Is Oxygen Consumption of Surviving Tissues Determined by the Oxygen Tension of the Suspension Medium?

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Oxygen Consumption, Isolated Tissue, Influence of Shaking Rate, Influence of Oxygen Tension

Oxygen tension in the suspension medium and oxygen uptake of the isolated diaphragm of the mouse have been determined simultaneously with systematic variation of shaking frequency and oxygen concentration of the gas phase. Oxygen tension in the suspension medium reaches its final value during the usual equilibration period of 10 minutes and remains constant for the whole measuring period. Each combination of shaking frequency and oxygen concentration of the gas phase results in a reproducible value of oxygen tension in the medium. Equal oxygen tension in the fluid phase can be attained by different combinations of shaking frequency and oxygen concentration of the gas phase. Oxygen uptake at the same oxygen tension of the suspension medium can be very different; it is the higher the higher the shaking rate in the combination. On the other hand the same oxygen consumption of the tissue samples can be observed with highly different oxygen tensions in the medium. It must be concluded from the data in this paper, that the influence of shaking rate per se on the oxygen uptake of isolated tissue can be of the same order as the influence of oxygen tension.

The oxygen uptake of isolated tissues in Warburg experiments increases under certain conditions with increasing shaking frequency. According to the accepted theory this can occur only if the oxygen tension of the suspension medium is below the critical level for total respiration. The increase in oxygen uptake in this situation is considered to be the consequence of the increase in oxygen tension of the medium due to increased convection by higher shaking frequencies. Above the critical level oxygen uptake is supposed not to react to further increases of oxygen tension. Maximum oxygen uptake reached by continuously increasing shaking frequency is considered as evidence that the critical level of oxygen tension in the medium has been reached [1–17].

The present concept is based on observations of the relation of shaking frequency to oxygen uptake at a given oxygen concentration of the gas phase only. The interpretation of this relation presupposes that shaking rate acts by changing the convection rate between gaseous and fluid phase of the respirometer vessels and in this way determines the O_2-tension of the suspension medium and consequently the oxygen uptake of tissue samples. Measurements of the actual changes of oxygen tension with increasing shaking frequency and of the relation of increase in oxygen tension to increase in oxygen uptake have not been performed to our knowledge. The possibility that shaking frequency per se influences the metabolic rate of isolated tissues has apparently not been considered.

We have systematically measured the oxygen tension of the suspension fluid simultaneously with the oxygen uptake of isolated diaphragms. Oxygen concentrations of the gas phase and shaking rates in these experiments were varied within wide limits. Under the conditions tested in this investigation the oxygen tension of the fluid phase reached a stable value during the equilibration period of 10 minutes. This value remained constant for the following 4 h during which oxygen uptake of tissue samples was measured simultaneously with the oxygen tension. Each combination of O_2-concentration in the gas phase and of shaking frequency resulted in a particular value of the oxygen tension in the suspension medium and of oxygen uptake by the tissue samples. The oxygen tension of the suspension medium existing with the different combinations of oxygen concentration in the gas phase and shaking frequency are connected by almost linear functions (Schmidt and Pichotka [18]).

The essential point of the results of the present investigation is that identical oxygen tensions of the suspension medium can be attained by widely different combinations of oxygen concentration of the gas phase and of shaking rate. This offers the possibility to decide whether the rate of oxygen uptake of isolated tissues is exclusively determined

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by the oxygen tension of the suspension medium or whether shaking rate per se is of importance.

It is the objective of this study to answer this question by comparing

1. the oxygen uptake of tissue samples at identical oxygen tensions of the medium resulting from different combinations of shaking frequency and oxygen concentration of the gas phase,

2. the oxygen tensions of the medium in experimental situations in which the same oxygen uptake of isolated tissues is observed with different combinations of shaking frequency and oxygen concentration of the gas phase.

Methods

The measurements were performed on isolated whole diaphragms of mice at 37 °C and in Medium II of Krebs [11] as suspension fluid. All technical details have been reported in former papers [19]. Thermostats of the type S 85 (Fa. Braun, Melsungen) were used, with special electronic equipment for the maintenance of constant shaking rates. Oxygen concentrations of the gas phase of approximately FO_2 = 0.4, 0.6, 0.8, and 1.0 were used; shaking frequencies varied from 30 – 100 cycles/min. The resulting oxygen tensions in the suspension fluid were measured with modified teflon-tipped platinum electrodes [20].

Oxygen uptake was measured at least for four hours. Readings of oxygen uptake were taken with 5 min intervals. Oxygen consumption was calculated with a computer programme at the G.M.D. Bonn. The results are given as mean values for 30 min periods; the first 30 min period is omitted from evaluation as explained formerly [21]. For technical reasons in most experiments O_2-tension could be measured for 3 h only. The simultaneous measurement of oxygen uptake and oxygen tension of the suspension medium was performed 3 to 8 times with each of the combinations of oxygen concentration in the gas phase and shaking frequency. Altogether 272 simultaneous measurements were performed. After the high reproducibility of the oxygen tension in the suspension medium had been established it was sufficient to measure oxygen uptake only.

The mean thickness of the whole diaphragm of adult mice was determined in a representative group with 0.25 ± 0.03 mm (S.D. [22]). In a later investigation a value of 0.27 ± 0.02 (S.D.) was obtained. The highest metabolic rate observed under steady state conditions was 30 ml O_2/(kg·min); in most cases 25 ml O_2/(kg·min) was not exceeded. The critical oxygen tension for total respiration of most samples used in this investigation was in the range of 100 Torr, the highest possible was as high as 150 Torr [$d = 0.3$ mm, $A = 30$ ml O_2/(kg·min), $D = 1.65 \times 10^{-5}$ cm^3/(cm·min·atm)]. In all experiments reported in the following the oxygen tension of the medium was far above the critical value.

In the four groups of experiments, described in the following, measurements on 89 diaphragms were made with at least 48 readings of oxygen uptake on each sample; that is altogether 3700 data on oxygen uptake.

Results

In Fig. 1 oxygen tension in the suspension medium (PO_{2Fl}) is presented as a function of shaking frequency (cpm) and of oxygen concentration in the gas phase (FO_2). The essential results in this diagram are:

1. At each shaking frequency oxygen tension of the medium increases almost linearly with the oxygen tension of the gas phase.

2. At each shaking frequency the oxygen tension of the medium reaches almost the same fraction of

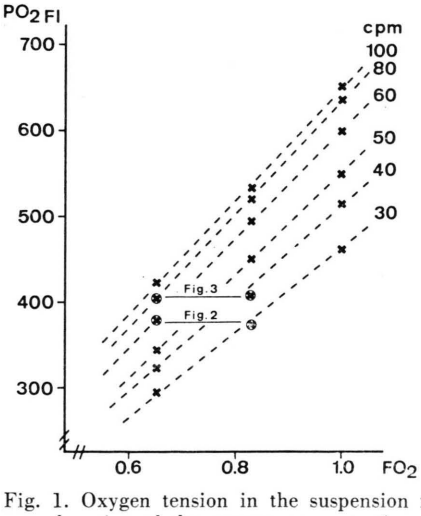

Fig. 1. Oxygen tension in the suspension medium (PO_{2Fl}) as a function of the oxygen concentration of the gas phase (FO_2) and of shaking frequency (cpm). Two pairs of points with the same oxygen tension of the medium (PO_{2Fl}) are connected; for these points the simultaneously measured oxygen tension and oxygen uptake are presented in Figs 2 and 3.

the oxygen tension of the gas phase; there is a slight increase of this fraction with increasing oxygen tension.

3. The combinations of oxygen tensions of the gas phase and of shaking frequencies which result in the same oxygen tension of suspension medium are located on parallels to the x-axis.

It is obvious that identical oxygen tensions of the suspension medium can be produced by many different combinations of shaking frequency and oxygen concentrations of the gas phase. This is especially evident in the upper range of O_2-concentrations in the gas phase ($FO_2 = 0.6 - 1.0$). With shaking frequency increasing from 30 to 100 cpm and $FO_2 = 0.2$ in the gas phase oxygen tension of the fluid phase increased from 87 to 123 Torr or by 36 Torr. With the same variation of shaking frequency and $FO_2 = 1.0$ in the gas phase the oxygen tension of the fluid phase increased from 465 to 655 Torr or by 190 Torr. Four groups of measurements in this diagram distinguished by special symbols are used for the construction of Figs 2 and 3.

In Fig. 2 the rate and time course of oxygen uptake of two groups of tissue samples is compared which were measured at the same oxygen tension of the fluid phase resulting from different combinations of oxygen concentrations of the gas phase and of shaking frequencies. The lower curve (I) of oxygen uptake [17 ml O_2/(kg·min)] has been recorded with $FO_2 = 0.83$ in the gas phase and a shaking frequency of 30 cycles/min; the upper curve [26 ml O_2/(kg·min)] was recorded with $FO_2 = 0.60$ in the gas phase and a shaking rate of 60 cycles/min. The mean oxygen tension of the suspension medium in the steady state for these two sets of conditions was 378 ± 10 and 382 ± 11 Torr respectively. With practically identical and constant oxygen tensions of the fluid phase for both groups the rate of

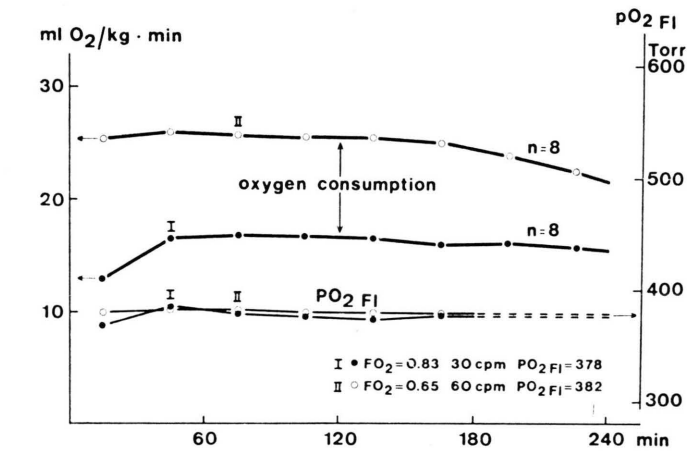

Fig. 2. The oxygen uptake of two groups of tissue samples during a measuring period of 4 hours. Oxygen tensions of the medium were the same for both groups but resulting from different combinations of shaking frequency (cpm) and oxygen concentration of the gas phase (FO_2). In each group 8 diaphragms with 48 data of oxygen uptake from each sample.

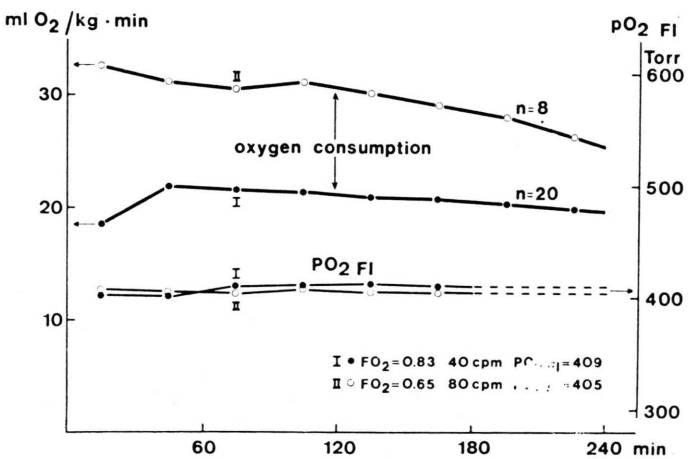

Fig. 3. The same situation as in Fig. 2, with higher oxygen tensions of the suspension medium. In the one group 20 and in the other group 8 diaphragms. 48 values of oxygen uptake from each sample.

oxygen uptake differs by roughly 50%. In the lower curve with the low shaking frequency (30 cpm), the oxygen uptake remains constant for four hours; in the upper curve with the higher shaking frequency (60 cpm) oxygen uptake decreases slowly in the first part of the measuring period and more rapidly after 2 h although oxygen tension has not measurably changed.

Fig. 3 displays the same result in another range of oxygen tension of the suspension medium. The lower curve of oxygen consumption [21 ml O_2/(kg·min)] is recorded with $FO_2 = 0.80$ in the gas phase and a shaking frequency of 40 cycles/min. The oxygen consumption demonstrated by the upper curve [30 ml O_2/(kg·min)] was recorded with $FO_2 = 0.65$ in the gas phase and a shaking frequency of 80 cycles/min. The mean oxygen tensions of the fluid phases reached 409 ± 12 and 405 ± 17 Torr respectively. In the same way as in Fig. 2 the oxygen uptake at the lower shaking frequency remains practically constant over the measuring period while the higher oxygen uptake combined with the higher shaking frequency at the same oxygen tension of the fluid phase is unstable from the beginning. Again for both groups oxygen tensions of the medium are absolutely constant, although oxygen uptake in one case decreases continuously.

From the results represented in Figs 2 and 3 it is obvious that with the same oxygen tension of the suspension medium the oxygen uptake of isolated diaphragms may be very different, depending on the different shaking frequencies existing in this situation. In the following diagrams (Figs 4 and 5) experiments are represented in which the same oxygen uptake has been recorded with widely different oxygen tensions of the suspension medium.

The two groups of experiments compared in Fig. 4 display an almost identical rate and time course of oxygen uptake. Oxygen uptake is constant during the first two hours thereafter it declines moderately in both curves. The mean values of oxygen uptake for the two groups are 25.62 ± 2.55 (S.D.) ml O_2/(kg·min) and 26.04 ml $O_2 \pm 2.87$ (S.D.) ml O_2/(kg·min). The values cannot be distinguished statistically. The oxygen tensions of the suspension medium measured with curve I is 382 ± 11 Torr arising from $FO_2 = 0.65$ in the gas phase and a shaking frequency of 60 cpm; the oxygen tension in the suspension medium with curve II is 232 ± 19 Torr originating from $FO_2 = 0.4$ in the gas phase and a shaking rate of 80 cpm. The values of oxygen tension in the suspension medium are constant during the measuring period and significantly different with $p \ll 0.001$.

In Fig. 5 the measurements of two experimental groups are shown with essentially the same result as in Fig. 4. The oxygen uptake for the two groups of measurements is identical during 3 periods of 30 min. Thereafter the oxygen uptake in the one group (I) remains stable, while the other declines moderately (II). The mean values of oxygen uptake are 21.40 ± 7.74 (I) and 20.90 ± 2.21 ml O_2/(kg·min) (II) respectively. The oxygen tension in the suspension medium belonging to curve I is

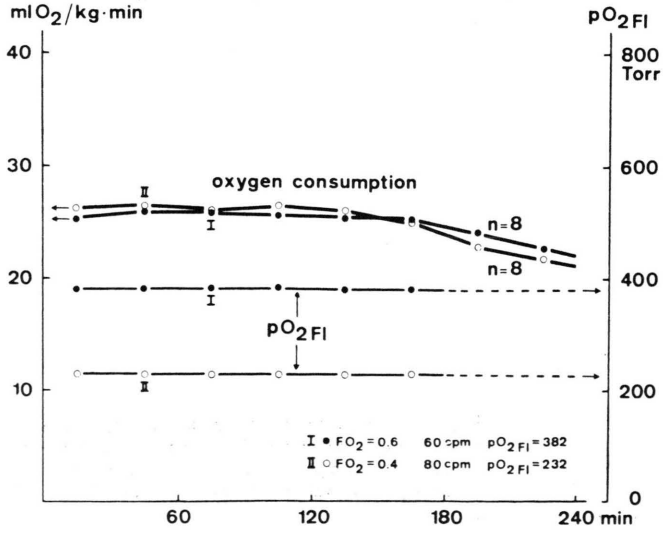

Fig. 4. The two groups of experiments display the same rate and time course of oxygen uptake, with different oxygen tensions of the suspension medium. Each curve represents the oxygen uptake of 8 diaphragms.

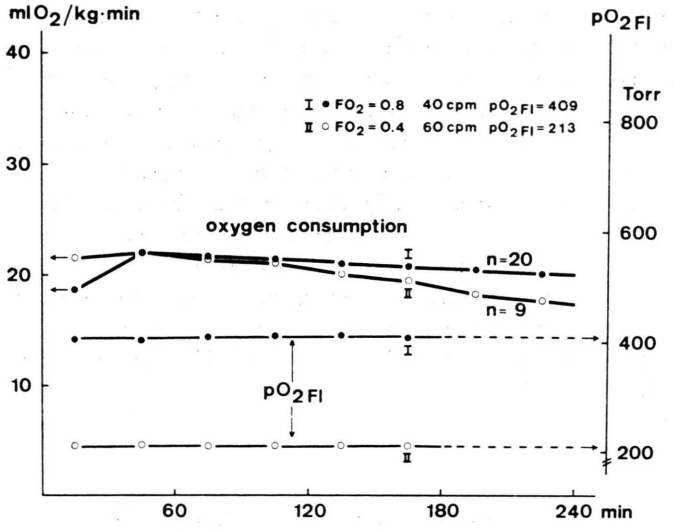

Fig. 5. Essentially the same observation as in Fig. 4. While the oxygen uptake remains constant in curve I all over the measuring period, curve II starts to decline moderately after 2 hours. Total oxygen uptake represented by the two curves is almost identical, but oxygen tension of the medium differs by a factor 2. In curve I measurements on 20 diaphragms and in curve II on 9 diaphragms.

409 ± 12 Torr originating from $FO_2 = 0.8$ in the gas phase and a shaking rate of 40 cpm; the oxygen tension of the suspension medium belonging to curve II is 213 ± 5 Torr originating from $FO_2 = 0.4$ in the gas phase and 60 cpm. The oxygen tensions of the medium differ almost by a factor 2, ($p \ll 0.001$).

Summary and Discussion

The experimental observations reported in this paper show clearly that there is no simple relationship between the oxygen tension of the suspension medium and oxygen uptake of isolated tissues. Under experimental conditions which result in the same oxygen tension of the suspension medium oxygen uptake of the diaphragms can be highly different (Figs 2 and 3). On the other hand the same oxygen uptake of isolated tissues can be observed with highly different oxygen tensions of the suspension medium (Figs 4 and 5).

As can be seen from Table I these statements are statistically well supported. For the two pairs of experimental groups compared in Figs 2 and 3 the oxygen tensions of suspension medium cannot be distinguished ($p > 0.1$), while the oxygen uptake is significantly different ($p \ll 0.001$) by approximately 50% of the lower value. The two groups of experiments compared in Figs 4 and 5 cannot be distinguished with regard to oxygen uptake ($p > 0.1$) but the oxygen tensions in both pairs differ significantly ($p \ll 0.001$) by 70 to 90% of the lower value.

Table I. The critical oxygen tension for total respiration of diaphragms of mice calculated according to Warburg from the thickness of the samples and maximum metabolic rate under steady state conditions was in most cases close to 100 Torr; the highest possible value was 150 Torr. As can be seen from the data in this table the oxygen tensions of the suspension medium (PO_{2Fl}) extend from 213—409 Torr.

Fig.	Experiment Nr.	n	FO_2	cpm	PO_{2Fl}	Significance	VO_2	Significance	VO_2/PO_{2Fl}	Significance
2	287	8	0.83	30	378 ± 11	$p > 0.1$	16.56 ± 1.75	$p \ll 0.001$	0.044 ± 0.003	$p \ll 0.0005$
	284	8	0.65	60	382 ± 10		25.62 ± 2.55		0.067 ± 0.003	
3	287	20	0.83	40	409 ± 17	$p > 0.1$	21.40 ± 1.74	$p \ll 0.001$	0.052 ± 0.0035	$p \ll 0.0005$
	284	8	0.65	80	405 ± 2		30.60 ± 3.64		0.076 ± 0.004	
4	284	8	0.65	60	382 ± 10	$p \ll 0.001$	25.62 ± 2.55	$p > 0.1$	0.067 ± 0.003	$p \ll 0.0005$
	283	8	0.40	80	232 ± 19		26.04 ± 2.87		0.112 ± 0.009	
5	287	20	0.83	40	409 ± 17	$p \ll 0.001$	21.40 ± 1.74	$p > 0.1$	0.052 ± 0.0035	$p \ll 0.0005$
	283	9	0.40	60	213 ± 5		20.90 ± 2.21		0.098 ± 0.004	

A quantitative expression for the different relation of oxygen tension of the medium to the simultaneous oxygen consumption is given by the quotient of these two values VO_2/PO_{2Fl}. This quotient for our data is presented in the last column of Table I. For the four pairs of experimental conditions compared in Figs 2 to 5 the quotients are significantly different with $p \ll 0.001$.

From the reported measurements it must be concluded that oxygen uptake of tissue samples does not depict the oxygen tension existing in the suspension medium. At the same oxygen tension of the medium oxygen uptake is the higher the higher the shaking frequency. Under normal conditions the contributions of changes in shaking rate to oxygen uptake of isolated tissues obviously can be of the same order as the possible changes in oxygen tension of the medium. It is therefore impossible to draw conclusions on the existing oxygen tension from changes in oxygen uptake induced by changes in shaking rate. It was already stated that the highest value of the critical oxygen tension for total respiration of isolated diaphragms does not exceed 150 Torr. All measurements reported in this paper have been performed with oxygen tensions of the medium far above the critical value of the Warburg equation. (See Table I.)

[1] H. Aebi, Helv. Physiol. Acta **8,** 525 (1950).
[2] H. Aebi, Die Bedeutung der Bestandteile des Serums für die Gewebsatmung (unter besonderer Berücksichtigung der Methodik der überlebenden Organschnitte nach O. Warburg), Habil. Bern 1951.
[3] W. R. Amberson, Biol. Bull. **55,** 79 (1928).
[4] A. Canzanelli and D. Rapport, Amer. J. Physiol. **127,** 296 (1939).
[5] F. N. Craig and H. K. Beecher, J. Neurophysiol. **6,** 135 (1943).
[6] M. Cutting and R. A. McCance, J. Physiol. **106,** 405 (1947).
[7] J. Field, Method of Med. Research **1,** 289 (1948).
[8] D. G. Friend and A. B. Hastings, Proc. Soc. exper. Biol. Med. **45,** 137 (1940).
[9] F. A. Fuhrman and J. Field, Arch. Biochem. **6,** 337 (1945).
[10] M. Ikeziri, Kunamoto Med. J. **9,** 22 (1956).
[11] H. A. Krebs, Biochim. Biophys. Acta **4,** 249 (1950).
[12] S. Minami, Biochem. Z. **142,** 334 (1923).
[13] J. R. Robinson, Biochem. J. **45,** 68 (1949).
[14] E. Shorr, Cold Spring Harbor Symposia **7,** 323 (1939).
[15] O. Warburg, Ergeb. Physiol. **14,** 253 (1914).
[16] O. Warburg, Biochem. Z. **142,** 317 (1923).
[17] O. Warburg, Biochem. Z. **152,** 51 (1924).
[18] H. J. Schmidt and J. Pichotka, in Vorbereitung.
[19] J. Pichotka, H. J. Schmidt, and L. Johannessen, XXVII. Int. Congress of Physiol. Sciences, Paris 1977.
[20] U. Gleichmann and D. W. Lübbers, Pflüg. Arch. **271,** 431 (1960).
[21] J. Pichotka u. H. J. Schmidt, Die Abhängigkeit der Stoffwechselgröße des isolierten Muskelgewebes (Mäusezwerchfell) von den Meßbedingungen. Kritik an der bisher üblichen Warburg-Methodik, in: Muskelstoffwechsel, körperliche Leistungsfähigkeit und Diabetes mellitus (K. Jahnke, H. Mehnert u. H. E. Reis, Hrsg.), S. 47–60, Schattauer-Verlag, Stuttgart 1977.
[22] H. Weigand, Die Beziehung zwischen Sauerstoffspannung und Sauerstoffaufnahme isolierter Gewebe, untersucht an ungeschnittenen Organen. Dissertation Bonn 1968.
[23] H. J. Schmidt, Über die Messung der O_2-Aufnahme des isolierten Gewebes. Kritik an den bisherigen Verfahren. Habilitation Bonn 1977.