# XCI. THE USE OF THE BARCROFT APPARATUS FOR THE MEASUREMENT OF TISSUE RESPIRATION.

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The Barcroft apparatus was originally designed to measure the total amount of gas evolved or absorbed by a given process. Recently however it has been widely used for the study of tissue respiration and similar processes, in which not so much the total amount of gas absorbed as the *rate* of the process is to be determined. In order that the results should be significant in such cases, it is essential to ensure that the process is not being limited by purely physical factors depending upon the apparatus itself. Two such factors are likely to introduce errors, namely, inability of the oxygen to diffuse sufficiently rapidly from the gas into the liquid phase, and insufficient rate of absorption of the carbon dioxide, produced in the respiration, by the alkali present for that purpose. In spite of the considerable amount of work which has been carried out by means of the Barcroft apparatus on tissue systems, no data on the limitations of the apparatus from this point of view have yet been published. This question is dealt with in the present paper.

## *Details of apparatus.*

The flasks of the apparatus were of the form illustrated in Fig. 1, this being the type used in this laboratory during the past seven years. The volume of the flask is 35 cc. and 3 cc. of liquid are used in all cases. The layer of liquid is about 1·5 mm. in thickness.

A considerable improvement in the reliability of the apparatus is effected by dispensing with the usual T taps, which were very liable either to leak or to become blocked, and substituting ordinary taps in the position shown in Fig. 1.

The liquid used in the manometers was paraffin coloured with Sudan III, which proved entirely satisfactory. Clove oil is to be avoided, as it is too viscous for use when rapid oxygen uptakes are being measured. The density

of the paraffin used should of course be accurately determined before calibrating the apparatus, so as to avoid the necessity of recalibration in the event of accidental loss of the liquid. The area of cross-section of the bore of the manometer tubes was about 0·5 mm.[2]

The usual type of shaking mechanism was employed. This was normally adjusted to give a rate of shaking of 120 complete oscillations per minute, with a distance of travel of 2·5 cm., these conditions being those adopted in previous work. The shaking was not interrupted when readings were taken.

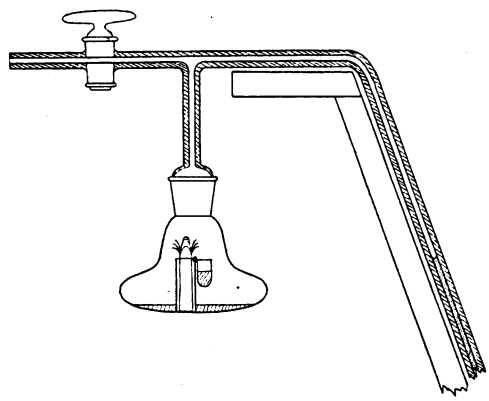

Fig. 1. Side view of apparatus. The drawing shows only one-half of the apparatus.

*Absorption of carbon dioxide.*

In using the Barcroft apparatus to measure the rate of oxygen uptake by tissue preparations, it is of course necessary that the effect of the $CO_2$ produced by the tissue shall be eliminated. For this purpose it has been customary to introduce about 0·3 cc. of a strong solution of NaOH or KOH into a small open tube sealed to the bottom of the flask, in order to absorb the $CO_2$. If any $CO_2$ accumulates in the gas phase, owing to incomplete absorption, the apparent oxygen uptake will be less than the true by a corresponding amount. If no alkali is added the movement corresponds to the difference between the oxygen absorbed and the $CO_2$ evolved, and this is made use of in determining the respiratory quotient (as described by Dixon and Elliott [1929]). In the course of the work just referred to we found that with the customary technique the rate of absorption of $CO_2$ was quite inadequate for experiments on rapidly respiring tissues. We therefore examined the efficiency of the absorption under various conditions.

For this purpose a Barcroft respirometer was set up containing in the flasks 1 mg. of sodium carbonate in the usual amount of liquid. In the right hand flask a small tube (as used by Keilin [1929]) containing 0·4 cc. of a strong solution of oxalic acid was suspended from the absorption tube by a small platinum hook as shown in Fig. 1. 40 % NaOH or KOH solution was placed in the absorption tube in the usual way. The apparatus was then

shaken in the thermostat for 5 minutes, the taps closed, and the zero reading taken. After shaking for a further period to make certain that the reading did not change, the apparatus was gently tapped so that the tube containing the acid was upset into the carbonate solution and the $CO_2$ (about 200 mm.$^3$) thus liberated. The $CO_2$ was rapidly evolved, and readings were then taken for some time after the evolution had ceased in order to follow the rate of absorption by the alkali. Results obtained for NaOH at 14° and for KOH at 40° are shown in Fig. 2, curves A and B.

It is evident from these curves that the rate of absorption of $CO_2$ is quite insufficient, when the customary technique is used.

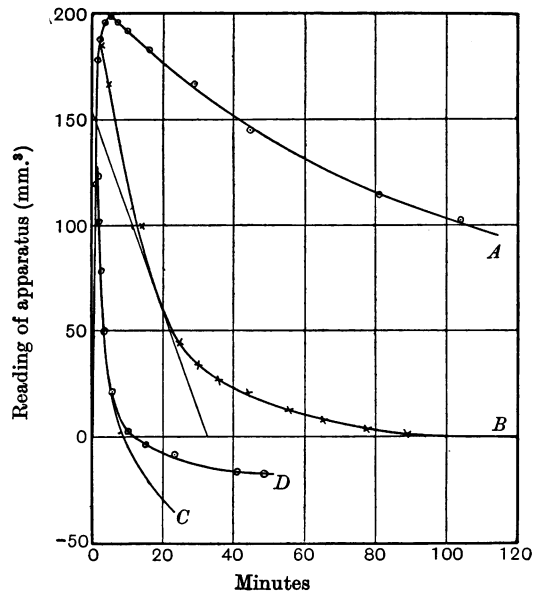


Fig. 2. Rate of absorption of $CO_2$ by various methods. Curve A; the absorbing agent was 40 % NaOH placed in the absorption tube; temperature 14°. (The curve for NaOH at 40° was slightly lower than this.) Curve B; 40 % KOH in the absorption tube; temperature 40°. Curves C and D; rolls of filter-paper were used in the right-hand flask only at 40°: Curve C; the paper was moistened with 40 % KOH; Curve D; 7 % KOH was used.

The tissue may be assumed to produce $CO_2$ at a constant rate. The rate of absorption on the other hand will depend upon the concentration of $CO_2$ in the gas phase. $CO_2$ will therefore accumulate in the gas phase until the rate of absorption becomes equal to the rate of production, and when this steady state is reached the movement of the manometer gives a true measure of the rate of oxygen uptake. Thus the error becomes smaller as the experiment progresses. It is possible to calculate the time required for attainment of the steady state and the magnitude of the error produced as follows.

Let $x$ = the volume in mm.$^3$ of $CO_2$ in the gas phase of the flask at any instant.

Let $R$ = rate of evolution of $CO_2$ by the tissue in mm.$^3$ per minute. $R$ may be assumed to be constant.

The rate of absorption of $CO_2$ will be proportional to the amount of $CO_2$ in the gas phase. This will be the case no matter whether the rate is determined by the diffusion of the gas along the absorption tube or directly by the number of molecules striking the absorbing surface. Therefore we can write

$$\text{Rate of absorption} = cx \text{ mm.}^3 \text{ per minute,}$$

$c$ being a constant depending on the form of the apparatus, temperature, etc.

The rate at which $CO_2$ accumulates is equal to the difference between the rate of evolution and the rate of absorption. Therefore

$$\frac{dx}{dt} = R - cx.$$

On integrating, and determining the constant of integration by putting $x = 0$ when $t = 0$, we get

$$x = \frac{R}{c}(1 - e^{-ct}) \qquad \text{.....(1).}$$

At $t = \infty$, $x_\infty = \frac{R}{c}$.

We may obtain the time required for $x$ to reach 99 % of its equilibrium value by putting $x = \frac{99}{100}x_\infty$.

Then $$\frac{99}{100}\frac{R}{c} = \frac{R}{c}(1 - e^{-ct})$$

whence $$e^{-ct} = 0{\cdot}01$$

$$-t_{99\,\%} = \frac{-2 \times 2{\cdot}3}{c}$$

$$t_{99\,\%} = \frac{4{\cdot}6}{c} \text{ minutes.}$$

We note that the time required for $x$ to reach 99 % of its final value is independent of $R$, the rate of evolution of $CO_2$.

By drawing a tangent to curve B, Fig. 2, at any given point we obtain the value of $cx$, the rate of $CO_2$ absorption. *E.g.* when $x = 75$ mm.$^3$ the rate of absorption $cx = \frac{153 \text{ mm.}^3}{34 \text{ mins.}}$, whence

$$c = \frac{153}{34 \times 75} = \frac{1}{16{\cdot}5}.$$

Therefore $t_{99\,\%} = 4{\cdot}6 \times 16{\cdot}5 = 76$ minutes.

Suppose $CO_2$ is evolved at the moderate rate of 300 mm.$^3$ per hour. Then $R = 5$ mm.$^3$ per minute, and $x_\infty = \frac{R}{c} = 5 \times 16{\cdot}5 = 82{\cdot}5$ mm.$^3$

Using equation (1) we can plot $x$ against $t$ as in Fig. 3. We see that using this method of absorption, approximate equilibrium between $CO_2$ evolution and absorption would not be reached for 76 minutes, at which time 82 mm.$^3$ of $CO_2$ would be present in the gas phase of the flask. Should the taps of the apparatus be closed after allowing say 10 minutes for the initial temperature equilibration, the curve shows that about 40 mm.$^3$ of $CO_2$ are already in the flask, and therefore during the next 60 minutes a further 40 mm.$^3$ of $CO_2$ will accumulate in the gas phase, so that the apparent oxygen uptake will be decreased by this amount.

Thus this method for $CO_2$ absorption is practically useless, and the following method, suggested to us by Dr Keilin, has been adopted.

A rectangle of filter-paper about 4 cm. by 2·5 cm. is rolled up fairly tightly into a cylinder, and tied with silk. The length of this roll should be about $\frac{1}{4}$ inch greater than that of the absorption tube in the flask. One end of the cylinder is then slit down by two cuts at right angles, and the ends spread out. This is then placed in the absorption tube as shown in Fig. 1, after lightly greasing the upper rim of the tube to prevent the alkali from creeping down the outside. The alkali solution is then run on to the paper.

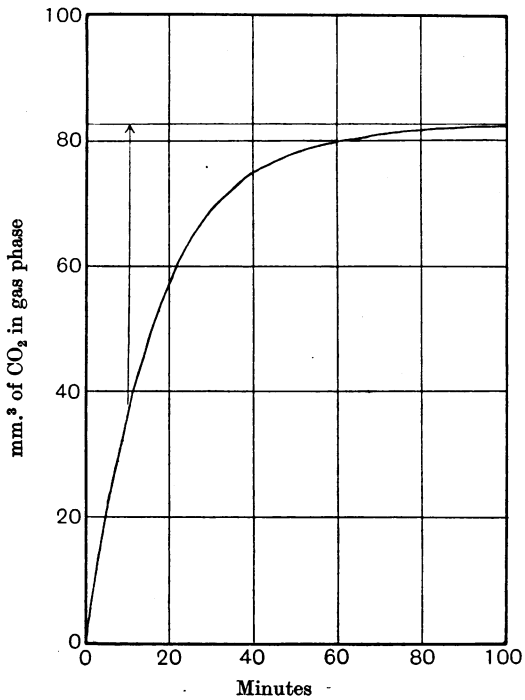


Fig. 3. The curve shows the accumulation of $CO_2$ in the gas phase with absorption conditions corresponding to curve B of Fig. 2, and with a $CO_2$ production of 300 mm.³ per hour. The length of the arrow represents the magnitude of the error produced when the taps are closed after 10 minutes.

The absorption of $CO_2$ is now very rapid, as is shown by curve C, Fig. 2. Using 40 % KOH at 40° it is evident however that a new factor has entered, for the amount of gas absorbed is greater than the amount of $CO_2$ present, and the pressure in the flask continues to fall after passing its original level. This was traced to the fact that under the action of the strong alkali the paper undergoes spontaneous oxidation with uptake of oxygen. With a weaker solution of alkali however the absorption of $CO_2$ is equally rapid, while the absorption of oxygen becomes nearly negligible. This is shown in curve D, Fig. 2, which was obtained using 7 % KOH at 40°. The inaccuracy due to

the oxidation of the paper can be entirely obviated by having the alkaline paper in both flasks of the apparatus. In curves A and B, Fig. 4, 6 % KOH on paper was used at 14°, the paper being in the right hand flask only in curve A and in both flasks in curve B.

By calculation similar to that given above it is found from curve B, Fig. 4, that using this method of absorption only 11 minutes is required to reach approximate equilibrium, at which time 12 mm.$^3$ of $CO_2$ are present in the flask if the rate of production of $CO_2$ is 300 mm.$^3$ per hour as before. If the taps are closed after 5 minutes there will be 10 mm.$^3$ already present, and the amount of $CO_2$ will increase by only 2 mm.$^3$ which is entirely negligible.

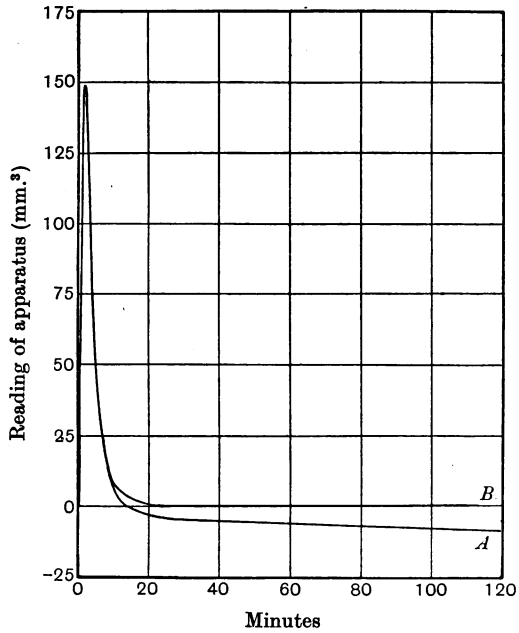


Fig. 4. This experiment was similar to that of Fig. 2. Curve A; 6 % KOH on filter-paper was placed in the right-hand flask only; Curve B; it was placed in both flasks of the apparatus. Temperature 14°.

Taking the high rate of $CO_2$ evolution of 1000 mm.$^3$ per hour, the same calculation shows that the error when the taps are closed after 5 minutes would only be 5 mm.$^3$

This method is therefore entirely satisfactory.

*Diffusion of oxygen as a limiting factor.*

In order to measure the true respiration rate of cells immersed in a liquid it is necessary to ensure that the rate at which oxygen can diffuse into the liquid is rapid in comparison with its utilisation. (This applies also to the measurement of oxygen absorption by other systems.)

The importance of this was early appreciated when adapting the Barcroft apparatus for measuring rates of oxygen uptake, and Dixon and Tunnicliffe [1923] pointed out the importance of using flasks of a form exposing a large surface of liquid to the gas and giving efficient mixing of the liquid, and also of making sure in carrying out experiments that the rate of uptake is independent of the rate of shaking of the apparatus, this being an indication that the uptake is not being limited by diffusion effects. In spite of the considerable amount of work which has since been carried out with the apparatus, no quantitative data have yet been published on the rates of uptake which the apparatus is capable of measuring without errors due to diffusion effects.

The essential condition which must be fulfilled if the experiment is to be significant is that the concentration of dissolved oxygen must not fall so low that its rate of utilisation by the respiring cells (or other system undergoing oxidation) falls appreciably below that which would be obtained if the pressure of oxygen in the liquid were identical with that in the gas.

In order to make this clear let us consider what takes place when the amount of respiring cells, *e.g.* yeast, present in the 3 cc. of liquid in the flask of the apparatus is gradually increased. With very small amounts of yeast the rate of oxygen uptake will be small, and hence the pressure of oxygen dissolved in the liquid will be only slightly below that in the gas phase; the observed rate of uptake will be proportional to the amount of yeast present, and will be independent of the rate at which the apparatus is shaken (*i.e.* of the degree of mixing of the liquid). As the amount of yeast added is increased the rate of uptake is proportionately increased and the tension of dissolved oxygen will fall lower; but so long as it does not fall below the point at which the respiration rate of the yeast depends upon the oxygen pressure the rate of uptake is still a true measure of the respiration, and remains proportional to the amount of yeast and independent of the rate of shaking. A point is ultimately reached however at which the oxygen pressure in the liquid falls so low that the actual respiration rate of the yeast is cut down, when further additions of yeast have much less effect, and since the rate of uptake is now to some extent determined by the rate at which the oxygen can get to the yeast, it begins to be dependent upon the rate of shaking. Finally with large amounts of yeast the rate of uptake reaches a limiting value which is practically independent of the amount of yeast, and is entirely dependent upon the rate of shaking.

Owing to the complicated mixing conditions a quantitative mathematical treatment is hardly practicable, but we shall give here a number of experiments illustrating the effects met with and the capabilities of the apparatus.

The actual results obtained with yeast are illustrated in Fig. 5, which shows the relation between the observed velocity of oxygen uptake and the amount of yeast taken at two rates of shaking. (In all these experiments large "KOH-papers" were used, capable of absorbing the $CO_2$ produced by large amounts of yeast.) It will be seen that up to the point $X$ on the curves

the apparatus measures the true respiration rate of the yeast, since the velocity of uptake remains proportional to the amount of yeast taken up to this point; also the rate of uptake observed is the same at both rates of shaking. Beyond this point the velocity becomes limited by diffusion, and practically independent of the amount of yeast, at the slower rate of shaking. At the higher rate of shaking however the respiration rate can be followed up to considerably higher values before diffusion limits the process. It appears therefore that with yeast the apparatus is capable of accurately measuring uptakes up to about 1000 mm.$^3$ per hour at the normal rate of shaking (120 per minute), at 15° and with the flasks filled with air. Correspondingly higher velocities can be measured at higher temperatures or with the flasks filled with pure oxygen instead of air.

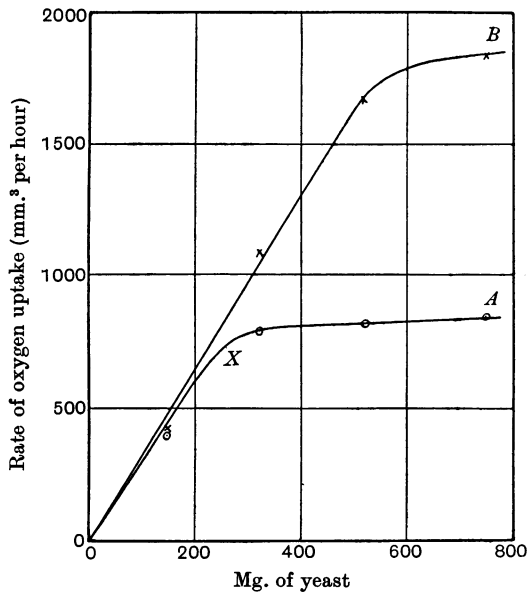


Fig. 5. Velocities of oxygen uptake observed with varying amounts of yeast. A suspension of 5 g. baker's yeast in 20 cc. phosphate buffer $p_H$ 7·6 was prepared, and mixtures of this suspension with phosphate buffer were taken so as to give the amounts of yeast stated in 3 cc. of liquid. In curves A and B the rates of shaking were 102 and 138 oscillations per minute respectively. Temperature 16°.

The maximum rate of uptake which can be measured varies greatly with the rate of shaking, as is to be expected. In order to obtain some idea of this rate of uptake at different rates of shaking, a number of observations were made with larger amounts of yeast, under conditions therefore where diffusion was the limiting factor. These results are shown in Fig. 6. The experimental variations are considerably greater when the process is limited by diffusion than when this is not the case, but the results are quite consistent in spite of the fact that several different respirometers were used. The true respiration rate of the amount of yeast used was about 3000 mm.$^3$ per hour

at 15°, as shown by determinations on small amounts with rapid shaking. This rate was reached at a rate of shaking of about 180 oscillations per minute.

Rates of shaking below 60 per minute do not cause much mixing of the liquid layer, which tends to oscillate as a whole from side to side of the flask. Definite mixing of the liquid begins at about 60, and thereafter becomes increasingly efficient. This is reflected in the form of the curve. Rates of shaking above 200 are not permissible, as there is then some risk of the liquid splashing on to the "KOH-paper," but there is no risk of this below this rate.

The position of the curve of Fig. 6 is not altogether invariable and independent of the nature and amount of the system absorbing oxygen. Even when the process is limited by diffusion the rate of uptake depends to a certain extent upon the amount and activity of the yeast taken (or, in the case of

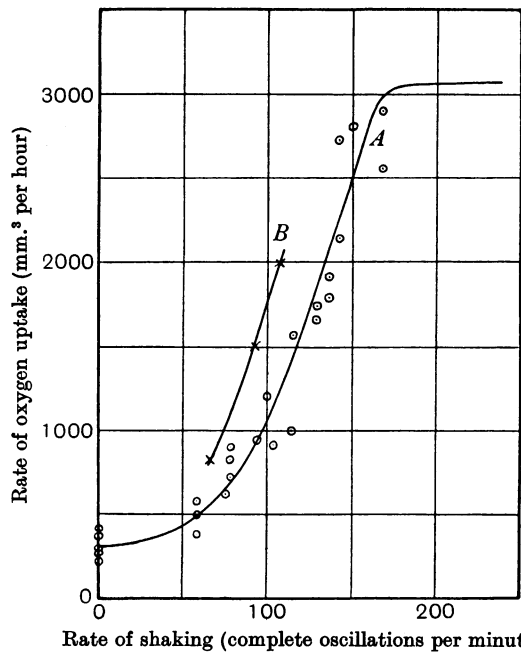


Fig. 6. Effect of rate of shaking on rate of oxygen uptake by large amounts of yeast. Each of the small circles represents an experiment with 600 mg. baker's yeast in 3 cc. phosphate buffer at 15°. The true respiration rate was slightly over 3000 mm.³ per hour. Curve B was obtained with 200 mg. of yeast at 37°.

autoxidation of a substance, the velocity constant of the reaction). In general, systems which would react rapidly with an adequate supply of dissolved oxygen tend to give higher curves than systems giving slower uptakes. That is to say, diffusion effects may influence the results at observed rates much lower in the case of systems giving only small rates of uptake (in the absence of such effects) than in the case of more rapidly reacting systems.

Thus with smaller amounts of yeast than those used in Fig. 6 the curves followed lie somewhat lower but are of similar form. As the rate of shaking

is increased the rate of uptake increases as before until the true respiration rate is reached, after which further increase of shaking has no effect. With decreasing amounts of yeast a series of curves such as E, F, B, C in Fig. 7 are thus obtained, the levels of the horizontal parts of the curves at the higher rates of shaking being proportional to the amount of yeast present. It is of course only on these horizontal parts of the curves that the true respiration rate is measured.

Conversely with more rapidly respiring cells the position of the curve is somewhat higher. In curve D of Fig. 7, for which the same amount of yeast was used as in Fig. 6, the yeast was previously incubated with a small amount of glucose, which increased its respiration about 5 times [cf. Dixon and Elliott, 1929]. The change in position of the curve is however comparatively small.

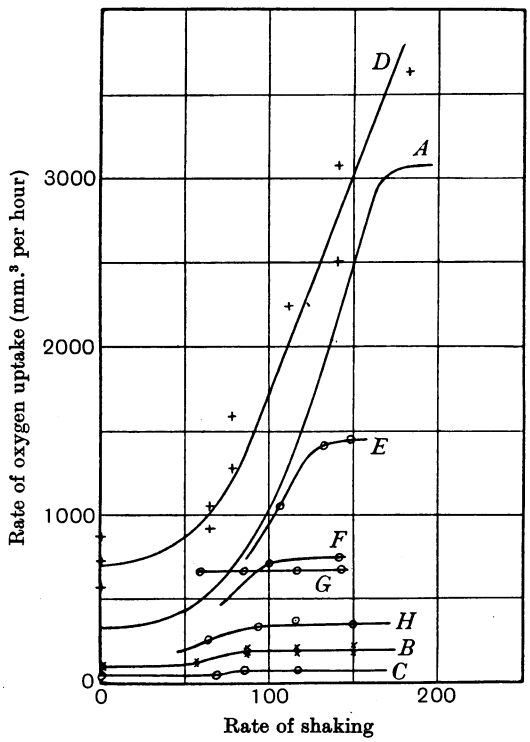


Fig. 7. Curve A is that of Fig. 5 reproduced for comparison. Curves A, B and C were carried out with the same sample of yeast, 600 mg., 60 mg., and 20 mg. being used respectively. Curve D was obtained with 600 mg. of the same yeast which had previously been treated with a small amount of glucose. Curves E and F were obtained with 500 mg. and 250 mg. of a different sample of yeast. Temperature 15° in all these curves. Curve G was obtained with 100 mg. of yeast at 37°, and curve H with 500 mg. of minced liver at 37°.

In the case of yeast the respiration rate is independent of the oxygen pressure within wide limits. In other cases, as for instance the autoxidation of cysteine, the reaction velocity depends upon the oxygen pressure.

A number of experiments were carried out on the oxygen uptake by cysteine in presence of varying amounts of iron as catalyst. The curves obtained were very similar to those given by yeast. It appears that with this system also velocities of the order of 1000 mm.$^3$ per hour can be measured at the usual rate of shaking (see Fig. 8).

This figure must not however be taken as representing the highest velocity measurable by the apparatus for all systems. With certain rapid reactions uptake rates of a much higher order can be measured without errors due to

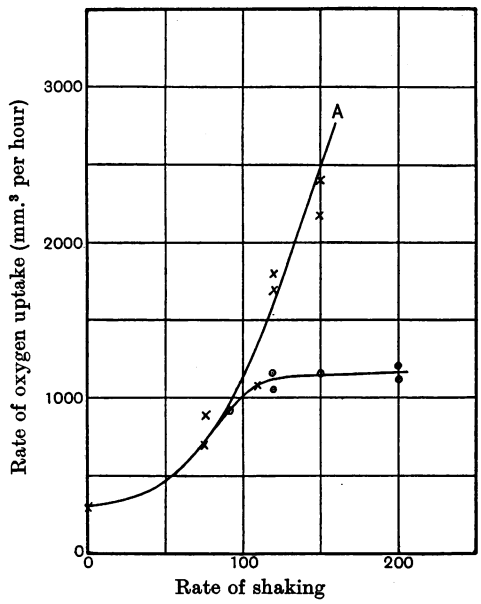


Fig. 8. Effect of rate of shaking on oxygen uptake by cysteine in presence of iron. The curve A is again reproduced from Fig. 6 for comparison. The crosses represent observations with 8 mg. cysteine + 0·04 mg. iron (as $FeSO_4$) in 3 cc. buffer. The circles were obtained with 8 mg. cysteine + 0·016 mg. iron. Temperature 14°.

diffusion effects. For instance in the case of oxygen uptake by alkaline pyrogallol solutions rates of the order of 10,000 mm.$^3$ per hour can be measured at the normal rate of shaking.

Fig. 9 shows the rates of uptake observed at different rates of shaking. In these experiments the pyrogallol solution was placed in the flasks, and the NaOH solution was placed in small hanging tubes as shown in Fig. 1. The manometers were then shaken in the thermostat for a few minutes, after which the NaOH was upset into the pyrogallol solution and readings commenced. By this means the rapid uptakes could be conveniently followed.

It will be seen that, although at the slower rates of shaking the reaction was evidently limited by diffusion, the velocity was quite unaffected by increasing the rate of shaking from 120 to 150, indicating that at 120 the true

velocity had been reached. This is also indicated by the fact that the velocity of uptake was exactly proportional to the pyrogallol concentration up to even higher velocities, as shown in Fig. 10. We have at present no wholly satisfactory explanation for the difference in behaviour between pyrogallol and the other systems studied.

It is now clear that the limiting rate of uptake which can be measured in the apparatus without being falsified by diffusion effects does not depend only upon the apparatus, but also upon the system under investigation. Our

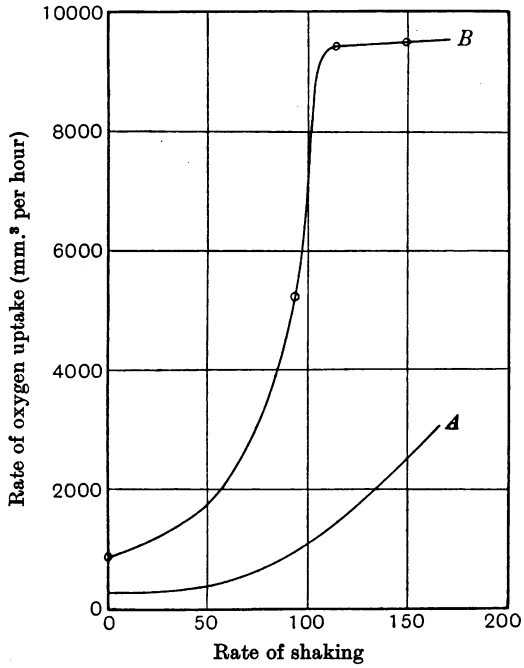


Fig. 9. Effect of rate of shaking on rate of oxygen uptake by alkaline pyrogallol. The curve A is again that of Fig. 6. Curve B was obtained as follows. 2·1 cc. water + 0·5 cc. of 0·5 % pyrogallol solution was placed in the flasks, and 0·4 cc. of 20 % NaOH in the hanging cups. After equilibration of the apparatus the NaOH was upset into the pyrogallol solution. Temperature 14°.

aim in this paper is not to provide a mathematical treatment which will account quantitatively for the effects observed, but rather to call attention to the absolute necessity, when studying any reacting system by means of the apparatus, of making certain that diffusion effects are not affecting the results, if the observations are to have any weight. Two main criteria are available. In the first place, it is obvious that if the reaction velocity is determined for a small amount with rapid shaking this will indicate the true value. If from this the value for a larger amount is then calculated, and the observed value corresponds with the calculated, it is clear that the true rate is still being measured. In other words, if the observed velocity is proportional to the amount of the system taken throughout the experimental range,

the experiments are valid. The second requirement is that the velocity of uptake must not be affected by the rate of shaking. Unless this is the case it is clear that diffusion effects are entering, and the significance of the results is doubtful. In studying a new system it cannot be predicted from the observed velocity alone whether diffusion effects are influencing the results or not, and one or other of the tests mentioned must be applied.

It can however be stated with some probability that with this type of apparatus at the usual rate of shaking and for reactions of average velocity,

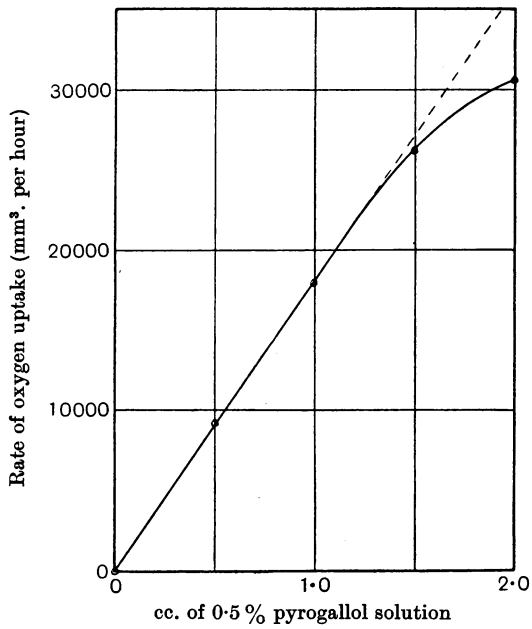


Fig. 10. Effect of amount of pyrogallol used on rate of oxygen uptake. The procedure was the same as in Fig. 9, except that the rate of shaking was maintained constant at 120, and the volume of 0·5 % pyrogallol solution taken was varied. The total volume of liquid was 3 cc. in each case as before.

diffusion effects do not play a considerable part with rates of uptake less than about 1000 mm.³ per hour at 15°. An examination of the literature shows that the rates of uptake measured with this type of apparatus have almost invariably been considerably lower than this—usually about 200–300 mm.³ per hour—and in that case it is unlikely that any considerable errors due to this cause have arisen. It is however not altogether uncommon in descriptions of work on oxygen absorption carried out in other types of apparatus to find such statements as "In order to obtain consistent results it was necessary to control carefully the rate of shaking." This of course indicates that such experiments are of doubtful significance.

Finally we may mention that none of our earlier work is affected by these results. We have always applied one or other of the above-mentioned tests

to ensure that diffusion errors were eliminated, and when we have worked with systems producing $CO_2$ we have employed the "KOH-paper" technique. Nevertheless in view of the large amount of work now being carried out with the apparatus it seemed desirable to publish the facts described in this paper.

SUMMARY.

1. The usual method for absorbing the $CO_2$ evolved in respiration experiments in the Barcroft apparatus is shown to be very inefficient, and an efficient method is described.

2. Experiments on the extent to which diffusion effects may limit the results obtained, when the Barcroft apparatus is used for measuring velocities of oxygen uptake, are described and discussed.

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Keilin (1929). *Proc. Roy. Soc. Lond.* B **104**, 206.