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Oxidative capacity of muscle and mitochondria: Correlation of physiological, biochemical, and morphometric characteristics

(mitochondrial membranes/enzymes/respiratory chain/stereology/maximal oxygen consumption)

KLAUS SCHWERZMANN, HANS HOPPELER, SUSAN R. KAYAR, AND EWALD R. WEIBEL*

Department of Anatomy, University of Berne, CH-3000 Berne 9, Switzerland

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ABSTRACT **The oxidative capacity of cat skeletal muscles (soleus, gracilis, and gracilis chronically stimulated for 28 days) was derived from the total mitochondrial content in the muscle, the surface area of mitochondrial inner membranes, and respiratory activities of isolated mitochondria. Mitochondrial content was estimated by standard morphometry. The surface area of mitochondrial inner membranes per unit volume of mitochondria was estimated by a stereological method. The respiratory activities of isolated mitochondria were measured biochemically, using pyruvate/malate, glutamate/malate, succinate, or cytochrome *c* as substrate. Structurally and functionally, mitochondria from the three muscle types showed nearly identical characteristics. Oxidative activity was dependent on substrate; with succinate, 5.8 ml of O_2 per min per ml of mitochondria was the rate most likely to represent physiological conditions. Oxidative activities of 3.1 ml·min^{-1}·ml^{-1} with pyruvate/malate and 14.5 ml·min^{-1}·ml^{-1} with cytochrome *c* as substrates were theoretical lower and upper bounds. The oxidative capacity of each of the three muscles was thus in direct proportion to the total volume of mitochondria in the muscle. The respiratory capacity of isolated mitochondria was very near to the maximal oxygen uptake rate of mitochondria that is commonly estimated in intact muscles of a wide variety of animals.**

In spite of the pivotal role of mitochondria in oxidative metabolism, their role in determining the oxygen flow through the respiratory system in mammals is still controversial. On the basis of evidence gained from human and animal training studies it is commonly held that mitochondrial oxidative capacity is vastly in excess of the capacity of the cardiovascular system to deliver oxygen (1). The currently most-accepted view is that the cardiovascular system limits maximal oxygen consumption ($\dot{V}O_{2\,max}$) during short-term heavy exercise (2) and that an increase in the quantity of mitochondria in muscle tissue with training is important mainly for an improved endurance capacity or fatigue resistance and for substrate selection (3, 4).

The results of comparative studies using allometric (5) and adaptive variation (6) of $\dot{V}O_{2\,max}$ are in marked contrast to the studies that used exercise training to modify the maximal transport capacity of the respiratory system. The comparative approach demonstrated that among species with severalfold differences in weight-specific $\dot{V}O_{2\,max}$ there was a close correlation between $\dot{V}O_{2\,max}$ and whole body mitochondrial content; consequently, the maximal *in vivo* oxygen consumption of mitochondria covered only a narrow range, 3–5 ml of O_2 per min per ml of mitochondria, in all species analyzed (7–9). This suggests the hypothesis that it is the total amount of mitochondrial respiratory enzymes in the cells that ultimately determines the oxidative capacity for muscle, and hence the capacity of whole body oxidative phosphorylation.

In the present study we measured values of mitochondrial oxygen consumption *in vitro* by isolated skeletal muscle mitochondria, under conditions assumed to yield maximal values due to optimal oxygenation and substrate supply. We compared these to values calculated for *in vivo* mitochondria at $\dot{V}O_{2\,max}$ elicited by whole body exercise (8). The common base of reference was the surface area of inner mitochondrial membrane, measured by stereological methods on both isolated and *in situ* mitochondria. We used mitochondria obtained from cat muscles differing in oxidative capacity by close to 2-fold: oxidative soleus, glycolytic gracilis, and gracilis transformed into an oxidative muscle by chronic electrical stimulation (10). It will be demonstrated that mitochondria *in situ* are able to operate close to their maximal oxygen consumption capacity *in vitro*, irrespective of their source.

MATERIALS AND METHODS

Animals. Experiments were performed on female cats with body weights of 3.2–4.5 kg. The procedures for the implantation of the chronic electric stimulator on one gracilis muscle were described previously (10); stimulation was for a total of 28 days, with a daily stimulation period of 8 hr. For the terminal experiment the animals were under pentobarbital anesthesia; the venous drainage of the muscle studied was isolated for measurement of oxygen uptake as reported elsewhere (10). At the end, the animals were killed by exsanguination, and the muscles were excised; a sample was taken for electron microscopy, and the rest was used for isolating mitochondria for biochemistry.

Biochemical Methods. Finely minced muscle tissue was incubated for 15 min at 4°C in a buffer containing 180 mM KCl, 10 mM EGTA, and 10 mM Hepes–KOH at pH 7.4, supplemented with 0.5 mg of Nagarse bacterial proteinase per g of tissue. Subsequently, the suspension was homogenized in a Polytron PT-10 tissue homogenizer for two 5-sec pulses and diluted with 1 vol of ice-cold buffer before centrifugation (10 min, 800 × *g*). The pellet was resuspended with a Teflon homogenizer and again centrifuged. The two supernatants were combined and centrifuged for 10 min at 8000 × *g*. The mitochondrial sediment was washed once in an isotonic sucrose medium (250 mM sucrose/0.1% defatted bovine serum albumin/10 mM Hepes–KOH, pH 7.4). The resulting mitochondrial fraction was then purified on a self-generating Percoll density gradient (11).

Oxygen consumption was measured polarographically (12) in a thermostatted (30°C) cuvette (2.3 ml) fitted with an oxygen electrode (Yellow Springs Instruments). The reaction

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Abbreviation: dbl, double-leaflet.

*To whom reprint requests should be addressed at: Department of Anatomy, University of Berne, Bühlstrasse 26, P.O. Box 139, CH-3000 Berne 9, Switzerland.

medium contained 220 mM mannitol, 70 mM sucrose, 1 mM $MgCl_2$, 2.5 mM potassium phosphate (pH 7.4), 2 mM Hepes–KOH (pH 7.4), and 2.5 mM respiratory substrate as indicated. State III respiration was started by the addition of 200–500 nmol of ADP. Under these conditions, the respiratory control index was usually higher than 8 with pyruvate/malate or glutamate/malate, and 4–6 with succinate as substrate. Uncoupled cytochrome-*c* oxidase was measured polarographically, with 2 μM cytochrome *c*, 0.2 mM *N,N,N′,N′*-tetramethyl-1,4-phenylenediamine, and 5 mM ascorbic acid as substrates in a hypotonic buffer as described by Schnaitman and Greenawalt (13). Cytochrome spectra were recorded exactly as described by Schwerzmann *et al.* (11). Protein concentrations were determined by a biuret method (14).

Electron Microscopy Preparations. Small strips of tissue were taken from the midportions of the muscles; random blocks were fixed in 6.25% (wt/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4, total osmolarity of 1.10), postfixed in 1% OsO_4, and block stained in uranyl acetate. After embedding in Epon, sections of ≈70 nm and <40 nm were cut, the latter being used for measuring inner membrane area (step 2 below).

Aliquots of isolated mitochondria were fixed in suspension (0.5 mg of protein per ml) in the isotonic sucrose medium to which glutaraldehyde was added to give a final concentration of 0.5%. After fixation on ice for at least 30 min, 0.6-ml aliquots of the suspension were filtered under positive pressure onto Millipore membrane filters (0.22-μm pore size). The sediment formed a pellicle of packed mitochondria attached to the filter. This was postfixed with 1% OsO_4 and dehydrated with ethanol, the filters were dissolved in propylene oxide, and the pellicle was embedded in Epon. Sections were cut perpendicular to the pellicle surface, again at ≈70-nm and <40-nm thickness.

Stereological Methods. For both the tissue samples and the pellicles the stereological analysis was carried out in two steps.

Step 1. The relative volume of mitochondria was estimated by point counting (15). For intact tissue we used transverse sections to estimate the volume density of mitochondria in the muscle tissue [V_V(mi, f)] by point counting on random micrographs at ×20,000 magnification (10). For the pellicles we followed the method described previously in detail (11) to obtain the volume of mitochondria related to 1 mg of protein, the reference parameters of the biochemical studies.

Step 2. To estimate the surface density of inner and outer mitochondrial membranes it was necessary to develop a better method. When the surface of the inner mitochondrial membrane is being estimated, standard stereological meth-

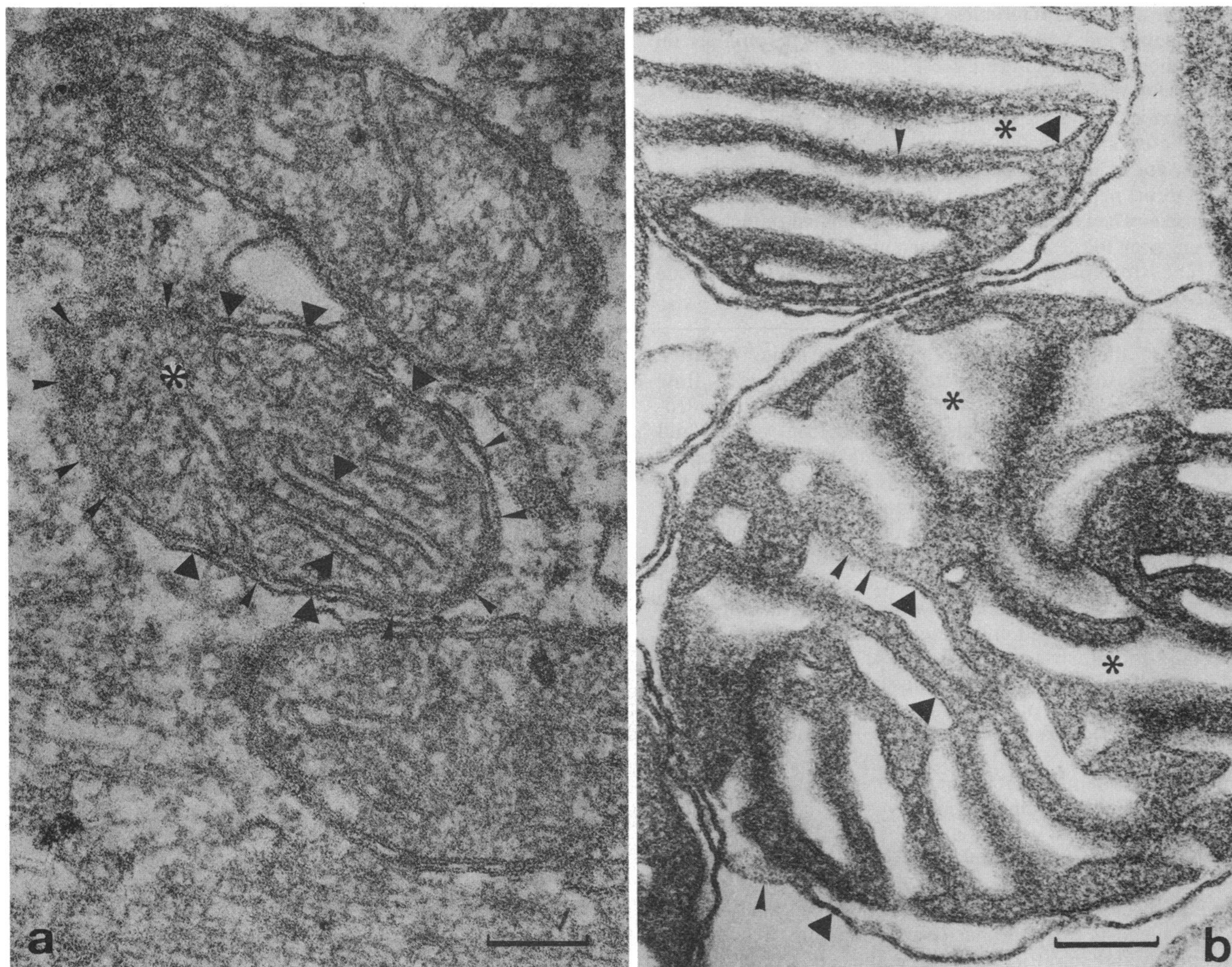

FIG. 1. Comparison of mitochondria fixed *in situ* (*a*) and after isolation (*b*). The isolated mitochondria were fixed in the condensed state; the intermembrane space (asterisk) appears wider and the matrix denser than *in situ*. Wide arrowheads point to membrane profiles exhibiting their double-leaflet (dbl) structure, whereas slender arrowheads indicate blurred membrane segments that are easily recognized along the outer membrane trace of mitochondria *in situ*. Note that about ⅓ of this trace shows dbl structure. (Scale marker = 0.1 μm.).

Table 1. Morphometry of mitochondria in intact muscle

Measurement	Soleus	Gracilis	
		Control	Stimulated
Muscle wet weight, g	4.37 ± 1.14	8.61 ± 1.14	8.34 ± 1.14
V_V(mi, f), %	6.22 ± 1.17	3.86 ± 0.62	6.06 ± 1.34
S_V(om, mi), $m^2 \cdot cm^{-3}$	16.23 ± 0.96	14.87 ± 0.65	15.35 ± 1.22
S_V(im, mi), $m^2 \cdot cm^{-3}$	36.03 ± 6.73	35.31 ± 3.62	34.97 ± 2.59
S(im)/S(om)	2.22	2.37	2.28
V(mi)/100 g, ml/100 g	5.28	3.28	5.15
S(im)/100 g, m^2/100 g	190.2	115.8	180.1

Results are mean ± SEM. V_V(mi, f) mitochondrial volume density in fibers; S_V(om, mi) and S_V(im, mi), surface density of outer and inner membrane in mitochondria; V(mi) and S(im), total volume and surface, respectively, expressed per 100 g (wet weight) of muscle.

ods yield unreliable results because only those membrane profiles can be unambiguously identified that are approximately perpendicular to the section plane so that their double-leaflet (dbl) structure is apparent (Fig. 1). We assume that the fraction of membrane oriented within a range of angles yielding a "dbl profile" on section is the same for outer and inner membrane. For the outer membrane it is easy to identify the *total* membrane trace as the envelope of mitochondrial profiles (Fig. 1). We can therefore estimate the fraction R_m of outer membrane detected as dbl profiles by counting intersections of a test line system with dbl profiles [I_{dbl}(om)] and with the *total* outer membrane trace [I_{tot}(om)]:

$$R_m = I_{dbl}(om)/I_{tot}(om). \quad [1]$$

This fraction was about ⅓ in our preparations.

The same test system was then used to count intersections with dbl profiles of the inner mitochondrial membrane [I_{dbl}(im)], and the number of test points hitting the mitochondrial profiles [P(mi)]. The surface density of outer and inner mitochondrial membrane in the mitochondrial volume is then obtained as

$$S_v(om, mi) = 2 \cdot I_{tot}(om)/[P(mi) \cdot k \cdot d] \quad [2]$$

and

$$S_V(im, mi) = 2 \cdot [I_{dbl}(im)/R_m]/[P(mi) \cdot k \cdot d], \quad [3]$$

where k and d are the characteristics of the test system defining test line length in relation to test point number (15).

This method was applied to both intact tissue and pellicles of isolated mitochondria. Transverse and longitudinal sections were used to compensate for a possible preferred orientation of inner membrane; mitochondrial profiles were selected by an unbiased sampling procedure, and electron micrographs were scored at ×200,000 magnification.

The total inner and outer membrane area contained in the unit volume of muscle tissue or in the pellicle unit corresponding to 1 mg of protein was obtained by multiplying the surface density by the mitochondrial volume per unit cell volume or per mg of protein obtained at the first step of stereological analysis.

RESULTS

Morphometry of Mitochondria in the Intact Muscles. As shown in Table 1, soleus muscle had a significantly higher volume of mitochondria per volume of muscle fiber (mitochondrial volume density) than gracilis muscle. Chronic electric stimulation of gracilis muscle for 28 days resulted in a mitochondrial volume density similar to that of soleus muscle, without change in muscle mass. The surface density of inner membrane (surface area per volume of mitochondria) was identical in the three muscles, as was the ratio of inner to outer membrane. The absolute mitochondrial volume and membrane area (Table 1) per muscle were calculated, assuming a density of 1.06 $g \cdot cm^{-3}$ (16) and a fiber volume per total muscle volume of about 0.9.

Structural Characteristics of Isolated Mitochondria. As shown in Table 2, the volume of mitochondria per unit protein mass was about $2.6 \cdot 10^{-3}$ $ml \cdot mg^{-1}$ for all three preparations. The membrane surfaces per unit volume of isolated mitochondria were likewise identical. Accordingly, we calculate that 1 mg of protein was associated with about 500 cm^2 of inner membrane in all preparations. There were no significant differences.

When comparing these data (Table 2) to those obtained on intact tissue (Table 1) we note that the ratio of inner to outer membrane area is approximately preserved, but both membrane surface densities are smaller in the isolated mitochondria by about a factor of 0.56. This appears to be the result of swelling of isolated mitochondria (Fig. 1) by a volume factor of 1.8.

Functional Analysis of Mitochondrial Fractions. Specific activity of oxygen consumption under phosphorylating conditions was measured with various substrates which cover different spans of the respiratory chain. As seen in Table 3, mitochondria from soleus and control gracilis muscles showed similar mean respiratory rates. The values for chronically stimulated gracilis muscle were consistently found to be higher; however, these differences did not reach statistical significance (see also Table 4).

Biochemical and morphometric data on isolated mitochondria were then combined to estimate the rate of O_2 consumption per unit volume and per unit inner membrane area of mitochondria elicited by the different substrates (Table 4).

Table 2. Morphometry of isolated mitochondria

Measurement	Soleus	Gracilis	
		Control	Stimulated
V(mi)/prot, $mm^3 \cdot mg^{-1}$	2.63 ± 0.43	2.58 ± 0.52	2.49 ± 0.53
S_V(om, mi), $m^2 \cdot cm^{-3}$	7.82 ± 0.07	8.05 ± 0.19	7.91 ± 0.38
S_V(im, mi), $m^2 \cdot cm^{-3}$	20.83 ± 2.93	19.28 ± 1.45	19.71 ± 4.49
S(im)/S(om)	2.66	2.40	2.49
S(om)/prot, $cm^2 \cdot mg^{-1}$	205.7	207.7	197.0
S(im)/prot, $cm^2 \cdot mg^{-1}$	547.8	497.4	490.8

For symbols see Table 1. V(mi), S(om), and S(im) are expressed per mg of protein.

Table 3. Maximal respiratory rates of isolated mitochondria with different substrates

	$\dot{V}O_{2\,max}$, nmol·min^{-1}·mg^{-1}		
		Gracilis	
Substrate	Soleus	Control	Stimulated
Pyruvate/malate	120.7 ± 51.8	122.7 ± 69.0	213.1 ± 76.9
Glutamate/malate	198.3 ± 61.5	187.6 ± 39.6	276.4 ± 87.4
Succinate	251.8 ± 70.5	253.8 ± 69.1	350.3 ± 55.2
Cytochrome *c*	838.0 ± 248	707.0 ± 222	616.0 ± 18*

Rates, expressed per mg of protein, were obtained on six preparations each for soleus muscle and gracilis muscle control and three preparations of stimulated gracilis muscle.
*Two preparations only.

For the three muscle types, these rates were statistically not different, with means of 3–14 ml of O_2 per min per m^2 of inner membrane with the different substrates. Correcting for the swelling of isolated mitochondria by a factor of 1.8, we estimate the rate of O_2 consumption of *native* mitochondria to be 3.1–14.5 ml of O_2 per min per ml of mitochondria and 87–408 μl of O_2 per min per m^2 of inner membrane, respectively, with these same substrates (Table 5).

DISCUSSION

Mitochondrial Isolation and Validity of Biochemical Measurements. The method employed for isolating mitochondria was designed to yield a representative sample of the entire mitochondrial population of the muscles. By combining mechanical homogenization with proteolytic digestion, we avoided preferential isolation of either subsarcolemmal or interfibrillar mitochondria (18). Further purification of the mitochondrial fraction on a density gradient did not result in more than one mitochondrial fraction, but it removed nonmitochondrial membranes (19) as well as disintegrated mitochondrial fragments (20). Our findings show that the isolation procedure leads to swelling of mitochondria by nearly a factor of 2 but preserves the ratio of inner to outer membrane surface (Tables 1 and 2).

The respiratory control index and the amount of cytochromes per mg of protein (Table 6) were high, attesting to the satisfactory functional integrity of the isolated mitochondria and the purity of the preparations (14). The high rates of ADP-dependent respiration of our preparations suggest that the conditions under which O_2 consumption was measured can be considered optimal (18).

It cannot be excluded, however, that, due to opening and resealing of mitochondrial structures in the course of isolation, matrix enzymes such as those of the tricarboxylic acid cycle may have leaked out. As a consequence, respiratory rates measured with substrates involving matrix-localized enzymes, namely pyruvate, malate, and glutamate, may underestimate the physiological oxidative capacity. On the other hand, the uncoupled rates measured with cytochrome *c* can only be considered a theoretical upper limit because the respiratory chain is bypassed. However, the rates measured with substrates such as succinate in the presence of ADP and phosphate should reflect the maximal physiological respiratory activity of mitochondria, since the rate-controlling steps of adenine nucleotide translocation and cytochrome *c* oxidation (21) are involved.

Table 5. Maximal respiratory rates of muscle mitochondria estimated from the oxidative capacity of isolated mitochondria related to inner membrane area and the surface density of mitochondria *in situ*

	$\dot{V}O_{2\,max}$	
Substrate	Per unit inner membrane area, μl·min^{-1}·m^{-2}	Per mitochondrial volume, ml·min^{-1}·cm^{-3}
Pyruvate/malate	87.3	3.1
Glutamate/malate	126.3	4.5
Succinate	163.3	5.8
Cytochrome *c*	408.1	14.5

Rates were calculated from the means of all muscles in Table 4 and the mean morphometric estimates in Table 1. The respiratory rates measured at 30°C were extrapolated to rates at physiological temperature (37°C), assuming a conversion factor of 1.3 (17). For the conversion of mol of O_2 into volume, a molar volume of 22.4 liters was assumed.

Validity of Morphometric Measurements. The measurement of inner mitochondrial membrane surface meets with two problems: (*i*) the uncertainty of measurements due to the resolution effect, which was overcome by performing the measurements at a magnification of over ×100,000 and by using very thin sections (22); and (*ii*) the failure to recognize membranes cut obliquely. To solve the second problem we have developed a method by which only traces of the inner membrane exhibiting a dbl structure had to be measured. These incomplete data were then corrected by a factor derived from measurements on the outer membrane. The validity of this method depends on the assumption that the fraction of dbl profiles is the same for outer and inner membranes. A number of tests indicated that errors introduced by this assumption are on the order of ±20% of the mean, or less, and that this should not affect the comparison between the different muscles.

Because of the morphometric method used the current morphometric data cannot be compared directly with previous measurements of inner mitochondrial surface area, either on intact muscle or on isolated liver mitochondria (11).

Comparing Different Muscles. We compared in this study three cat muscles, the highly oxidative soleus muscle (>95% slow-twitch, oxidative fibers), the predominantly glycolytic gracilis muscle (>70% fast-twitch, glycolytic fibers), and the gracilis muscle converted into an oxidative muscle by chronic stimulation (10). It had been found that the values of $\dot{V}O_{2\,max}$

Table 4. Oxidative capacities of isolated muscle mitochondria related to unit inner membrane area, measured with different substrates

	Oxidative capacity, μmol·min^{-1}·m^{-2}			
		Gracilis		
Substrate	Soleus	Control	Stimulated	Mean of all muscles
Pyruvate/malate	2.20 ± 0.94	2.47 ± 1.39	4.34 ± 1.57	3.00
Glutamate/malate	3.62 ± 1.12	3.77 ± 0.80	5.63 ± 1.78	4.34
Succinate	4.60 ± 1.29	5.10 ± 1.39	7.14 ± 1.13	5.61
Cytochrome *c*	15.30 ± 4.53	14.21 ± 4.46	12.55 ± 0.37*	14.02

Oxidative capacities were calculated from maximal respiratory rates of isolated mitochondria in Table 3 and the means of the corresponding surface densities of inner membranes in Table 2.
*Two measurements only.

Table 6. Densities of respiratory complexes in the inner membrane and the maximal turnover rates of respiratory complexes

Respiratory complex	Concentration, mol per mg of protein	Density, no. per μm^2	Maximal turnover rate, molecules of O_2 reduced per complex per sec
Complex I	0.16	1610	19.3
Complex II	0.33	3220	17.4
Complex III	0.57	3352	16.7
Complex IV	0.97	9468	14.9

The concentration of respiratory complexes was estimated from cytochrome spectra of isolated mitochondria exactly as indicated by Schwerzmann *et al.* (11). The density was calculated from the concentration and the mean inner membrane area of isolated muscle mitochondria. The turnover rate is the number of O_2 molecules consumed per complex calculated from the mean respiratory rates in Table 4, with pyruvate/malate for complex I, succinate for complex II and III, and cytochrome *c* for complex IV, respectively.

per unit muscle mass, measured under maximal electrical stimulation on the perfused muscle, was proportional to the volume density of mitochondria (10). The present biochemical and morphometric study revealed neither qualitative nor quantitative differences between the mitochondria collected from the three muscles (Tables 2–4). The consistently higher respiration rates with various substrates observed in stimulated muscles did not reach statistical significance. For the subsequent considerations we shall therefore use for each of the substrates mean respiratory rates, averaged over the three muscle preparations (Table 5), as well as a mean surface density of inner mitochondrial membrane of 35.4 $m^2 \cdot ml^{-1}$ for mitochondria *in situ* (Table 1).

Estimating the Rates of Enzyme Activities of the Respiratory Chain. The different rates of O_2 consumption per unit inner membrane surface elicited by different substrates appear intriguing. These substrates serve the respiratory chain at different sites so that these rates may be determined, at least in part, by the number of enzymes of each complex contained in the membrane.

From the concentrations of cytochromes measured spectrophotometrically, we estimated the density of respiratory complexes in the unit area of inner membrane (Table 6). We calculate that 1 μm^2 of inner membrane contains 1610 units of complex I, 3220 units of complex II, 3352 units of complex III, and 9468 units of complex IV. Of the substrates used here, pyruvate/malate activates the chain at complex I, glutamate/malate and succinate at complexes II and III, and cytochrome *c* at complex IV. Calculating the total number of complexes from the measured membrane surface and dividing the measured values of O_2 consumption by the appropriate number of complexes, we estimate that each complex must perform 15–19 "reactions" per second when the mitochondrion is maximally stimulated, irrespective of the substrate used. The rate of "maximal" O_2 consumption elicited by the different substrates therefore appears to be determined by the rate at which the activated complexes can operate as well as by their number.

Estimating Oxidative Capacity of Muscle. Since we estimated maximal O_2 consumption with respect to a consistent common reference, the inner mitochondrial membrane area, we can now compare *in vivo* mitochondrial O_2 consumption values with those obtained on isolated mitochondria under optimized conditions. As shown in Table 5, we estimate that the unit volume of intact mitochondria should achieve a specific $\dot{V}O_{2\,max}$ of 5.8 $ml \cdot min^{-1} \cdot ml^{-1}$ when succinate is used as substrate. This comes quite close to the 3–5 $ml \cdot min^{-1} \cdot ml^{-1}$ estimated consistently as average mitochondrial $\dot{V}O_{2\,max}$ for mammalian muscle *in vivo* (7, 9). On the other hand, it appears that this mitochondrial $\dot{V}O_{2\,max}$ is only about ⅓ of the theoretical upper limit of mitochondrial O_2 consumption estimated with cytochrome *c* as substrate.

The values of $\dot{V}O_{2\,max}$ measured directly on the isolated muscle by electrical stimulation were lower by about a factor of ½ (10); this reduction, however, was attributed to the specific conditions prevailing in isolated perfused muscles.

In conclusion, this integrated study has shown that *in vivo* physiological estimates of mitochondrial $\dot{V}O_{2\,max}$ are in good agreement with *in vitro* biochemical estimates of mitochondrial oxidative capacity. Muscles operating at their maximal aerobic capacity appear to exploit 60–80% of the oxidative capacity of their mitochondria.

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1. Blomqvist, C. G. & Saltin, B. (1983) *Annu. Rev. Physiol.* **45,** 169–189.
2. Astrand, P. O. & Rodahl, K. (1977) *Textbook of Work Physiology: Physiological Bases of Exercise* (McGraw-Hill, New York), 2nd Ed.
3. Davies, K. J. A., Packer, L. & Brooks, G. A. (1981) *Arch. Biochem. Biophys.* **209,** 539–554.
4. Gollnick, P. D. (1977) *Ann. N.Y. Acad. Sci.* **301,** 64–71.
5. Weibel, E. R., Taylor, R. C., Gehr, P., Hoppeler, H., Mathieu, O. & Maloiy, G. M. O. (1981) *Respir. Physiol.* **44,** 151–164.
6. Taylor, C. R., Weibel, E. R., Karas, R. H. & Hoppeler, H. (1987) *Respir. Physiol.* **69,** 117–127.
7. Hoppeler, H. & Lindstedt, S. L. (1985) *J. Exp. Biol.* **115,** 355–364.
8. Hoppeler, H., Kayar, S. R., Claassen, H., Uhlmann, E. & Karas, R. H. (1987) *Respir. Physiol.* **69,** 27–45.
9. Kayar, S. R., Hoppeler, H., Lindstedt, S. L., Claassen, H., Jones, J. H., Essen-Gustavsson, B. & Taylor, C. R. (1989) *Pflügers Arch.* **413,** 343–347.
10. Hoppeler, H., Hudlicka, O. & Uhlmann, E. (1987) *J. Physiol. (London)* **385,** 661–675.
11. Schwerzmann, K., Cruz-Orive, L. M., Eggmann, R., Saenger, A. & Weibel, E. R. (1986) *J. Cell Biol.* **102,** 97–103.
12. Estabrook, R. W. (1967) *Methods Enzymol.* **10,** 41–47.
13. Schnaitman, C. & Greenawalt, J. W. (1968) *J. Cell Biol.* **38,** 158–175.
14. Pedersen, P. L., Greenawalt, J. W., Reynafarje, B., Hullihen, J., Decker, G. L., Soper, J. W. & Bustamante, E. (1978) *Methods Cell Biol.* **20,** 411–481.
15. Weibel, E. R. (1979) *Stereological Methods: Practical Methods for Biological Morphometry* (Academic, London), Vol. 1.
16. Mendez, J. & Keys, A. (1960) *Metabolism* **9,** 184–188.
17. Chappell, B. J. (1964) *Biochem. J.* **90,** 237–248.
18. Palmer, J. W., Tandler, B. & Hoppel, C. L. (1977) *J. Biol. Chem.* **252,** 8731–8739.
19. Mickelson, J. R., Greaser, M. L. & Marsh, B. B. (1980) *Anal. Biochem.* **109,** 255–260.
20. Bolender, R. P., Paumgartner, D., Losa, G., Muellener, D. & Weibel, E. R. (1978) *J. Cell Biol.* **77,** 565–589.
21. Tager, J. M., Wanders, R. J., Groen, A. K., Kunz, W., Bohnensack, R., Kuster, U., Letko, G., Bohme, G., Durzynski, J. & Wojtczak, L. (1983) *FEBS Lett.* **151,** 1–9.
22. Paumgartner, D., Losa, G. & Weibel, E. R. (1981) *J. Microsc.* **121,** 51–63.