Effect of Mechanical Traumatization on Rate and Constancy of Oxygen Uptake of Isolated Diaphragms

H. J. Schmidt, M. Sommer, and J. Pichotka

Physiologisches Institut I der Universität Bonn, Nussallee 11, D-5300 Bonn 1

Z. Naturforsch. **34 c**, 400–405 (1979); received June 27, 1978/February 7, 1979

Isolated Tissues, Oxygen Consumption, Mechanical Traumatization, Division of Tissue Samples

Diaphragms of mice were traumatized by division parallel or transversally to the muscle fibres. The extent of traumatization was varied by the degree of subdivision. Oxygen uptake was measured by the Warburg technique. Undivided diaphragms as control displayed constant oxygen uptake for the four hours of observation. With divided diaphragms oxygen uptake was unstable. Division parallel to the muscle fibres resulted in continuous decrease of oxygen uptake after a normal initial phase; the decline was significantly steeper with increasing extent of traumatization. Division transversally to the muscle fibres resulted in an initial increase of oxygen uptake followed in a second phase by a continuous decrease. The initial increase as well as the final decrease of oxygen uptake was more pronounced with higher extent of subdivision.

Introduction

The determination of metabolic rates of isolated tissues by the Warburg technique requires samples which are thin enough that the necessary oxygen supply can be provided by diffusion from the surface. There are few organs or tissues which are naturally below the limiting thickness of the Warburg formula, such as the diaphragm, the intestinal wall and some skeletal muscles of small laboratory animals. In most cases the organs under investigation have to be cut to slices with traumatization of a large number of cells. This is especially true for the parenchymatous organs such as liver and kidney. It is still a matter of discussion in what direction and to what extent the oxygen uptake of isolated tissues is changed by this traumatization.

The diaphragm of small animals is used in many investigations on intermediary metabolism and on factors influencing metabolic rate [1, 2]. It can be easily divided into equal halves, one half serving as control for the changes induced in the other by experimental conditions. While examining this procedure we observed that rate and stability of oxygen uptake may already be significantly influenced by the division of diaphragms into two or four parts. We interpreted this as effect of traumatization. Under this hypothesis we used the subdivision of diaphragm into smaller parts as a model of quantifiable mechanical injury on rate and constancy of oxygen uptake.

Reprint requests to Dr. H. J. Schmidt.

0341-0382 / 79 / 0500-0400 $ 01.00/0

Methods

Adult male mice of an inbred strain (Agnes Bluhm), weighing from 22 to 27 g were used. They had been raised and kept at 22 ± 1.0 °C room temperature with 15 h daytime ($6^{00} - 21^{00}$ h) and free access to standard food and water supplemented with ascorbic acid.

The preparation was performed under sterile conditions. After decapitation the animals were completely skinned. Both cavities were opened in the midline and the contents removed. The diaphragms were carefully dissected along the insertion line at the thoracic wall and the crura cut close to the vertebral column. In this way traumatization of the muscular tissue by preparation was minimized. The mean thickness of the diaphragms determined from weight and surface was 0.25 ± 0.03 mm. Experimental traumatization was performed in two ways. In the first group the diaphragms were divided by radial sections, that is parallel to the muscle fibres, into 4, 8, 16 and approximately 32 parts of similar size. Up to 16 parts the dissection could be performed rather exactly; in the last step the 16 segments were usually divided uncontrolled into 32 or more pieces. By sections parallel to the muscle fibres the muscle cells are damaged as little as possible. The preparation of the second group was intending maximal traumatization of the muscle fibres by

transverse sections. In order to obtain four parts the diaphragms were divided along the lines of an equilateral triangle pointing towards the sternum. Eight parts were obtained by dividing the four parts again transversely to the muscle fibres. In both groups all segments belonging to one diaphragm were given into the same respirometer vessel. Measurements on undivided diaphragms served as controls.

The oxygen consumption was measured by the direct Warburg technique [1, 3, 4], in Medium II of Krebs [5], with 100% oxygen in the gas phase and carbon dioxide being absorbed by 2 N KOH in the center well. Temperature was 37.0 ± 0.02 °C and shaking rate 40 cycles per minute. This low shaking rate was chosen after it had become obvious, that shaking rate itself highly influences rate and constancy of oxygen uptake [6–8]. Readings of oxygen uptake were taken with intervals of 5 minutes. Under the described conditions undivided diaphragms regularly presented constant oxygen uptake for 4 hours or more.

The high shaking rates of the Warburg technique had been introduced to insure equilibration of gas tensions between the gaseous and liquid phases. With low shaking rates as in this investigation the equilibration of O_2-tension between the two phases will not be attained. The O_2-tension of the liquid phase had therefore to be determined polarographically. Under the conditions used in this investigation and in the presence of normally respiring diaphragms in the respirometer vessels the mean oxygen tension of the liquid phase was 534 ± 27 Torr ($n = 288$). Even under the most unfavourable conditions in single experiments ($d = 0.3$ mm, $A = 50$ ml O_2/kg·min) critical oxygen tension according to the Warburg formula did not exceed 250 Torr.

Oxygen consumption in this paper is given in ml O_2 per kg and minute. This unit can easily be transformed into the other units used in literature.

$$\text{ml } O_2/\text{kg} \times \text{min} = 0.06 \times \text{ml } O_2/g \times h = 3.3 \times QO_2 .$$

The results reported are based on measurements on 142 diaphragms with approximately 6000 data on oxygen uptake. Less than 3% of the primary readings of oxygen uptake had to be discarded. The failures were mainly due to the spilling of KOH from the center well into the suspension medium. In this way the pH of the suspension medium was changed to the alkaline side and oxygen uptake of the tissue samples reduced. We excluded all measurements with pH-values of the suspension medium $\geqq 7.8$ at the final control. Calculations were performed with the IBM 370 of the RHRZ Bonn.

Results

The time course of oxygen consumption of undivided diaphragms and of diaphragms divided into 4 to 32 pieces by sections parallel to the muscle fibres is presented in Fig. 1. The essential data of these experiments are compiled in Table I.

In the initial phase of the measurements the time course of oxygen uptake of untraumatized and traumatized diaphragms is almost indentical. The regular slight increase in oxygen uptake from the first to the second 30 min period (+5 to 10%) is apparently the same in both groups. With this initial increase untraumatized diaphragms (controls) reach the final level of oxygen uptake which is kept constant for 4 to 6 hours. Oxygen uptake of the traumatized diaphragms starts to decline soon after reaching the level of the controls. The steepness of the decline

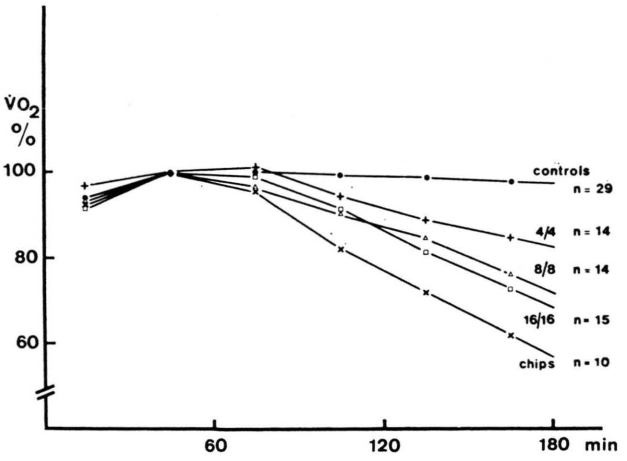

Fig. 1. Time course of oxygen uptake of diaphragms divided parallel to the muscle fibres into 4 to 32 pieces; undivided diaphragms as control. The values are given in per cent of the mean oxygen uptake from 30 to 60 min for each group. These means are almost identical, extending from 32.0 to 34.2 ml O_2/kg×min with a common mean of 32.9 ml O_2/kg×min. During the first two 30 min periods oxygen uptake of the traumatized diaphragms is essentially not different from the controls. After this initial adjustment oxygen uptake of the undivided diaphragms remains constant at this level while oxygen uptake of the traumatized diaphragms starts to decline. The steepness of decline is proportional to the extent of traumatization. For mean values and significance see Table I. n = number of experiments; mean values of oxygen uptake (30 min) are derived from $6 \times n$ recorded values.

Table I. Oxygen uptake of undivided diaphragms and diaphragms divided into 4 to 32 pieces parallel to the muscle fibres. Initial (30 to 60 min) and final (150 to 180 min) mean values of oxygen consumption for the different degrees of traumatization (Col. I). Extent and significance of the decline in oxygen uptake for the different degrees of traumatization (Col. II). Significance of the correlation of increasing degrees of traumatization and increasing steepness in the decline of oxygen uptake (Col. III). n = number of experiments; each mean value (30 min) is derided from $6 \times n$ recorded values.

Mean oxygen uptake [ml O_2/kg·min] ± SD of diaphragms undivided or divided parallel to the muscle fibres

Degree of dissection	n	I. Mean oxygen uptake ± SD [ml O_2/kg·min]		II. Decrease of oxygen uptake in 2 hours		III. Significance of difference between neighbouring groups
		30.–60. min	150.–180. min	%	significance	(χ^2-test)
0 = control	29	32.0 ± 1.6	31.4 ± 1.5	− 1.9	n.s.	$> p \ll 0.001$
4/4	14	32.8 ± 1.6	27.6 ± 1.6	−15.9	$p \ll 0.001$	$> p < 0.001$
8/8	14	32.3 ± 2.3	24.6 ± 2.3	−23.8	$p \ll 0.001$	$> 0.01 < p < 0.05$
16/16	15	34.0 ± 2.2	24.8 ± 2.4	−27.1	$p \ll 0.001$	$> p < 0.001$
32/32	10	34.2 ± 2.2	21.0 ± 1.8	−37.4	$p \ll 0.001$	

is proportional to the degree of traumatization and proceeds almost linearly with time. In this way oxygen uptake of traumatized diaphragms appears to pass a maximum during the second and sometimes during the third 30 min period. Within two hours (60. – 180. min) mean oxygen uptake of the diaphragms divided into 4 pieces decreases by 15%; for the diaphragms divided into 8, 16 and 32 pieces oxygen uptake falls by 24%, 27% and 37% respectively. It is evident that the differences in oxygen uptake between controls and traumatized diaphragms increase proportional to time. The values given in Table I refer to total measuring periods of 180 minutes.

The results of these measurements were submitted to statistical treatment. The reality of the decrease in oxygen uptake separately for each degree of traumatization was tested by the Wilcoxon-test for paired differences [9]. Compared was the oxygen uptake in the period from 30 to 60 min to that in the last period of observation (150. to 180. min). The oxygen uptake of the control group of undivided diaphragms remained constant (−1.9%). For each of the four degrees of traumatization the decline in oxygen uptake was significant with $p \ll 0.001$. The significance of the differences between the neighbouring groups of increasing traumatization was tested by the χ^2-method. Basis for comparison was the oxygen uptake of the different groups in the interval from 150. to 180. minutes. For three of the four pairs of neighbouring values oxygen uptake from 150. to 180. minutes was significantly different with $p < 0.001$ (undivided to 4/4, 4/4 to 8/8, 16/16 to 32/32). For the fourth pair (8/8 to 16/16) oxygen uptake in the final period of observation was different with $0.01 < p < 0.05$. This result necessarily includes the statement that in comparison to untraumatized diaphragms the decrease in oxygen uptake increases significantly with each degree of traumatization ($p \ll 0.001$).

In the second series of experiments oxygen consumption of diaphragms divided transversally to the muscle fibres was compared to that of diaphragms divided parallel to the muscle fibres. Two groups of measurements have been performed; in the first the diaphragms were divided into four pieces in the second into 8 pieces. Measurements on undivided diaphragms were again used as controls.

Oxygen uptake of the untraumatized diaphragms (controls) and of the diaphragms divided by sections parallel to the muscle fibres behaved in the same way as described in the first part (Table I – Fig. 1). For the control groups oxygen uptake remained constant for the 4 hours of observation on the level reached in the second 30 min period. For the two groups subdivided parallel to the muscle fibres oxygen uptake reached the normal level between 30 and 60 minutes and started to decline thereafter. The steepness of the decline was again proportional to the degree of subdivision. So far the results are the same as presented in Fig. 1.

Oxygen uptake of diaphragms divided transversally to the muscle fibres behaved very differently (Fig. 2). The initial values started at much higher levels than the controls and remained elevated for about two hours before they declined. The initial oxygen uptake was higher and the following decline steeper for the group with more extensive traumati-

Table II. Oxygen uptake of undivided diaphragms and diaphragms divided parallel or transversally to the muscle fibres. Initial (30 to 60 min) mean values of oxygen consumption for the different groups. Increase in oxygen uptake of the initial values of traumatized groups in comparison to controls (Col. I). Final mean values (210. to 240. min) extent and significance of the decline in oxygen uptake for different groups during the measuring period (Col. II). Significance of the steeper decline in oxygen uptake after transverse section of the muscle fibres (Col. III).

Mean oxygen uptake [ml O_2/kg·min] ± SD of diaphragms divided to the same extent transverse or parallel to the muscle fibres

Degree and type of dissection	*n*	I			II			III
		Mean initial oxygen uptake ± SD 30.—60. min	Increase of oxygen uptake in traumatized groups		Mean final oxygen uptake ± SD 210.—240. min	Decrease in three hours		Significance of difference between parallel and transverse dissection
			Increase and Significance			Decrease and Significance		
		[cm^3 O_2/kg·min]	%		[cm^3 O_2/kg·min]	%		χ^2-test
0 = control	10	30.5 ± 2.4	+2.3		30.6 ± 3.1	0	n.s.	
4/4 parallel	9	31.2 ± 5.8	$p < 0.01$	+12.1% $p \ll 0.001$	27.2 ± 2.8	−12.9	$p < 0.001$	$p < 0.001$
4/4 transverse	9	34.2 ± 2.8			27.5 ± 4.3	−19.6	$p < 0.001$	
0 = control	13	28.4 ± 2.1	+7.7		28.0 ± 2.8	− 1.4	n.s.	
8/8 parallel	14	30.6 ± 4.0	$p < 0.001$	+36.3% $p \ll 0.001$	25.5 ± 2.5	−16.7	$p \ll 0.001$	$p \ll 0.001$
8/8 transverse	9	38.7 ± 3.4			22.0 ± 5.8	−43.2	$p \ll 0.001$	

zation. The maximum initial oxygen uptake (second 30 min period) was 110% and 136% of the controls for diaphragms divided into 4 or 8 parts respectively. The decline of oxygen uptake in the second half of the measuring period was 20% and 43% of the maximum value for diaphragms divided into 4 or 8 parts respectively. The deviations in both directions from the constant oxygen uptake of intact diaphragms are highly significant.

Summary and Discussion

Under the described experimental conditions carefully prepared intact diaphragms maintained constant oxygen uptake for the whole period of observation (4 hours). Division of the diaphragms into several parts induced oxygen uptake to become unstable. Sections parallel to the muscle fibres caused a continuous decrease of oxygen uptake, the steepness of the decrease being significantly correlated to the extent of subdivision. Traumatization of the diaphragms by sections transversal to the muscle fibres produced a distinct increase of oxygen uptake in the initial phase followed by a continuous decrease in the second phase. In this group the initial increase of oxygen uptake as well as the steepness of the secondary decline were correlated to the extent of traumatization.

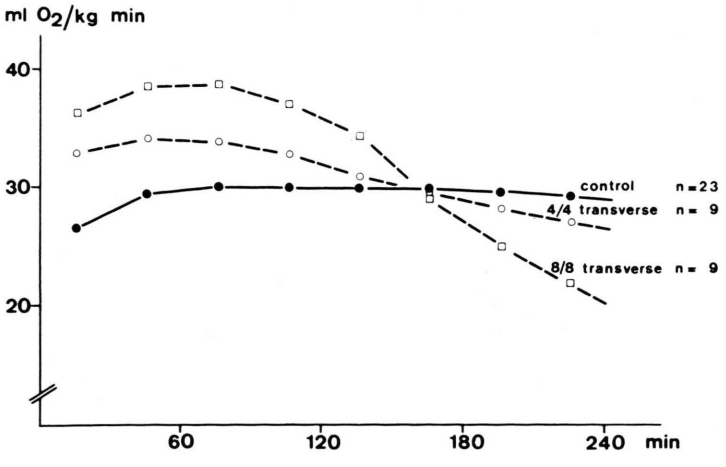

Fig. 2. Time course of oxygen uptake of diaphragms divided transversally to the muscle fibres. Oxygen uptake of the undivided controls remains constant after the short initial phase of adjustment. Oxygen uptake of the traumatized diaphragms starts with much higher initial values and declines from the elevated level after 2 hours. The increase of oxygen uptake in the initial phase is more pronounced with the higher extent of traumatization. Significance of the observations see Table II.

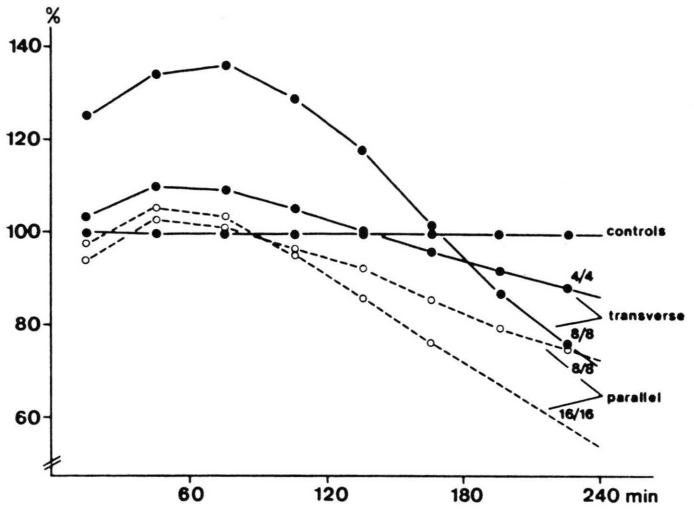

Fig. 3. Time course of oxygen uptake of diaphragms divided parallel and transversally to the muscle fibres. The values are given in per cent of the corresponding controls. Parallel division leads to a small but significant initial increase of oxygen uptake preceding the dominating phase of decline. After transversal division diaphragms start with higher initial values of oxygen uptake followed by a steeper decline.

The seemingly different reactions to sectionning parallel or transverse to the muscle fibres are linked by continuous transitions. With less extensive parallel subdivision of the diaphragms oxygen uptake of the traumatized tissue starts to decline without exceeding the level of oxygen uptake of controls. With more extensive traumatization of the same type (8/8 or more) the decline of oxygen uptake is preceded by a moderate but significant initial increase; the time course of oxygen uptake in these groups becomes very similar to that after transversal sections of the muscle fibres. The diagram in Fig. 3 represents this analogy. It appears safe to conclude that the time course of oxygen uptake in the different experimental groups is determined by the extent of injury to the muscle fibres.

This interpretation is in good agreement with the observations reported in literature. Oxygen uptake of strip preparations of skeletal muscle with uninjured muscle fibres remains constant for definite periods [2, 10–12]. Oxygen uptake of slices of muscle tissue with the unavoidable limited traumatization of muscle fibres decreases continuously from the beginning [2, 10–12]. Latapie mince of skeletal muscle with its extensive traumatization of muscle fibres displayed a higher initial oxygen uptake than muscle strips and a steep decline of oxygen uptake in a second phase [3, 12]. The analogy between these observations and those reported for the diaphragm in this paper is obvious. Similar observations are reported on heart muscle. Smyth [13] observed that the oxygen uptake of Latapie mince of heart muscle is much higher than that of slices of heart muscle.

There are few observations in the literature on the effect of mechanical traumatization of parenchymatous organs on the rate of oxygen uptake. Druckrey [14, 15] reported that very thin slices of rat liver in comparison to thicker slices still below the limiting thickness display a higher initial rate of oxygen uptake with secondary decline. Field *et al.* [10,16] observed a lower than normal oxygen uptake with thin slices of rat liver. In both groups of publications the higher proportion of traumatized cells with thin slices is considered responsible.

The authors thank Mrs. H. Körner, R. Schieffer, and U. Isenberg for skillful technical assistance.

[1] A. Kleinzeller, Manometrische Methoden, VEB G. Fischer Verlag, Jena 1965.
[2] E. Shorr, A Symposium on Respiratory Enzymes Madison, Wis., University of Wisconsin Press 1942, p. 268–271.
[3] W. W. Umbreit, R. H. Búrris, and J. F. Stauffer, Manometric Techniques, 4th Edition 1964, Burgess Minneapolis 1964.
[4] O. Warburg, Biochem. Z. **152,** 51 (1924).
[5] H. A. Krebs, Biochim. Biophys. Acta **4,** 249 (1950).
[6] F. Kreuzer, Persönliche Mitteilung.
[7] J. P. Pichotka, L. Johannessen, and H. J. Schmidt, Pflüg. Arch. **343,** Suppl. R 13, Nr. 25 (1973).
[8] H. J. Schmidt and J. Pichotka, Pflüg. Arch. **368,** R 18 (1977).

[9] B. van der Waerden and E. Nievergelt, Tafeln zum Vergleich zweier Stichproben mittels X-Test und Zeichentest, Springer-Verlag, Berlin-Göttingen-Heidelberg 1956.
[10] J. Field, Methods of Med. Res. **1,** 289–307 (1948).
[11] H. B. Richardson, E. Shorr, and R. O. Loebel, J. Biol. Chem. **86,** 551 (1930).
[12] E. Shorr, The Relation of Hormones to Carbohydrate Metabolism in vitro, Cold Spring Harbor Symposia **7,** 323–348 (1939).
[13] D. H. Smyth, Biochem. J. **36,** 1046–1056 (1940).
[14] H. Druckrey, Naturwissenschaften **XXIII,** 796–799 (1935).
[15] H. Druckrey, Arch. exp. Zellforsch. **22,** 587–591 (1939).
[16] F. A. Fuhrman and J. Field, Arch. Biochem. **6,** 337–349 (1945).
[17] J. P. Pichotka, H. J. Schmidt, and L. Johannessen, Conditions for the Stability of the Metabolic Rate of Isolated Tissues, in publication 1978.