the bacteria with carbon and nitrogen sources other than those on which the cultures were grown routinely. It was therefore necessary to make sure that the small quantities of nutrients washed off the culture slants along with the organisms were removed before the experiment proper began. To accomplish this, the suspension of organisms obtained by washing off the plates into 0.07 M potassium

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phosphate buffer at pH 7.0 was aerated at 30 C for approximately 3 hours before the respiratory experiment was begun. A small quantity of citrate was added at the beginning of the aeration. In the presence of this excess of citrate the organisms removed all of the nitrogen source (ammonia), and in due course the citrate itself disappeared. At this point the rate of oxygen consumption fell to the low value that is characteristic of cells deprived of both carbon and nitrogen sources. This rate is generally called the endogenous rate.

Each respirometer vessel contained: (1) 1-ml aliquot of a bacterial suspension the respiration of which had been reduced to the endogenous level as just described (it contained approximately 10^9 bacteria); (2) 0.5 ml of 0.4 per cent magnesium sulfate; (3) 0.25 ml of the carbon source as a 4 to 8 per cent solution; and (4) 0.25 ml of the nitrogen source which contained 0.025 to 0.05 mg N_2.

Whenever necessary the pH of the solutions was adjusted to 7.2 by adding hydrochloric acid or sodium hydroxide. After the equilibration period the rate of oxygen consumption was followed, and when it had become constant (after 30 to 90 minutes) at the "resting rate," the nitrogen compound was added from the onset and the oxygen uptake was observed for several hours more.

RESULTS

Preliminary experiments. The medium in which the organisms were suspended at the start of these experiments, containing, as it did, phosphate buffer, magnesium sulfate, and a carbon source, lacked only a source of nitrogen in order to support the growth of the organisms. If the nitrogen-containing compound which was added could be utilized by the organisms, then growth ensued upon the addition of this substance and the mass of bacterial protoplasm in the vessel began to increase. This in general causes the rate at which oxygen is taken up in the respirometer to increase, and, as we and others have shown, under suitable circumstances the rate of growth can actually be determined from the rate of the oxygen uptake in the vessel.

The addition of a number of possible nitrogen sources to the resting bacteria in the respirometer did not cause a rise in the rate at which oxygen disappeared. In these instances, therefore, it was considered that growth had not occurred and, consequently, that the respective combinations of carbon and nitrogen sources employed would not support rapid growth. It was ascertained in this way that, with the mixture of citrate and glycerol used in our previous work as the carbon source, no growth took place in a 3-hour period after the addition of any one of the following as the sole source of nitrogen: creatine, glycine, leucine, D-glutamic acid, L-histidine, L-cystine, methionine, phenylalanine, tyrosine, or tryptophan. On the other hand, with ammonium chloride as the nitrogen source, growth was not supported by the carbon sources tartrate, acetate, succinate, alcohol, or lactate.

Rapid growth was found with various combinations of the nitrogen sources—asparagine, urea, and DL-alanine—and the carbon sources—citrate, glycerol, and pyruvate, or the mixture of citrate and glycerol.

With aspartic acid as the nitrogen source only very slow growth took place.

However, asparagine, the amide of aspartic acid, supported rapid growth. Analyses of the medium in the asparagine experiment showed, too, that all of the nitrogen of asparagine (i.e., both the amine and the amide nitrogen) was used. It was therefore of interest to find that, even in the presence of added ammonia, the amine nitrogen was still taken up very slowly.

Nitrogen and carbon sources. We pass now to a detailed consideration of the observations made using compounds that supported growth. These data are best represented by curves that show the cubic millimeters of oxygen taken up

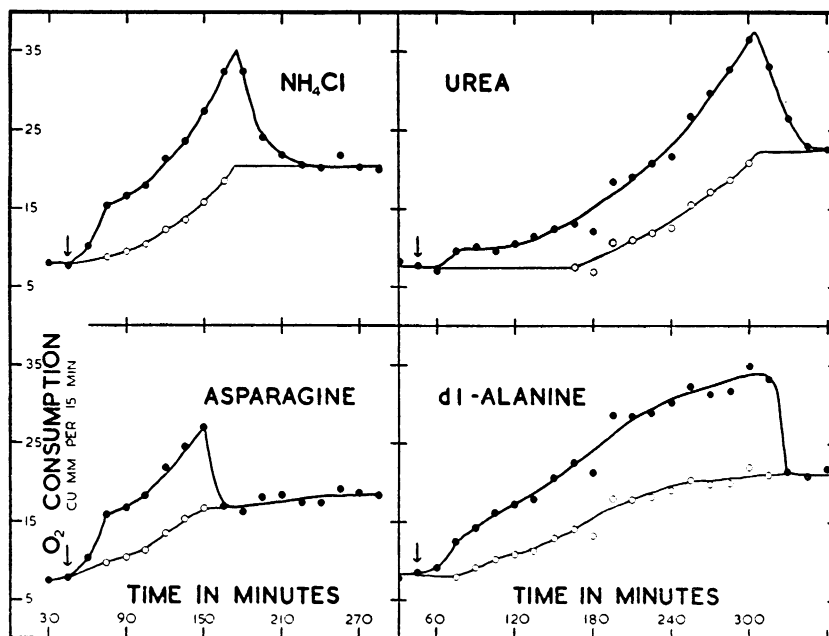

Figure 1. The time course of the rate at which oxygen is taken up in a respirometer vessel containing *S. marcescens* suspended in the nonnutrient medium to which 0.05 mg of nitrogen were added as ammonium chloride, urea, asparagine, or DL-alanine at the time marked by the arrow. Each experimentally determined point (indicated by a dot) is the average of the values from 8 to 10 separate experiments. The open circles indicate the calculated resting rates corresponding to the rates observed on the growing cells. This calculation was made exactly as described by McLean and Fisher (1947).

per vessel per 15 minutes plotted against time. Typical data using ammonium chloride (these from our previous work), urea, asparagine, or DL-alanine as the nitrogen source, with the mixture of citrate and glycerol as the carbon source, are shown as dots in figure 1. The results for the carbon sources, citrate, glycerol, and pyruvate, with ammonium chloride or asparagine as the nitrogen source are not shown as they are qualitatively identical with the ones using citrate plus glycerol and differ only quantitatively from them.

The course of the oxygen uptake following the addition of the nitrogen source is strikingly similar in all these cases. It may be seen (figure 1) that during the

20 to 30 minutes following the addition of the nitrogen source the rate of oxygen uptake increased by 50 to 100 per cent. Thereafter the rate of oxygen continued to rise, but it did so somewhat less rapidly. The initial rapid rise followed by the more gradual one was so constant and striking a feature of these experiments that to emphasize it the lines have been drawn through the points in figure 1 as if the transition from one phase to the other occurred instantaneously at a definite moment in time (which, of course, was undoubtedly not strictly the case). The rate of oxygen uptake continued to rise during the second phase and finally it reached a maximum from which it fell sharply to a reasonably constant lower value. The sharpness of the fall was so striking (it frequently occurred between successive readings of the respirometer) that again the lines have been drawn through the points as if the transition from the rising rate of oxygen consumption to the falling one occurred instantaneously, which undoubtedly was not quite the case.

In our previous experiments with ammonium chloride it was shown that, as nearly as one could see, the disappearance of the last traces of the ammonia from the medium coincided with the occurrence of the maximum rate of oxygen uptake. Chemical analyses of the medium in the present experiments indicated precisely the same thing: exhaustion of the nitrogen source was associated with a fall in the rate at which oxygen is taken up in the vessel. Now growth, as indicated by the uptake of the nitrogen source, must have ceased at the moment that all of the nitrogen was taken up, so that from this moment the quantity of bacterial protoplasm present in the vessel must have been constant. We have already noted that the rate of oxygen uptake fell to a lower constant value following the cessation of growth. This must mean that the bacterial protoplasm consumed oxygen at two rates, depending upon whether it was actively growing or was in a resting condition.

The relative magnitudes of the two rates, the resting and the growing, are indicated by the maximum rate of oxygen uptake found in the respirometer and the lower relatively constant rate found some time after this. It is convenient to express the resting rate as a percentage of the growing one. Its value is given by $\frac{\text{constant rate which follows maximum rate}}{\text{maximum rate}} \times 100$. It was shown in our earlier investigation (McLean and Fisher, 1947), in which ammonium chloride was used as the nitrogen source and a mixture of citrate and glycerol as the carbon source, that the resting rate averaged 57 per cent of the growing one. This value was obtained even when the amounts of growth in the respirometers were varied by varying the amount of ammonia added to the initially resting cells. This observation was of particular significance because it indicated that for each growing rate of oxygen consumption there was a corresponding resting rate to which the oxygen consumption would fall if the ammonia present were suddenly withdrawn. Therefore it is possible to include in addition to the measured rates (the dots) in figure 1 a set of calculated rates (the circles), which show the resting rate to which the oxygen consumption would have fallen if at any moment the ammonia in the medium had been suddenly removed. It will be

noted that the line traced out by these calculated points indicates the way the rate of oxygen uptake in the respirometer would have changed with time if the amount of bacterial protoplasm in the respirometer had been gradually increased without the participation of the normal chemical processes of growth, as, for example, by the gradual addition of more and more resting cells.

The area that is bounded by the line showing the course of the observed growing rate and the calculated line describing the resting rate represents a quantity of oxygen. It is the amount of oxygen that the bacterial protoplasm consumed during growth in excess of that which would have been used by the same protoplasm at rest. More specifically still, it is the amount of oxygen associated with a definite amount of growth, namely, that growth which involved the assimilation of a known quantity of a known nitrogen-containing compound. As was pointed out in our earlier paper, it is of interest to consider this quantity of oxygen in relation to the quantity of nitrogen assimilated, for it represents the *cost* of growth under these conditions in terms of oxygen.

These considerations relating to the growing and resting rates were developed initially with respect to our earlier experiments in which only ammonia was used. It appears, however, that they must apply equally well to these new experiments in which nitrogen was supplied as urea, asparagine, or alanine. The time course of the resting rates shown in figure 1 for the urea, asparagine, and alanine experiments was in fact calculated in exactly the same way as in the original work using ammonium chloride.

Although these observations are qualitatively the same as the previous ones, they differ quantitatively from them. A glance at figure 1 reveals the areas to be different. Actually, for quantitative comparisons of the results observed under the various circumstances described above, the data permit four measurements to be made (McLean and Fisher, 1947): (1) the generation time, (2) the resting rate as a percentage of the maximum, (3) the difference between the resting rate before the nitrogen source was added and the resting rate after the nitrogen source was exhausted, and (4) the area that measures the extra oxygen associated with assimilation of the nitrogen source. These will be discussed in turn.

The generation time is the time for the bacterial mass to double. It was taken as the time required during the logarithmic phase for the rate of oxygen consumption to double. This procedure is justified by our previous observation (McLean and Fisher, 1947) that, under these conditions at least, the rate of increase in either the growing or the resting rate of oxygen consumption is strictly proportional to the rate of increase of the bacterial mass. The average values of the generation time found using various nitrogen and carbon sources are shown in table 1, column 3. They are clearly not identical: with ammonium chloride, citrate, and glycerol growth was very rapid, i.e., the generation time was short, whereas with pyruvate or asparagine and the same carbon sources the growth was considerably slower. Marked differences in the generation times do not appear here since compounds in which growth was very appreciably slower were intentionally excluded. Special mention must be made of DL-alanine

TABLE 1

Quantitative characteristics of the growth of S. marcescens as functions of the nature of the carbon and nitrogen sources

N SOURCE (1)	C SOURCE (2)	GENERATION TIME Minutes (3)	RESTING RATE OF O_2 CONSUMPTION As % of growing rate (4)	INCREASE IN RESTING RATE ml O_2/hr/mg N (5)	O_2 ATOMS Per atom N (6)
Ammonium chloride	Citrate + glycerol	72 ± 6 (10)	57.0 ±1.8 (1)	1.02 ±.06 (10)	2.17 ± .14 (10)
	Citrate	98 ± 8 (10)	60.0 ±3.6 (7)	1.31 ±.34 (6)	3.05 ± .35 (7)
	Glycerol	88 ±13 (7)	56.5 ±5.4 (6)	1.43 ±.31 (6)	3.65 ± .47 (6)
	Pyruvate	100 ± 7 (10)	71.6 ±3.6 (10)	1.14 ±.26 (10)	1.90 ± .16 (10)
Asparagine	Citrate + glycerol	78 ± 8 (10)	68.3 ±4.3 (10)	0.92 ±.14 (10)	1.06 ± .14 (10)
	Citrate	95 ±17 (10)	62.0 ±2.8 (10)	1.20 ±.14 (10)	2.14 ± .19 (10)
	Glycerol	88 ±15 (10)	69.1 ±2.6 (10)	1.00 ±.20 (10)	1.57 ± .26 (10)
	Pyruvate	116 ±11 (9)	70.0 ±4.2 (9)	0.94 ±.17 (9)	1.80 ± .18 (9)
Urea	Citrate + glycerol	99 ±15 (10)	57.6 ±3.6 (9)	1.23 ±.10 (10)	3.38 ± .11 (8)
Alanine	Citrate + glycerol	approx. 66 (10)	61.9 ±3.7 (9)	1.03 ±.07 (10)	3.80 ± .12 (8)

The standard deviation of each quantity is given following the ± sign. The number of experiments made to obtain each quantity is given in parentheses following it. The value for the generation time with alanine as the nitrogen source is estimated, since, as indicated in the text, a logarithmic phase of growth was not observed with this compound.

(figure 1), for with this mixture of isomers no phase was observed during which the logarithm of the rate of oxygen consumption was a linear function of time. Nevertheless the rate of oxygen consumption did increase rapidly when alanine was added, and after increasing to a maximum it fell to a relatively constant resting rate. As chemical analyses showed that both of the enantiomorphs were utilized, the departure from logarithmic growth was probably due to a difference in the rates at which the two were consumed.

The final resting rate of oxygen consumption (i.e., that relatively constant value observed when nitrogen assimilation is finished), when expressed as a percentage of the maximum rate of oxygen consumption in a given respirometer, provides an indication of the relative magnitudes of the growing and resting rates of oxygen consumption (Armstrong and Fisher, 1947; McLean and Fisher, 1947). The values found for the different nitrogen and carbon sources are given in column 4 of table 1. They vary from 57 to 71 per cent, indicating that the value of the resting rate was a function of the carbon and nitrogen sources being utilized. Speculation concerning the reason for this does not seem warranted at present, however, since the actual values may not be as accurately determined as the standard deviations would tend to indicate. This follows from the fact that the curves relating the rate of oxygen consumption to time vary slightly from one compound to another. For example, after the assimilation of asparagine (figure 1), the resting rate rose gradually for a time, whereas after the assimilation of ammonia it fell gradually. Such differences in the shapes of these curves may conceivably have led to small systematic errors in the calculations made from them.

Since the resting rate of oxygen consumption has been found to be proportional to the amount of bacterial nitrogen present (McLean and Fisher, 1947) the increase in this rate after the assimilation of a certain quantity of a nitrogen source provides an estimate of the increase in the bacterial protoplasm. The values for this increase after the assimilation of 1 mg of nitrogen are given in column 5 of table 1. Although they are not all identical (those for asparagine, for example, were generally slightly lower than the others), one is much more impressed with the similarity of the values than with the differences between them. This is especially true when it is recalled again that there were small differences in the shapes of the curves from which the resting rates were determined. At least to a first approximation then, one may conclude that the increase in level did not depend on the source of either the carbon or the nitrogen. The presumption is therefore that all of the nitrogen in each case was incorporated into protoplasm and not stored in any relatively inert form.

The atoms of oxygen consumed per nitrogen atom assimilated were calculated in each instance, as described earlier, from the area bounded by the lines representing the change of the growing and resting rates (see figure 1). This area is, of course, a function of the generation time, of the difference between the growing and resting rates of oxygen consumption, and of the increase in the resting rate due to the assimilation of a given quantity of nitrogen. Other things being equal, an increase in the generation time will result in an increase in the area.

If the difference between the growing and resting rates increases, this too will increase the area. Also, if the increase in the resting rate is greater with one compound than with another, the area will increase because a longer time will be required to consume the necessary oxygen.

The individual factors upon which the experimentally determined areas depend have already been found to vary from one compound to another. It follows that unless the variations of the factors occurred in a reciprocal fashion the areas will also vary. The latter have been determined in the present research. They have been expressed in terms of the number of oxygen atoms consumed for each atom of nitrogen assimilated and will be found in table 1, column 6. Large differences between the values are apparent immediately, so that the factors that determine the areas certainly did not vary with one another in a reciprocal fashion. It is evident that the oxygen consumed during the assimilation of a particular nitrogen source varied with the carbon source available, and conversely that with a given carbon source, the mixture of citrate and glycerol for example, the amount of oxygen consumed varied with the nitrogen source. It is therefore clear that both the nitrogen and the carbon source had a marked influence on the quantity of oxygen involved in the growth process.

Although the values recorded in the table appear to vary somewhat randomly, two regularities are suggested. First, the number of oxygen atoms consumed per nitrogen atom assimilated as asparagine was always equal to or less than that required if the nitrogen was supplied as ammonia. Second, the oxygen consumed per nitrogen atom assimilated was greater when either citrate or glycerol alone was the carbon source than it was when both of these compounds were present simultaneously. Evidently some sort of interaction was possible when both carbon sources were present.

Temperature. To test the effect of temperature on the growth that resulted when the nitrogen source was added, experiments exactly like those reported in figure 1 were conducted simultaneously on aliquots of the same bacterial suspension in baths at 26, 34, and 38 C, respectively. As before, citrate, glycerol, $MgSO_4$, and the bacteria suspended in buffer were in the main space of the vessel, and ammonium chloride or asparagine was added from the onset. Since the observations discussed earlier were made at 30 C, there are available, in all, measurements at four temperatures, 26, 30, 34, and 38 C. As would be expected, the generation time varied with the temperature (table 2, column 3), and the data suggest that the growth rate reached a maximum under these conditions at approximately 34 C.

The fact that the resting rate of oxygen consumption would become a larger fraction of the growing rate as the temperature rose (table 2, column 5) probably could not have been predicted a priori. It will be noted that this effect of temperature was much more marked when the nitrogen source was ammonia than when it was asparagine.

The values for the increase in the resting rate of oxygen consumption due to the assimilation of 1 mg of nitrogen are given in column 6 of table 2. As would be expected, the amount by which the resting rate increased at a given tempera-

TABLE 2

Quantitative characteristics of growth of S. marcescens as functions of temperature with each of two sources of nitrogen

N SOURCE	TEMP. (C)	GENERATION TIME		RESTING RATE OF O_2 CONSUMPTION	INCREASE IN RESTING RATE		O_2 ATOMS PER ATOM N
(1)	(2)	Minutes (3)	Q_{10} (4)	As % of growing rate (5)	ml O_2/hr/mg N (6)	Q_{10} (7)	(8)
Ammonium chloride	26	98 ±17 (9)	3.3	54.4 ±3.6 (9)	6.4 ±0.8 (9)	3.1	2.32 ±0.25 (9)
Ammonium chloride	30	72 ± 6. (10)	0.15	57.0 ±1.8 (10)	10.1 ±0.43 (10)	1.3	2.17 ±0.14 (10)
Ammonium chloride	34	71 ± 3.5 (9)		62.8 ±2.6 (9)	11.3 ±1.43 (9)	1.0	1.95 ±0.22 (9)
Ammonium chloride	38	90 ±14.5 (9)		73.1 ±3.8 (9)	12.3 ±1.25 (9)		1.61 ±0.30 (9)
Asparagine	26	102 ±23. (8)	2.9	66.4 ±2.9 (9)	6.2 ±0.66 (9)	4.2	1.13 ±0.17 (9)
Asparagine	30	78 ± 7.5 (10)	0.6	68.3 ±4.3 (10)	9.1 ±1.28 (10)	1.6	1.06 ±0.14 (10)
Asparagine	34	74 ±12. (10)		69.5 ±3.0 (9)	10.5 ±0.91 (9)		1.28 ±0.25 (9)
Asparagine	38	78 ± 5. (9)		73.0 ±3.6 (8)	10.1 ±1.94 (8)		1.18 ±0.21 (8)

As in table 1, the figures in parentheses indicate the number of separate determinations averaged to obtain the value shown, and the standard deviation of the separate determinations about the average is given following the ± sign.

ture was approximately the same whichever nitrogen source was involved. It therefore follows that the data given in the table represent actually the resting rate of oxygen consumption per mg of bacterial nitrogen as a function of temperature. The temperature coefficients in table 2, column 7, indicate therefore the effect of temperature on the resting rate of oxygen consumption.

The values for the extra oxygen consumed for each nitrogen atom assimilated, which will be found in table 2, column 8, were essentially independent of temperature when asparagine was the nitrogen source, but varied somewhat when

TABLE 3

Quantitative characteristics of the growth of S. marcescens in the presence and absence of sulfathiazole (ST)

(The ST concentration was 0.05 per cent)

QUANTITY DETERMINED	AMMONIUM CHLORIDE			ASPARAGINE		
	No ST	ST		No ST	ST	
		Absolute	% Control		Absolute	% Control
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Increase in resting rate due to growth (ml O_2/hr/mg N)	1.02 (0.05)	0.92 (0.08)	90	0.92 (0.14)	0.78 (0.10)	84
Generation time (minutes)	72 (6)	124 (14)	168	78.0 (7.5)	114 (17)	
Relative growth rate (from generation time)	100	58	58	100	68	68
Level as % peak	56.4 (0.9)	71.0 (1.2)	—	68.0 (4.2)	78.5 (6.0)	—
$\frac{\text{Growing-resting}}{\text{resting}} \times 100$	77.3	40.8	53	47	27.5	59
Oxygen atoms per nitrogen atom	2.19 (0.14)	1.61 (0.11)	73.5	1.17 (0.31)	0.83 (0.17)	71

The quantities of the nitrogen sources added were sufficient to provide initially 0.05 mg of nitrogen per respirometer vessel. Each control (i.e., no ST) value is the average of 10 determinations, and each value with ST present is the average of 6. The standard deviation of the separate determinations from each average is given in parentheses following it.

the nitrogen source was ammonium chloride. Although the changes with temperature in the latter case were small, their regularity leaves little doubt about their reality. It seems necessary to conclude that the chemical mechanism by which ammonia was assimilated varied slightly with the temperature.

Sulfathiazole. The increased rate of oxygen consumption with the assimilation of nitrogen, i.e., with growth, in *E. coli* has already been found to be inhibited by sulfathiazole (Armstrong and Fisher, 1947). Since a similar increase in the rate of oxygen consumption takes place when *S. marcescens* is growing, the effect of sulfathiazole on the oxygen consumption of this organism was also

investigated. The present experiments were primarily designed to show the effect of sulfathiazole on the number of oxygen atoms needed in the ammonia assimilation, as well as the effect on the rate of oxygen consumption during assimilation, which was already known for *E. coli*. In these experiments the bacteria were prepared with citrate plus glycerol as the carbon sources. Sulfathiazole was added with these when desired to give a final concentration of 0.05 per cent. After the resting level had been established, a limited amount of ammonium chloride or asparagine was added and the rate of oxygen consumption was followed as usual. The quantitative aspects of the curves showing the rates of oxygen consumption with and without sulfathiazole are compared in table 3. The data for ammonium chloride and asparagine are essentially identical. They show that, while there was only a slight inhibition (10 per cent) of the resting rate, the generation time was markedly increased (i.e., the rate of growth was decreased) by sulfathiazole and that the rate of oxygen consumption during growth was much lower in the presence of sulfathiazole. These results confirm those of Armstrong and Fisher for *E. coli* under similar conditions.

It will be noted in table 3 that the amount of oxygen consumed for each nitrogen atom assimilated was less in the presence of sulfathiazole than in its absence. One must conclude from this new observation that sulfathiazole caused some qualitative change in the sequence of chemical reactions by which ammonia and asparagine were taken up.

DISCUSSION

It is of interest to consider the quantity of oxygen consumed during the assimilation of the various nitrogen sources in terms of some of the chemical changes that might be involved. Suppose, for example, that the first step in the assimilation of the nitrogen in alanine is an oxidative deamination. This would, of course, require 1 atom of oxygen for each molecule of alanine destroyed. The utilization of each molecule of ammonia thus set free would require, according to column 6 of table 1, 2.2. atoms of oxygen, if carbon were supplied as the mixture of citrate and glycerol. In all, therefore, 3.2 atoms of oxygen would be taken up for each atom of nitrogen utilized. From column 6 of table 1 it may be seen that 3.8 atoms of oxygen were actually required. The difference between 3.2 and 3.8 seems appreciable, although it may not be very significant.

In the case of urea it appears possible that ammonia is first formed by the hydrolytic action of urease, and that the only oxygen needed is that used during the assimilation of this ammonia. If so, no more oxygen would be needed for the assimilation of urea than for the assimilation of ammonia itself. However, the data indicate that, in the citrate and glycerol mixture, 2.2 atoms of oxygen are required to assimilate 1 nitrogen atom in the form of ammonia, whereas 3.4 are required if the nitrogen is supplied as urea. One therefore infers that an oxidative breakdown of the urea must take place, rather than hydrolysis.

The assimilation of asparagine is of particular interest since less oxygen was taken up here than in any other of the cases examined (table 1, column 6). If

the amine nitrogen of this molecule were utilized in the manner already considered for alanine, then 3.2 atoms of oxygen would be taken up for each amine group which disappeared. As a consequence, even if no oxygen were required for the assimilation of the amide nitrogen, 1.6 atoms of oxygen would disappear for each nitrogen atom taken up. Actually, in the citrate and glycerol mixture, only 1.1 atoms of oxygen were required, so that oxidative deamination presumably is not the actual mechanism.

It is of course possible that the utilization of asparagine involved first a hydrolysis of the molecule to ammonia and aspartic acid, a reaction known to occur in a variety of organisms. Provided that the aspartic acid, left perhaps in a particularly reactive state, did not require any oxygen, and that the utilization of the ammonia required the usual 2.2 atoms per nitrogen atom, this would result in the consumption of 2.2 atoms of oxygen per asparagine molecule, or 1.1 atoms per nitrogen atom. This would account for the observations with asparagine in the citrate and glycerol mixture but clearly not when other carbon sources were used.

These considerations thus make it clear that it is not practical at present to propose a complete chemical scheme that will summarize the new observations recorded in this paper.

SUMMARY

The oxygen consumption of *Serratia marcescens* was studied during growth and also with the cells in a resting condition.

The rate of oxygen consumption by growing cells of *Serratia marcescens* is significantly higher than that of resting cells when growth is supported by such nitrogen sources as ammonia, alanine, urea, or asparagine with carbon supplied as citrate, glycerol, or pyruvate.

The quantity of oxygen associated with the assimilation of known quantities of each nitrogen source was determined. It was found to vary over a threefold range depending upon the temperature and the nature of both the nitrogen and carbon sources. It was likewise modified by adding sulfathiazole.

The observations are discussed briefly in relation to some of the current ideas about the chemical changes which might be involved.

REFERENCES

ARMSTRONG, F. H., AND FISHER, KENNETH C. 1947 The oxygen consumption associated with growth in *Escherichia coli* and the effect of sulfathiazole and of *n*-propyl carbamate on it. J. Gen. Physiol., **30**, 279–289.

McLEAN, DOROTHY J., AND FISHER, KENNETH C. 1947 The relation between oxygen consumption and the utilization of ammonia for growth in *Serratia marcescens*. J. Bact., **54**, 599–607.