dioxide is of a competitive nature would account for such variation as occurs in the level of carbon dioxide required to bring about succinate accumulation in different plant tissues.

Whether or not very high concentrations of carbon dioxide in the atmosphere around a plant tissue could in fact result in a sufficiently high bicarbonate concentration in the cytoplasm to bring about the inhibition of DPNH-cytochrome *c* reductase and cytochrome *c* oxidase is not known. If this did occur the combined inhibition of these enzymes together with that of the succinic dehydrogenase would go a long way towards explaining the onset of carbon dioxide zymasis (Thomas, 1925) in atmospheres containing carbon dioxide in concentrations approaching 50% and abundant oxygen.

SUMMARY

1. The rates of three reactions concerned in the oxidation of succinate and reduced diphosphopyridine nucleotide by *Ricinus* mitochondria were determined in reaction mixtures in equilibrium with various concentrations of carbon dioxide at pH 7.4 and 25°.

2. Succinic-cytochrome *c* reductase was markedly inhibited by relatively low concentrations of carbon dioxide-bicarbonate but only slightly by corresponding concentrations of sodium chloride. The inhibition was competitive with respect to succinate concentration. For this and other reasons it is inferred that the carbon dioxide-sensitive component of the reductase is succinic dehydrogenase.

3. Reduced diphosphopyridine nucleotide-cytochrome *c* reductase was inhibited by high concentrations of sodium bicarbonate and other salts.

4. Cytochrome *c* oxidase was stimulated by low

concentrations and inhibited by high concentrations of sodium bicarbonate and other salts.

5. The results have been discussed in relation to the observed effects of carbon dioxide on the oxidation of succinate and other acids by intact mitochondria and the effects of carbon dioxide in the environment around intact plant tissues.

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The Measurement of the Diffusion of Oxygen through Respiring Tissue

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The diffusion coefficient of oxygen through non-respiring tissue has been measured by Krogh (1919). The figure he obtained was a little lower than that through water. Similar values have been obtained by other workers (Kirk & Johnsen, 1951). In general the diffusion coefficient of oxygen through tissue appeared to be equal to that through water multiplied by the fraction of the tissue composed of

water. It seemed that the transport of oxygen through tissues consisted of simple diffusion through the aqueous part uninfluenced by the solid part. That this is not universally true was shown by Longmuir & Roughton (1952), who found that the diffusion coefficient of nitrogen and carbon monoxide through haemoglobin solutions of the concentration found in red cells was about one-

tenth of that through water. We have found some evidence to suggest that there might be an increase in the diffusion coefficient of oxygen through respiring tissue, perhaps as a result of 'protoplasmic streaming'. It was necessary therefore to determine the diffusion coefficient of oxygen through respiring tissue. This has been done by Hill (1928), who measured the uptake of oxygen by anoxic muscle and obtained a figure similar to Krogh's. There were two possible sources of error. If the affinity of the tissue for oxygen was not great enough, Hill's equation might not be valid, and he was measuring the diffusion of oxygen through tissue that had been anoxic. Seifriz & Epstein (1941) have shown that 'protoplasmic streaming' is not resumed until some time has elapsed after the application of an inhibitory influence.

The application of Warburg's equation (Warburg, 1923) to the measurement of the diffusion coefficient of oxygen through respiring tissue has been considered previously (Longmuir, 1958; Longmuir & Bourke, 1959).

Warburg's equation states that

$$C_1 = C_0 - \frac{A d^2}{D 8},$$

where C_1 is pO_2 in the deepest cell layer of the slice; C_0 , pO_2 at the surface of the slice; A , oxygen uptake of the slice in ml./min./ml. of tissue; D , diffusion coefficient expressed as ml. of oxygen/cm.²/min. with a partial pressure gradient of 1 atm./cm.; d , slice thickness. This equation assumes that the respiration rate is independent of the partial pressure of oxygen. It has been shown (Longmuir, 1957) that this is true for cells and mitochondria above the critical oxygen concentration (the critical oxygen concentration is the lowest concentration that will produce the maximum respiration rate). In the present work we have found that it is true also for tissue slices. It seems reasonable to assume that when any part of the slice contains less oxygen than is necessary to maintain the maximum respiration rate of that region the slice as a whole will respire at less than its maximum rate. In other words, the critical oxygen concentration for the slice (C_s) will occur when the critical oxygen concentration for the terminal oxidase (C_c) in the middle of the slice has been reached. Thus it is possible to take C_s and C_c as particular values of C_0 and C_1 respectively. Below C_s Warburg's assumption does not hold and presumably his equation is invalid. We wished to ascertain if it is valid above this value. We found that the C_s of liver slices was about 20 times as great as the C_c of individual cells, so as a first step we neglected C_c and Warburg's equation reduced to

$$C_s = \frac{A d^2}{D 8}.$$

If this is true then C_s should increase as the square of the slice thickness. Experimentally we found if we increased the thickness from 0.1 to 0.5 mm., C_s increased at most only twofold, instead of the 25-fold rise required by this equation.

This finding might have been due to a structural factor; for example, the correct value of d to use in the equation might have been the thickness of the liver muralium. However, we obtained the same kind of results with liver, kidney and heart slices. As the histological pattern of all these tissues is different it seems unlikely that we are concerned with a problem of tissue architecture.

It is known that the metabolism of individual liver cells is different from that of slices (Laws & Stickland, 1956), so we were probably not justified in assuming that the C_c for cells in the slice is identical with that of individual cells and that therefore this figure should not be neglected. Warburg's equation could be rewritten as

$$d^2 = \frac{8DC_s}{A} - \frac{8DC_c}{A}.$$

If this equation is valid then a plot of d^2 against C_s/A should yield a straight line of slope $8D$ and intercept on the y axis of $-8DC_c/A$. The intercept on the x axis should be C_c/A . The experiments to be described were designed to determine to what extent the relationship between d^2 and C_s/A could be regarded as linear. If linearity holds then the value of the diffusion coefficient of oxygen through respiring tissue could be determined, and also the C_c of that terminal oxidase which has the lowest affinity for oxygen.

EXPERIMENTAL

Solutions. The slices were suspended in a solution of the following molarities: 0.05 M-KCl; 0.05 M-potassium phosphate buffer, pH 7.3; 0.12 M-potassium succinate. The water used was glass distilled, since it had been found that deionized water contained the substances responsible for the electrode poisoning found in earlier work (Longmuir, 1954). The slices respired fairly well in the absence of succinate, but the respiration rate decreased a little with time. The object of this work was to determine the oxygen concentration at which the respiration rate began to diminish, so it was necessary to ensure that the Q_{O_2} in the presence of enough oxygen, did not fall. This was achieved by adding succinate. It was found that a high concentration (0.12 M) was the minimum necessary to produce the maximum Q_{O_2} in the thickest slices used. The solution has the same freezing point as serum so it is presumably iso-osmotic.

Slices. These were cut with the McIlwain tissue slicer (McIlwain & Buddle, 1953). The tissues were obtained from 6-week-old rats. Slices of liver were cut from near the periphery of a lobe in order to avoid regions containing large blood vessels. At the same time very narrow slices were discarded as in the derivation of Warburg's equation diffusion

from the edges is neglected. Before cutting the kidney slices the organ was decapsulated and bisected. Heart-muscle slices were cut from the wall of the left ventricle.

The thickness of an individual slice was determined by dividing its weight by the specific gravity and the area. The area was measured by cutting out a piece of Paxalin of the same area as the slice. From the weight of this and its area/unit wt., the area of the slice was calculated. We found that the slices were slightly thinner than expected from the setting of the ratchet on the tissue slicer. The thickness of the slices did not alter during the period of measurement in the polarographic cell.

Methods

The polarographic method used for measuring oxygen concentrations and respiration rates is essentially that of Longmuir (1957) but with some modifications. It was necessary to file the gold electrode flush with the glass to prevent the slices becoming entangled. It was found possible to introduce the slices through the side arm but, as this obviously distorted them, we placed them in the solution in the cell before introducing the rubber disk and electrode. Measurements of oxygen concentration were carried out at 37°. We made the following observations: (1) Except in a few experiments the slices remained intact despite the rapid rotation of the electrode. When the slices did break up they did so at once and those experiments were abandoned. If the slices survived the first few minutes they remained intact for the rest of the period of measurement. (2) Provided that not too many slices were put in the cell at once they did not tangle with one another; nor did they produce any 'noise' on the recorder of the polarograph. In practice it was found that approximately 100 mg. dry wt., was a suitable amount. The cell contained 20 ml. of solution in which was dissolved approximately 300 µl. of oxygen. This quantity of oxygen was taken up by the slices in 5-10 min. However, with this rate of uptake the slope of the current-time curve was too low to permit the satisfactory use of the

Table 2. *Relation of thickness of rat-kidney slices to Q_{O_2} and the critical oxygen concentration*

C_s represents the critical oxygen concentration for rat-kidney slices and d their thickness.

d (mm.)	Q_{O_2}	C_s (mm)
0.11	34	0.19
0.15	31	0.18
0.20	37	0.31
0.26	30	0.24
0.26	60	0.50
0.35	22	0.37
0.35	19	0.22
0.44	19	0.35
0.44	23	0.30
0.44	17	0.30
0.52	13	0.29
0.52	20	0.24
0.52	15	0.28
0.52	21	0.37

Table 3. *Relation of thickness of rat-heart slices to Q_{O_2} and the critical oxygen concentration*

C_s represents the critical oxygen concentration for rat-heart slices and d their thickness.

d (mm.)	Q_{O_2}	C_s (mm)
0.20	73	0.28
0.20	53	0.21
0.26	56	0.34
0.26	41	0.22
0.26	59	0.27
0.35	50	0.28
0.35	75	0.26
0.52	38	0.35
0.52	43	0.39

differential circuit to demonstrate breaks in the respiration rate-time curves. The trace we obtained on the polarograph showed a straight line initially and then the slope began to diminish progressively becoming zero at zero oxygen concentration. Duplicate measurements were made after fresh solution was run in. Provided that this was done slowly enough no slices were washed out. Duplicate values of C_s , the oxygen concentration at which the trace ceased to be linear, agreed with one another. From the slope of the linear portion the respiration rate could be determined. After sufficient replicate measurements had been made the slices were washed out of the cell with a solution of known oxygen concentration and a calibration point was obtained on the recorder of the polarograph. The slices were then dried for 2 hr. at 105°. From the dry weight and the slope of the linear portion of the trace the Q_{O_2} (µl. of O_2 /mg. dry wt./hr.) was calculated.

RESULTS

The results are shown in Tables 1-3. When the square of the thickness is plotted against C_s/Q_{O_2} the points should lie on a straight line, if Warburg's equation is valid. Fig. 1 shows the results obtained with rat-kidney slices. The correlation coefficients between these two variables were 0.661, 0.846 and 0.885 for liver, kidney and heart respectively. Each

Table 1. *Relation of thickness of rat-liver slices to Q_{O_2} and the critical oxygen concentration*

C_s represents critical oxygen concentration, and d thickness. The slices marked (a) came from the same liver and similarly for those marked (b).

d (mm.)	Q_{O_2}	C_s (mm)
0.11 (a)	58	0.26
0.11 (b)	56	0.20
0.11	38	0.16
0.15	50	0.31
0.15	45	0.22
0.15	63	0.27
0.15	49	0.27
0.22	44	0.26
0.22	43	0.29
0.22	68	0.32
0.22	45	0.24
0.26	62	0.25
0.26 (a)	43	0.26
0.26 (b)	46	0.19
0.26	41	0.34
0.36	20	0.22
0.52	42	0.33
0.52	27	0.35
0.52	32	0.22
0.52	26	0.24

of these values would occur less than once in 100 times by chance if there were no association.

These results are compatible with Warburg's equation and the regression lines and intercepts give measures of the diffusion coefficients and the critical oxygen concentrations of the terminal oxidase. The diffusion coefficients and critical oxygen concentrations are shown in Table 4.

In Warburg's equation the oxygen consumption and the concentration of oxygen are expressed in the units given in the introduction. We have expressed our experimental results for oxygen consumption in Q_{O_2} and oxygen concentration in moles/l.

Fig. 1. A plot of the square of the thickness of rat-kidney slices against the ratio of the critical oxygen concentration to the Q_{O_2} . The middle line is the regression line and the others show the 95% confidence limits of the intercept.

DISCUSSION

The diffusion coefficients given in Table 4 are much higher than those found by Krogh (1919) and the critical oxygen concentrations are higher than those found in individual cells (Longmuir, 1957). These high figures suggest two things. First, even very thin slices only two or three cells thick have a critical oxygen concentration that is much higher than that of individual cells. Secondly, an increase in the thickness of a slice has a very small effect on its critical oxygen concentration, so it appears that oxygen can permeate rather freely.

It seems unlikely that the high concentration of succinate we used could have had an appreciable effect since, in the absence of any substrate, results of the same order were obtained. The C_c values were lower in the absence of succinate but approximately in proportion to the decrease in Q_{O_2} . Both sets of values were approximately halved. We have not quoted any figures obtained without added substrate as it was impossible to obtain exact replicates owing to the continued slow fall in the Q_{O_2} .

If the C_c truly is high, although this is not established since the standard error is so large, then cells in slices may contain a terminal oxidase in addition to cytochrome oxidase. Laws & Stickland (1956) have shown that liver cells have a greater respiratory activity than that due to the mitochondria they contain.

Since the figures we have obtained for the diffusion coefficient of oxygen through respiring tissues are higher than those found for non-respiring tissues the explanation must lie in some property associated with respiration. Some biological systems show a rhythmic ebb and flow of protoplasm (Seifriz, 1937). Such a movement in tissues might be enough to transport oxygen actively. It is well known that in the measurement of diffusion coefficients very slight vibration can increase enormously the apparent value. It seems likely that the mitochondria which appear to imbibe water continuously while they are actively extruding it may set up considerable microturbulence, particularly if they take up water over their whole surface and extrude it at certain 'spots'.

Seifriz & Epstein (1941) have shown that protoplasmic streaming can be stopped by a number of agents, such as mechanical violence, carbon dioxide and anaesthetics. In preliminary experiments we

Table 4. Diffusion coefficients of oxygen through the three tissues and the critical oxygen concentration of the terminal oxidase calculated from the slopes and intercepts of the regression lines (d^2 on C_c/Q_{O_2})

Organ	$10^{-4} \times \text{Slope}$	$10^{-4} \times \text{s.e. of slope}$	$10^4 D_{O_2}$ (Krogh's units)	Intercept	s.e. of intercept	C_c (mm)
Kidney	1.52	0.29	0.63	-0.048	0.120	0.093
Liver	3.92	0.88	1.64	-0.140	0.141	0.168
Heart	3.74	0.74	1.54	-0.093	0.108	0.126

have found that carbon dioxide increases the C_s value by up to 50%. We have not yet sufficient results to publish but they do lend support to the concept of an active intracellular transport of oxygen.

SUMMARY

1. The validity of Warburg's equation for diffusion of oxygen through tissue slices is discussed.

2. A method of studying the respiration of tissue slices is described which can be used to measure both their Q_{O_2} and their critical or limiting oxygen concentration.

3. The results obtained with this method support the validity of Warburg's equation.

4. The equation is used to calculate values for the diffusion coefficient of oxygen through respiring liver, heart and kidney slices, and values for the minimum concentration of oxygen necessary to saturate a terminal oxidase in those tissues.

5. Unexpectedly high values for the diffusion coefficients and possibly for the critical oxygen concentration of the terminal oxidase were obtained. Their significance is discussed.

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Properties of L- α -Glycerophosphate Oxidase and its Role in the Respiration of *Trypanosoma rhodesiense*

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An aspect of host-parasite relationships of significance in chemotherapy concerns the biochemical differences which exist between pathogenic trypanosomes and the tissues of their vertebrate and invertebrate hosts. One important difference is that the blood-stream forms of many trypanosomes do not contain detectable cytochrome pigments (Brand, 1951; Ryley, 1956; Fulton & Spooner, 1959). Despite this, such parasites are notable for their high rates of respiration; for example, it has been calculated that the Q_{O_2} (glucose) of the African trypanosome, *Trypanosoma rhodesiense*, is about 50 times that of the tissues of its mammalian host (Brand, 1951).

The present work deals with hydrogen-transport systems present in a strain of the human parasite, *T. rhodesiense*, which has been maintained by syringe-passage in rats for many years. As far as is known the metabolism of this strain does not differ

from that of the parasite freshly isolated from a human patient (Grant & Fulton, 1957; Fulton & Spooner, 1959). The results are mainly concerned with the properties of a L- α -glycerophosphate oxidase whose reaction with oxygen does not appear to be mediated either by pyridine nucleotides or a cytochrome system. The high activity and substrate specificity of this oxidase indicates that it can account for most of the respiration of the intact trypanosome *in vitro*.

A brief account of some of these results has already been presented (Grant & Sargent, 1959).

EXPERIMENTAL

Organism. The 'Liverpool' strain of *T. rhodesiense*, its maintenance in rats and the isolation of the parasite from infected rat blood have been described previously (Grant & Fulton, 1957).