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Respiration Rate of Bacteria as a Function of Oxygen Concentration

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The respiration rate of an organism is measured by determining the rate at which it absorbs oxygen. It is usual to relate this value to some measurement of the size of the organism such as weight or surface area. Variations in the rate of respiration have been determined in this work, so absolute measurements will not be mentioned. In the absence of oxygen, respiration cannot take place and it is known that when the concentration of oxygen surrounding the organism is progressively reduced, the respiration rate falls as the concentration becomes sufficiently low. In this paper is described a study of the respiration rate of some bacteria as a function of oxygen concentration.

When bacteria are suspended in a medium containing a suitable supply of nutrient they use up the oxygen in solution. If the solution is exposed to the air, oxygen will go into solution, tending to bring the system back to equilibrium. After a time a steady state is set up when the rate of solution of oxygen equals the rate at which the organisms are respiring. By using a manometric device the rate of solution can be measured and thus the respiration rate. It is possible to determine the concentration of oxygen in solution by techniques of this sort. One method is to employ very small numbers of bacteria so that the rate of uptake of oxygen is so slow that in the steady state the oxygen in solution is very nearly in equilibrium with that in the gas phase.

In papers published some years ago (Warburg & Kubowitz, 1929; Meyerhof & Schulz, 1932; Shoup, 1929; Cook, 1930) manometric methods of determining the oxygen concentration at which yeast and various bacteria respire at half their maximum rates were described. The figures so obtained were for a few organisms only and there appeared to be some disagreement, even allowing for the large

temperature coefficients. It seemed advisable to redetermine this oxygen concentration for a number of organisms in the hope of finding some fundamental relationship so that the measurements could be predicted for any organism at any temperature and pH.

An attempt was made to repeat the previous manometric techniques and an improved method suggested by Dr D. Herbert was also tried. It was soon apparent that while these methods might yield results in the hands of highly skilled workers, in fact they involved working at the limits of usefulness of manometric techniques where diffusion from the gas to the liquid phase becomes limiting (Dixon, 1943). It was therefore decided that direct measurement of the oxygen concentration in solution by a polarographic method should be tried.

The use of the conventional dropping-mercury electrode was first investigated. It was found that a slowing of the respiration rates of bacterial suspensions at low concentrations of oxygen could be shown, but that this occurred at the limits of sensitivity of the instrument.

Harris & Lindsey (1948) described a vibrating platinum electrode which gave very much higher diffusion currents for the same concentration of oxygen when compared with the dropping-mercury electrode. This was tried, but for mechanical reasons it could not be used conveniently in a cell free from a gas phase.

During a visit here, Prof. Chain suggested a very ingenious rotating platinum electrode, which easily overcame this difficulty. Several experiments showed that measurements of the concentration of oxygen at which bacteria respire at half their maximum rates could be obtained with this apparatus. Duplicate determinations, however,

varied rather badly and investigation showed that this was due to overlapping of the oxygen and hydrogen waves. Kolthoff & Jordan (1952) demonstrated that there was a wider separation of two waves using gold instead of platinum. A gold electrode was employed and gave results agreeing well with one another, and this type of electrode was used for all the determinations reported here.

MATERIALS AND METHODS

Solutions. Potassium chloride (0.1 M) was prepared from Polaritan Reagent (Hopkin and Williams Ltd.) using distilled water that had been further purified by passage through a column of Amberlite. MB-1 (British Drug Houses Ltd.).

Phosphate buffer 0.1 M pH 7.3 was prepared from AnalaR phosphoric acid, Polaritan KOH (Potassium Hydroxide Pellets Polaritan) and pure water by the method described by Slater (1952) so as to be free of pyrophosphate.

A 1% solution of glucose in a mixture of equal volumes of the above solutions was used as the suspending medium in all the experiments. The pH was 7.3 except when otherwise stated, when it was altered by adding Polaritan KOH till the required pH was obtained.

Organisms. In general the organisms were grown on tryptic meat-digest agar at 37°. The overnight growth was usually sufficient but some required 36 hr. *Micrococcus candicans*, *Acetobacter suboxydans* and *Azotobacter indicum* grew best at 25°. Some of the cultures of *Bacillus megatherium* were grown on this medium to which 1.5% glycine or 1% LiCl had been added in order to produce cells larger than normal.

Acetobacter suboxydans was grown on malt wort liquor with added chalk.

Azotobacter indicum was grown on a medium containing KH_2PO_4 (0.1%), MgSO_4 (0.02%), FeSO_4 (0.02%), Na_2MoO_4 (0.0002%), glucose (0.5%), sucrose (1.0%), agar (2%) and water to 100%. The organisms were washed off the agar with the suspending medium. The pig- and ox-heart preparations were made by the method of Slater (1949). The baker's yeast was purchased locally.

Method. The polarographic method used continuously records the concentration of O_2 in solution. The gold electrode (Fig. 1) consists of a small sphere on the end of a wire sealed into a piece of soda-glass tubing, and electrical connexion between this and the wire to the polarograph is made with Wood's metal. The electrode is inserted into the cell through a hole in a rubber disk. The fit of the electrode into the disk, and of the disk into the cell, is so tight that leakage of air cannot occur even when the pressure difference between the cell and the atmosphere is 760 mm. of mercury. The other end of the electrode is connected by a piece of rubber tubing to an iron rod which engages through a journal bearing concentrically situated in a brass disk rotated at 1300 rev./min. by a constant-speed motor. The lower end of the cell is closed by a sintered glass disk, on top of which is a layer of 4% agar in saturated KCl. This end is immersed in a solution of saturated KCl which is connected with a saturated calomel electrode by means of an agar bridge. This electrode and the bridge must be large enough not to polarize with the passage of 150 μA . In practice a 3 lb. bottle of 10 cm. diameter and tubing of 1 cm. bore satisfied this requirement. The cell is filled with the suspending medium, all air bubbles

being removed. The volume of liquid in the cell is about 20 ml.

The gold electrode is made the cathode, and the calomel cell the anode in their connexions with a Cambridge pen-recording polarograph. The model used is type no. 91331 and it has a sensitivity range of 400 to 0.4 mm./ μA .

Theoretical basis of method. When a constantly increasing potential is applied across the electrodes, O_2 is reduced at the cathode, and a current flows which is proportional to the rate of reduction. It is postulated that rapid rotation of the electrode results in the retention of a very thin layer of stationary liquid around the moving part, while the bulk of the liquid is fairly uniformly mixed. If now a sufficiently great potential difference is applied to reduce all the O_2 at the gold surface, a diffusion gradient will be set up across the stationary layer. The rate at which O_2 diffuses through this layer will be proportional to its concentration in the bulk of the solution, and thus the current flowing through the cell will be proportional to the concentration of oxygen in solution.

The current flowing will be proportional to the area of the electrode and inversely proportional to the thickness of the stationary layer. These two variables were investigated in order to find the optimum conditions. Increasing the rate of rotation increased the diffusion current, but above 1000 rev./min. this increase tends to a plateau, and in practice a speed of 1300 rev./min. was found convenient, as changes in speed produce relatively small changes in current at this point. Theoretically, increasing the size of the gold sphere should increase the diffusion current indefinitely. In practice the internal resistance of the polarograph becomes the limiting factor, and it was found convenient to use an

Fig. 1. Diagram of the apparatus. 1, gold electrode; 2, Wood's metal; 3, wire to polarograph; 4, cell containing suspending medium; 5, inlet; 6, outlet; 7, 4% agar in sat. KCl; 8, sintered glass; 9, sat. KCl; 10, agar bridge; 11, saturated calomel half-cell of 10 cm. diam.; 12, constant-speed motor.

electrode of 1.5 mm. diameter, which gives a current of $150\mu\text{A}$ when the O_2 concentration is that of an air-saturated solution. This current gives full-scale deflexion on the recorder of the instrument at minimum sensitivity.

The selection of the potential at which all the O_2 at the gold surface is reduced gave some difficulty. When a constantly increasing potential is applied across the electrodes, the current flowing increases in such a manner as to produce two steps or waves (Fig. 2).

Electrode reactions. The reactions giving rise to these waves differ from those which produce the two waves when O_2 is reduced at the dropping-mercury electrode. In the latter case the first wave is due to the reduction of O_2 to H_2O_2 and the second to the reduction of H_2O_2 to OH^- (Kolthoff & Miller, 1941). Laitinen & Kolthoff (1941) studied the two waves formed when O_2 is reduced at the platinum electrode. They found that the first wave is due to the reduction of O_2 to H_2O_2 and the second to the reduction of H^+ to H_2 . The large hydrogen overvoltage found with mercury does not occur with platinum and so the hydrogen wave occurs at a very much more negative potential and presumably masks the peroxide wave. In their discussion on the reactions occurring at the gold electrode Kolthoff & Jordan (1952) stated that they occur as follows:

They then state that if H_2O_2 is added to the solution the following reactions then take place:

These authors utilized these second reactions to devise a method of exalting the diffusion current of oxygen so that very small quantities of O_2 could be determined. However, it seemed that even if no H_2O_2 was added, that which is formed in reaction (3) might react in the same way. If all the H_2O_2 reacts in this way then the overall reaction will be

If, on the other hand, none of the H_2O_2 undergoes this further reaction, the overall reaction will be

It seemed to be desirable to determine the overall reaction occurring at the surface of the electrode described here. If it is reaction (7), then the H_2O_2 formed might interfere with bacterial metabolism, or if the organism produced catalase this might result in an increase of the diffusion current for the same concentration of O_2 .

In order to test whether or not the H_2O_2 formed at the electrode undergoes further reduction, the coulombs passing for each g.mole of oxygen disappearing were determined. In the first experiment the apparatus was set up as shown in Fig. 1. Some difficulty arose over the loss of O_2 by diffusion into the agar layer. Normally this loss is insignificant, but it is comparable in amount to that being reduced at the electrode. When a correction had been made for this loss it was found that $3.8 F$ (coulombs) had passed for each g.mole of oxygen reduced. As the correction for the loss of oxygen by diffusion was rather large, it was considered desirable to devise an experimental arrangement in which this loss could not occur. This was done by replacing the agar by a pool of mercury which then acted as the anode. Electrical contact with this pool was made by immersing the cell in

Fig. 2. Polarograms obtained by applying a progressively increasing potential between the gold cathode and the anode. As the potential increases the current increases in the manner shown in these curves. The first wave, (a), is due to the reduction of oxygen and the second, (b), to the reduction of hydrogen ions. Curve 1 is obtained after the initial preparation (Kolthoff & Jordan, 1952) and curve 2 after the application of about 0.8 v for 30 min. The potential at x where the two curves begin to overlap is the one chosen for current measurements. It will be seen that if a point on the first curve to the left of x were chosen, the diffusion current would appear to diminish with time.

a second pool of mercury; the pores of the sintered glass disk being filled with mercury. Connexion with the polarograph was made by placing a copper wire in this second pool. It was found in this experiment that $3.69 F$ (coulombs) passed for each g.mole of oxygen reduced.

These experiments show that most if not all the H_2O_2 is reduced at the electrode. Chemical determination of H_2O_2 free in the solution in the cell after experiments of this kind showed that less than one-tenth of the amount formed by reaction (3) remained. This quantity is much too small to interfere with bacterial metabolism. The addition of excess of catalase did not affect the diffusion current.

From these results it appears that the overall reaction is not stoichiometric and the possibility that the number of faradays passing for each g.mole of oxygen reduced might vary with different concentrations of oxygen must be considered. If this occurs the diffusion current may not vary linearly with the O_2 concentration. In calibration experiments it has been found that by plotting the diffusion current against O_2 concentration determined by Van Slyke's (Peters & Van Slyke, 1948) and Winkler's (Snell & Snell, 1949) methods a straight line is obtained running through the origin. The range of concentrations used was 10^{-3} – $10^{-7} M$.

If the electrode is allowed to rotate for 30 min. at an applied potential of just less than 1 v and the potential is then varied between 0 and 1 v, the oxygen wave will be found to have been shifted towards the hydrogen wave. The two waves are still distinct enough to enable a potential to be selected at which all the O_2 but none of the hydrogen ions were reduced, and thus the diffusion current is unaltered. It is important to 'age' the electrode in this way before beginning any determinations. The ageing process is associated with a change in the surface appearance of the electrode, which loses its brightness and becomes matte. Usually the potential selected was 0.7 v.

The current flowing at 0.7 v is not entirely due to the reduction of oxygen; the diffusion current of the reduction of some metal ions, the condenser current and the residual current all contribute. If the oxygen is all removed by a reducing agent such as Na_2SO_3 or a respiring biological system these other currents can be measured. In practice, using Polaritan reagents, they are very small.

It appeared probable that some experiments might take a long time to perform, and so it was necessary to determine the stability of the diffusion current. The apparatus was run for several periods of 3 days and the greatest change in one period was a fall of 5%, but in the others the change was less than 2%. The changes were linear with time so corrections can be made.

Errors can arise from the loss of O_2 by reduction at the cathode and by diffusion into the agar and the rubber disk, but in the time during which individual determinations are made these errors are negligible.

The diffusion current has a temperature coefficient of about 2%/1°, approximately the same as that of the diffusion coefficient of O_2 in water. Thermostatic temperature control is therefore essential. The temperature of the room used in this department is kept so constant that temperature variations in the apparatus, without the use of special precautions, were less than 0.1° during the course of any one experiment. The experiments at higher temperatures were conducted in a hot room.

Experimental protocol. When a suspension of organisms is introduced into the cell the O_2 is all used up and the diffusion current falls to zero. In this way an initial calibration point is obtained. The next stage is to introduce some more O_2 in solution and record its disappearance at a higher instrumental sensitivity. It is found that the O_2 is used up at a constant rate at first and later more slowly, the curve finally becoming horizontal when no O_2 remains (Fig. 3). The paper of the recorder is carried on a curved carriage with the pen arm pivoted at the centre of curvature. Thus a constant

Fig. 3. The effect on the diffusion current when a bacterial suspension is added to the cell. In (a) the solution is initially saturated with O_2 at atmospheric pressure and finally it is O_2 free. In this way the current flowing at a known concentration of oxygen is determined and the apparatus calibrated. Fig. 3 (b) is similar to (a), except that it is obtained at a higher instrumental sensitivity and represents a 1000-fold magnification of region x of curve (a). 1, line drawn to touch curve, slope of line half maximum slope of experimental curve; 2, Km , the concentration of oxygen at which the bacteria respire at half their maximum rate.

rate of uptake produces a straight line. The maximum slope is now measured and a line of half this slope is drawn so as to touch the curve. The distance between the point of contact and the bottom of the curve (zero O_2) is a measure of the O_2 concentration at which the bacteria respire at half their maximum rate.

In practice it was found convenient to adjust the amount of bacteria present so that the initial slope of the curve was about 2, and the sensitivity selected was such as to give a measurement of the half rate less than 10% of full-scale deflexion.

In previous work the object has been to determine the O_2 concentration at which bacteria respire at half their maximum rate. The method described above produced a continuous recording of the O_2 consumption of a bacterial suspension at continually varying O_2 concentration, so it seemed that more information might be obtained by inspection of the whole curve rather than consideration of one point only. The results actually obtained from the recorder of the polarograph consist of a plot of O_2 concentration against time, and the shape of these curves appeared to be similar to substrate concentration $\times$ time curves for enzymes acting on their substrates. If this is so the experimental relationship between O_2 concentration and time ought to fit the Michaelis-Menten relationship. This is normally stated in the

form $-\frac{dS}{dt} = \frac{k'S}{Km + S}$, but for convenience in the consideration of the relationship of concentration and time it may be integrated, when it yields

$$\frac{S_0 - S}{t} = k' + \frac{Km}{t} \times \ln \frac{S}{S_0},$$

where S_0 = the initial oxygen concentration;

S = the oxygen concentration at time t ;

k' = the maximum respiration rate;

Km = the Michaelis constant (i.e. the substrate concentration giving $-ds/dt = \frac{1}{2}k'$).

When $\frac{S_0 - S}{t}$ is plotted against $\frac{1}{t} \times \ln \frac{S}{S_0}$ an approximately straight line of slope Km is obtained. Values of Km so obtained agree well with the concentrations of oxygen at which the bacteria respire at half their maximum rate as read from the experimentally derived curves.

Alternatively, by determining values of t from this form of the integrated equation

$$t = \frac{S_0 - S}{k'} + \frac{Km}{k'} \times \ln \frac{S_0}{S}$$

at various values of S read off 8-fold enlargements of the experimental curves obtained from any of the organisms, Km and k' being similarly derived, and then plotting them against the appropriate values of S , curves are obtained which fit the experimental

ones (Fig. 4). The fit is relatively poor at the bottom of the curves, the experimental curve lagging behind the theoretical curve by 0.3 mm. at the worst point. This could be due to friction between the pen and the paper.

Thus it is not unreasonable to conceive of the bacterium standing in the same relationship to O_2 as an enzyme does to its substrate, and therefore the concentration of oxygen at which it respire at half its maximum rate may be designated the K_m .

Since the Michaelis constant is theoretically an inverse measure of the affinity of an enzyme for its substrate, by this analogy the concentration of oxygen at which bacteria respire at half their maximum rate is an inverse measure of their affinity for oxygen.

Fig. 4. The continuous curve is obtained experimentally. The straight line touching it has half the maximum slope of the curve. The crosses are points calculated from the equation $t = \frac{S_0 - S}{k'} + \frac{K_m}{k'} \times \ln \frac{S_0}{S}$.

RESULTS

In Table 1 are a number of results for various organisms. Measurements on yeast and pig- and ox-heart preparations have been included for comparison. The heart preparations were oxidizing succinate and not glucose as was the case with the bacteria.

It will be noted that there are fairly wide variations, the larger organisms having a larger K_m . At this stage in the experimental work it was realized that size and K_m were related. It looks as though the relationship is of the form $K_m = Ad^x$, where A is the proportionality factor, d is the cell diameter and x is a constant which is a function of cell shape. This theory was tested by plotting $\log K_m$ against $\log d$. The value of d was obtained from standard textbooks of bacteriology, e.g. Bergey (1948), Topley & Wilson (1946) except in the case of the *B. megatherium* cultures in different media where the values of d were estimated by comparison with one another. More precise measurements would have been desirable, but the resultant plot was so nearly a straight line of slope 2.6032 and intercept of -7.0460 that it was felt there was something worth looking into. The fiduciary limits of the $\log K_m$ calculated from the cell diameter are ± 0.2605 , which are sufficiently narrow to permit calculations of K_m from cell size accurately enough for the practical consideration of growing organisms.

It is obvious that a variation of K_m which is a function of size is not likely to be due to a variation in the affinity of the respiratory enzymes for oxygen. An explanation based on diffusion seems more probable. Attempts were therefore made to measure the K_m of cell-free preparations containing the respiratory system in contact with the bulk of the suspending fluid. In the intact cell these two are presumably separated by a layer of cell substance across which there is a diffusion gradient.

Table 1. The K_m values of various organisms showing the effect of cell size

The K_m 's of baker's yeast and heart-muscle preparations are shown for comparison. The bacteria and yeast were suspended in a solution of 1% glucose in a mixture of equal parts of 0.1 M-KCl and 0.1 M phosphate buffer of pH 7.3. For the heart-muscle preparations the glucose was omitted and the solution instead made 0.004 M with respect to succinate.

Organism	Diameter (μ)	Temperature ($^{\circ}$)	K_m (molar O_2) $\pm$ coefficient of variation
<i>Aerobacter aerogenes</i>	0.6	19.0	$3.10 \times 10^{-8} \pm 9.0\%$
<i>Micrococcus candidans</i>	0.5	20.2	$1.10 \times 10^{-8} \pm 9.1\%$
<i>Bacillus megatherium</i>	2.0	19.2	$5.97 \times 10^{-7} \pm 3.3\%$
<i>B. megatherium</i> (glycine)	4.0	20.6	$3.12 \times 10^{-6} \pm 7.0\%$
<i>B. megatherium</i> (LiCl)	2.4	20.0	$7.07 \times 10^{-7} \pm 1.8\%$
<i>Azotobacter indicum</i>	1.6	19.6	$3.00 \times 10^{-7} \pm 7.3\%$
<i>Acetobacter suboxydans</i>	2.7	19.2	$1.57 \times 10^{-6} \pm 4.5\%$
<i>Serratia marcescens</i>	0.7	18.8	$3.60 \times 10^{-8} \pm 5.0\%$
<i>Escherichia coli</i>	0.6	19.2	$2.22 \times 10^{-8} \pm 3.2\%$
Baker's yeast	—	19.0	$6.45 \times 10^{-7} \pm 3.3\%$
Pig-heart preparation	—	17.3	$1.86 \times 10^{-8} \pm 6.4\%$
Ox-heart preparation	—	19.8	$2.39 \times 10^{-8} \pm 5.4\%$

Table 2. *Comparison of the K_m values of intact cells and cell-free preparations made from them*

Both the cells and the cell-free preparations were suspended in 1% glucose in a mixture of equal parts of 0.1M-KCl and 0.1M phosphate buffer pH 7.3.

Organisms	Temp. (°)	K_m (molar O_2)	
		Intact cells	Cell-free preparation
<i>Aerobacter aerogenes</i>	18.0	4.01×10^{-8}	3.83×10^{-8}
<i>Bacillus megatherium</i>	18.8	7.13×10^{-7}	2.87×10^{-8}
<i>B. megatherium</i> (glycine)	18.2	1.34×10^{-6}	3.68×10^{-8}

Table 3. *Effect of temperature on the K_m values of some bacteria and a heart muscle preparation*

The bacteria were suspended in 1% glucose in a mixture of equal parts of 0.1M-KCl and 0.1M phosphate buffer pH 7.3. For the heart-muscle preparation the glucose was omitted and the solution instead made 0.004M with respect to succinate.

Organism	Temp. (°)	K_m (molar O_2)
<i>Aerobacter aerogenes</i>	19.0	3.10×10^{-8}
	35.0	3.72×10^{-7}
<i>Bacillus megatherium</i>	21.2	9.02×10^{-7}
	32.2	2.77×10^{-6}
<i>B. megatherium</i> (glycine)	20.2	1.21×10^{-6}
	34.0	3.57×10^{-6}
Ox-heart preparation	19.8	2.39×10^{-8}
	33.2	4.10×10^{-8}

Using the method of Tissières (1952) to make the cell-free preparations, experiments were conducted on organisms at both ends of the size scale. It will be seen from the results given in Table 2 that the K_m 's of the cell-free preparations are approximately the same for the organisms investigated and that they are not very much less than the K_m of the smallest. This indicates that the K_m of the small organisms is not appreciably affected by diffusion whereas in the larger ones it is a most important factor. This would be expected to produce a marked effect on the temperature coefficient of K_m . Determinations of the K_m of *Aero. aerogenes* at a higher temperature shows there is a 12-fold increase with a temperature rise of 16°. If the temperature coefficients of the K_m 's of the respiratory systems of *Aero. aerogenes* and *B. megatherium* are the same and the temperature coefficient of the diffusion constant of oxygen through bacterial substance is taken as 2% per degree it is possible to calculate that a rise in temperature of the order of 15° would produce a rise in the K_m of intact *B. megatherium* of 3.6-fold. Experimentally this rise was found to be 3-fold (Table 3).

The effect of pH change on K_m was determined in the case of *Aero. aerogenes*. Determinations at pH's lower than 7 are impracticable for polarographic reasons, but determinations at pH's 8 and 9 showed a small but not significant rise with increasing pH.

Presumably the pH of the contents of the cell is not much affected by a change in the pH of the suspending medium.

DISCUSSION

Despite the wide variations in the K_m 's of different organisms and in their temperature coefficients, all the oxygen concentration/time curves fit the theoretical ones. In the larger organisms there is, however, a concentration gradient between the bulk of the liquid and the location of the respiratory system.

It is tempting to visualize the oxygen concentration in the bulk of the liquid falling in parallel with that in the interior of the cell. To demonstrate this it would be necessary to show that the oxygen concentration both outside and inside reach the appropriate K_m values at the same time. This has not yet proved possible for technical reasons.

If some information can be obtained about the distribution of the respiratory system within the cell it should be possible to calculate the diffusion coefficient of oxygen through cell substance by the methods of Nicholson & Roughton (1951). The fact that K_m 's of both intact *Aero. aerogenes* and the cell-free preparation from it are so nearly the same suggest that there is a very small diffusion gradient between the bulk of the suspending fluid and the respiratory system, whereas in the case of *B. megatherium* this gradient is much larger. This suggests there is a fairly uniform distribution of the system in *Aero. aerogenes* while in *B. megatherium* the difference in K_m 's could be due to a localization of the system in the centre of the cell.

Investigations into the nature and distribution of the respiratory system in bacteria are being conducted along the lines suggested above.

It will be seen that the K_m 's of cell-free preparations and mammalian mitochondria are substantially the same although there are differences in structure and in the cytochrome systems. There is however a big difference in the temperature coefficients (Table 3).

In Table 1, yeast, the largest organism, has not got the highest K_m . This means it does not fit the equation $K_m = Ad^2$ and thus the expression is not generally valid.

SUMMARY

1. A polarographic method of measuring and continuously recording the concentration of oxygen in solution down to the order of $10^{-9}M$ has been described.

2. This method has been used to determine the respiration rate of bacteria as a function of oxygen concentration.

3. The results so obtained have shown that the respiration rate of bacteria is related to the oxygen concentration by the Michaelis-Menten equation, and the characteristic K_m 's of a number of organisms have been determined.

4. The effect of temperature and pH changes on the K_m have been investigated.

5. Reasons for the differences in the K_m 's of different organisms have been discussed and an empirical equation relating K_m to the size of the organism has been established.

I wish to thank Dr D. Herbert, at whose suggestion this work was undertaken, for advice and criticism. I am indebted to Prof. Chain for very kindly describing to me the type of rotating electrode devised by Dr G. Gualandi. I am very grateful to Dr D. W. Henderson for his enthusiastic support and encouragement, to Mr L. J. Bardwell and Miss B. J. Clarke for technical assistance and to Mr S. Peto for assistance with the statistics. Acknowledgement is made to the Chief Scientist, Ministry of Supply, for permission to publish this communication.

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The Role of Magnesium in the Hydrolysis of Sodium Pyrophosphate by Inorganic Pyrophosphatase

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Inorganic pyrophosphatase has been found to be widely distributed in living organisms. It has been demonstrated in various organs and in tissue fluids (Kay, 1928; Lohmann, 1933; Roche, 1931; Minniti, 1939; Nagana & Menon, 1948; Gordon, 1950; Bloch-Frankenthal & Back, 1951). Bauer (1937), who showed it to be present in yeast, obtained it free from phosphomonoesterase. More successful attempts at its purification from yeast were made by Bailey & Webb (1944) and by Heppel & Hilmoe (1951). Kunitz (1952) obtained it from the same source in crystalline form.

Pyrophosphate, the specific substrate of this enzyme, was found in baker's yeast (Lindahl & Lindberg, 1946), insect tissue (Heller, Karpiak & Zurikowa, 1950), and in moulds (Mann, 1944). Kornberg (1950) reported recently on the produc-

tion of pyrophosphate in the reversible synthesis of diphosphopyridine nucleotide from nicotinamide mononucleotide and adenosine triphosphate (ATP) in the presence of hog-liver or beer-yeast preparations. He also proposed a mechanism for pyrophosphate accumulation in tissues and fungi. The enzymic splitting of pyrophosphate from ATP has also been demonstrated (Hitchings & Fuller, 1939; Zeller, 1950; Heppel & Hilmoe, 1953).

Pyrophosphatases, so far described, are dependent upon the presence of magnesium for their ability to catalyse the hydrolysis of inorganic pyrophosphate according to the reaction:

(Bailey & Webb, 1944).