Identification of Myoglobin in Human Smooth Muscle*

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Myoglobin (Mb) has been believed to be absent generally from mammalian smooth muscle tissue. Examination of human rectal, uterine, bladder, colon, small intestine, arterial, and venous smooth muscle by immunohistochemical techniques shows that each of these tissues is immunopositive for both smooth muscle myosin and human Mb. Mb-specific primers were used for the polymerase chain reaction to generate cDNA from smooth muscle tissues. Southern hybridization with a Mb-specific probe gave a very strong signal with the cDNA from rectum, weaker signals from small intestine and uterus, a faint signal from colon, and no signal from bladder tissue. High performance liquid chromatography analysis coupled with sequence determination has shown that containing heme-binding serum albumin as well as hemoglobin in extracts of smooth muscle seriously compromise any heme-based or spectrophotometric assay of Mb. Combined affinity and size exclusion chromatography, however, provide the necessary resolution. The cDNA-derived amino acid sequence of human smooth muscle Mb was found to be identical to that of Mb from striated muscle.

Ray Lankester (2, 3) first observed intracellular hemoglobin (myoglobin, Mb) in mammalian striated muscle. He also reported Hb (Mb) in the rectal smooth muscle (mshinter) of man, but it appeared to be absent from other mammalian smooth muscle. Later, Björck (4) found Mb in human uterine smooth muscle, and Jaisle and Huber (5) reported a 3-fold increase in Mb in human rectal smooth muscle. Later, Bio¨rck (4) found Mb in human uterine smooth muscle, and Jaisle and Huber (5) reported a 3-fold increase in Mb in human rectal smooth muscle. He also reported Mb from striated muscle.

Intrigued by Lankester’s early observation, we have reexamined smooth muscle tissue from the human rectum and from other human tissues. We reasoned that if Mb did occur generally in smooth muscle, even at a low concentration, it might affect the kinetics of the dynamic cycle in which S-nitrosohemoglobin delivers NO to vascular tissues and causes vasodilation (11).

MATERIALS AND METHODS

mRNA Extraction from Smooth Muscle—Samples of human smooth muscle from rectum, uterus, small intestine, colon, and bladder were frozen in liquid nitrogen and stored at −80 °C. Each of these samples was obtained during surgery with the permission of the patient. All of the surgical procedures involved removal of cancerous tissue, but the muscle cell samples studied here were not themselves cancerous. About 0.4 g of smooth muscle from each of these tissues was used for the mRNA preparation with the Fast Track mRNA Isolation Kit version 3.5 from Invitrogen (San Diego, CA).

cDNA Preparation—cDNA (10 µg) from each tissue was combined with an oligo dT 28-mer that has an Xba I site at its 5’ end (500 ng) in diethylpyrocarbonate sterile deionized water (DEPC-dH2O) prepared by adding 100 µl of DEPC (Sigma, catalog number D-5758) to 100 ml of deionized water, shaken, and incubated at 37 °C overnight and autoclaved. The annealing reaction was carried out by incubating the mixture at 65 °C for 5 min followed by cooling on ice. Next, 1 µl of RNasin (40 units/µl, Promega), 8 µl of 2.5 mM dNTP mix, 10 µl of 5× reverse transcription buffer from U. S. Biochemical Corp., 5 µl of 0.1 M dithiothreitol (Life Technologies, Inc.), 14 µl of DEPC-dH2O, and 2 µl of 200 units/µl Moloney murine leukemia virus reverse transcriptase (U. S. Biochemical Corp.) were added to the annealing reaction solution. After incubating at 37 °C for an hour, 5 µl of a 3 M sodium acetate solution and 125 µl of 100% ethanol were added, and the resulting solution was kept at −20 °C overnight. After ethanol precipitation, the pellet was dissolved in 20 µl of DEPC-dH2O.

Amplification of Mb cDNA—A polymerization chain reaction (PCR) was carried out to amplify Mb cDNA. The coding sequence of Mb was obtained by using 2 µl of the mRNA-cDNA from the reverse transcription as template and two synthesized primers, 5′-GACTCTAGAATGG-GGCCTACGCGAGGG-3′ and 5′-AGTTCTAGACTAGCCCTGGAAGCC-CAG-3′, which are complementary to the 5′ and 3′ ends of the known human Mb coding sequence (12) and contain an Xba I site at its 5′ end. The Mb cDNA (see above) was digested with Xba I and inserted into pUC19 vector (New England Biolabs) cut with Xba I and Smal. The resulting PCR product was analyzed after running an aliquot on a 1.0% agarose gel.

cDNA Cloning and Sequencing of Human Smooth Muscle Mb cDNA—The PCR product (480 base pairs) obtained by amplification of Mb cDNA (see above) was digested with Xba I and inserted into pUC19 vector (New England Biolabs) cut with Xba I. The ligated DNA was cloned into a DNA ligation kit from Takara Shuzo Co. (catalog number 6021). The resulting ligation solution was used to transform MAX efficiency DH5α competent cells (Life Technologies, Inc.). Plasmid DNA was extracted from positive clones, and the DNA insert was sequenced by the dideoxy chain termination method (U.S. Biochemical Corp. Sequenase™ version 2.0 DNA Sequencing kit).

Northern Hybridization of Multiple Human Tissues—A blot of mRNA from multiple human tissues (catalog number 7760-1), supplied by CLONTECH Laboratories, Inc., was used to test for Mb expression in other tissues. The blot included mRNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. The human Mb cDNA from the PCR amplification was used as a probe, and the manufacturer’s protocol was followed.

Quantification of Mb by Affinity Chromatography and Spectrophotometric Adsorption of Mb from Human Smooth Muscle Tissue—Samples of human smooth muscle from rectum, uterus, small intestine, colon, and bladder were frozen in liquid nitrogen and stored at −80 °C. Each of these samples was obtained during surgery with the permission of the patient. All of the surgical procedures involved removal of cancerous tissue, but the muscle cell samples studied here were not themselves cancerous. About 0.4 g of smooth muscle from each of these tissues was used for the mRNA preparation with the Fast Track mRNA Isolation Kit version 3.5 from Invitrogen (San Diego, CA).

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metric Assay—The procedure of Schuder et al. (13) was used in an attempt to quantify Mb in muscle tissue after removal of Hb by affinity chromatography. The affinity column was made by coupling the αβ dimer of human Hb to CNBr-activated Sepharose 4B. Hb in the extract binds to the αβ dimer on the column, and Mb passes through unretarded. The percentage of Mb extracted is given as (1 – Aαβ/Aαβ + Aαβ) (12). The buffer used was 100 mM KPO4, 1 mM 2-mercaptoethanol (2 g, Pharmacia Fine Chemicals) was used as described (13). Human Hb (8 µmol) was used for the coupling reaction. The capacity of the column was determined by applying pure human Hb and then measuring the absorbance of the eluate at 420 nm. The column retained all of 0.3 µmol of Hb applied but 0.7 µmol of Hb exceeded the capacity and was detected in the eluate. Therefore, quantities of extract set to be under 15 ml/h. The fractions containing apparent Mb were collected. The spectra of both deoxy-Mb and CO-Mb were measured as absorbance of the eluate at 420 nm. The column retained all of 0.3 µmol of Hb. The column was regenerated with 207 mM 2 M NaCl, which removes bound Hb. The extraction of rectal skeletal muscle, rectal smooth muscle, and uterine tissue generally followed Schuder et al. (13). Frozen tissue (0.4–1.0 g) was cut into small pieces and ground under liquid nitrogen. The resulting tissue powder was weighed and extracted with 10 ml of 100 mM potassium phosphate and 2% SDS, and 100 mM of Hb applied but 0.7 µmol of Hb exceeded the capacity and was detected in the eluate. Therefore, quantities of extract set to be under 15 ml/h. The fractions containing apparent Mb were collected and pooled. Extract I of rectal skeletal muscle was similarly processed. The spectra of both deoxy-Mb and CO-Mb were measured as described (13). The millimolar extinction coefficients were taken to be εαβ = 907 mM cm−1 at 424 nm (MbCO) and εαβIII = 121 mM cm−1 at 435 nm (deoxyMb), the values given for horse Mb (14). We measured the absorbance ratio, A435/A424 = 0.820 for horse deoxy-Mb and then calculated the εαβ value at 424 nm: 0.820 × 121 = 99.2 mM cm−1. The apparent concentration of Mb was calculated from the following equation.

\[
D_{ab} = \frac{A_{COO\text{-dimer}} - A_{deoxyMb}}{A_{Mb-\text{dimer}} - A_{Mb-deoxyMb}}
\]  

(Eq. 1)

where the εαβ values are for 424 nm. All HPLC was performed on a SynChropak RP-P C18 reverse phase column (250 × 4.6 mm, SynChrom, Inc., Lafayette, IN) driven by a Beckman model 332 gradient chromatography system. Extract I of rectal muscle and the eluate from the affinity column (see above) were applied separately to the HPLC column with the following gradient program: Buffer A 0.1% trifluoroacetic acid in water, Buffer B, 0.1% trifluoroacetic acid in acetonitrile, 0–5 min, 0% B; 5–15 min, 0–30% B; 15–115 min, 30–55% B; 115–25 min, 55–0% B. Absorbance of the eluate was monitored at 220 nm with an Hitachi model 10–10 spectrophotometer. Absorbing fractions were collected and lyophilized. The protein was redissolved in water and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (10%) resolving gel and 3% stacking gel (15).

Size Exclusion Chromatography—The separation of Mb from serum albumin in Extract I (after affinity chromatography) was examined with a Bio-Gel SEC 40xl size exclusion column (300 × 7.8 mm; Bio-Rad catalog number 125-0604). The buffer used was 100 mM KPO4, 1 mM NaEDTA at pH 7.0 running at 25 °C. The column eluate was monitored at either 280 or 415 nm with an Hitachi spectrophotometer (model F-2266, diluted 1:200) and rhodamine-conjugated goat anti-rabbit IgG, F(ab′)2 fragment (Cappel, Durham, NC, catalog number 55671, diluted 1:200) as immobilized specific antibodies followed by a mixture of mouse anti-smooth muscle myosin and rabbit anti-Mb antibodies followed by a mixture of FITC-conjugated anti-mouse and rhodamine-conjugated anti-rabbit secondary antibodies. Slides were examined with a Nikon Optiphot epifluorescence microscope equipped with rhodamine and fluorescein filters. Images were acquired with an integrating CCD camera connected to a Macintosh computer containing a frame grabber and NIH image software. Images were cropped and labeled by use of Adobe Photoshop and Canvas software and printed with a Sony color printer.

RESULTS

Occurrence of Mb mRNA in Different Smooth Muscle Tissues—cDNA encoding human Mb was amplified by PCR from the total cDNA prepared from different tissues. Specificity was achieved by using primers constructed on the basis of the gene-derived NH2- and COOH-terminal sequences (12). The same mass of tissue (0.4 g) was used from each source for Southern hybridization (Fig. 1) so that the signal should reflect approximately the relative quantity of Mb mRNA in each tissue. The strongest signal was clearly from rectal smooth muscle tissue with much less from tissue of the small intestine and uterus. Only a very weak signal was obtained from colon tissue, and none was detected from bladder tissue.

Northern hybridization of mRNA from diverse tissues probed with cDNA for human Mb gave signals only for skeletal muscle and heart mRNA (Fig. 2). Although no signal was detected in the other tissues, the Northern hybridization is much less sensitive than the Southern hybridization prepared with PCR-amplified cDNA, so the negative finding could mean only that an insufficient quantity of mRNA was present in the experiment.

Immunohistochemistry of Smooth Muscle Tissue—We examined tissues with smooth muscle from rectum, uterus, colon, small intestine, bladder, arteries, and veins with immunohistochemical techniques. Differently labeled fluorescent antibodies to both human smooth muscle myosin and Mb were used. The results reveal that human rectal muscle is immunopositive for both smooth muscle myosin and for Mb (Fig. 3a). A cryostat section passing longitudinally (panels A and B) and transversely (panels C and D) showing sections passing orthogonally (panels E and F) through a bundle of smooth muscle fibers demonstrates double-immunolabeled immunoreactivity to smooth muscle myosin was detected by using an FITC-labeled second antibody and to Mb by using a rhodamine-conjugated secondary antibody. Views through the fluorescein filter set are shown in panels A, C, and E, and views through the rhodamine filter set are shown in panels B, D, and F (Fig. 3a). The tissue sections in panels A, B, C, and D, and
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Fig. 1. Southern blot of PCR products from various human smooth muscle tissues hybridized with a Mb-specific 32P-labeled probe (oligomer 5’-AGTTCAAGCAGTTAAGTCAAGGACG-3’ corresponding to codons 45 to 54 of human Mb). Lane 1, 1-kilobase DNA ladder; lane 2, 0.5 µl of PCR-amplified cDNA for Mb from rectal smooth muscle; lane 3, blank; lanes 4–7, 10 µl of PCR-amplified cDNA for Mb from colon, small intestine, bladder, and uterine tissue, respectively. The last wash of the membrane was in 6 × SSPE at room temperature. Rectal smooth muscle (lane 2) gave the strongest Mb signal, followed by weaker Mb signals for small intestine and uterine tissue (lanes 5 and 7). Only a faint signal was evident for colon tissue (lane 4), and no signal was detected for bladder tissue (lane 6). bp, base pair.

Fig. 2. Northern blot of mRNA from multiple human tissues. Lanes 1–8 are mRNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas, respectively. Human Mb cDNA was used as a probe. Last wash of membrane was in 0.1 × SSC at 55 °C. The blot indicates expression of Mb only in human heart (lane 1) and skeletal muscle (lane 6) but not the other tissues. kb, kilobases.

(Fig. 3a) received a mixture of anti-smooth muscle myosin and anti-Mb antibodies, followed by a mixture of the two secondary antibodies. The sections in panels E and F received no primary antibody but were incubated with the mixture of the two secondary antibodies. Thus, the sections in panels E and F serve as negative controls. Although some labeling of connective tissue is obvious in the absence of primary antibody, the muscle fibers themselves are unlabeled. Additional controls (also not shown) showed that these smooth muscle fibers were unreactive with an antibody to sarcomeric myosin and that rat skeletal muscles were unreactive with an antibody to sarcomeric myosin and that rat skeletal muscles were unreactive with the anti-smooth muscle myosin antibody. Very similar results were obtained for artery and vein tissue (Fig. 3b), for uterine tissue (Fig. 3c), and for colon, small intestine, and bladder (data not shown).

Amino Acid Sequence of Smooth Muscle Mb—The PCR-amplified cDNA for Mb from human rectal smooth muscle was cloned into pUC19 and sequenced. The deduced amino acid sequence was found to be identical to that of Mb from striated muscle (12). The same tissue preparation also yielded PCR-amplified cDNA encoding part of the heavy chain of smooth muscle myosin, thus confirming the identification of the tissue.

Spectrophotometric Quantification of Mb after Hb Removal—The principle of the procedure (see “Materials and Methods”) is to use an affinity column, which can bind at least 0.3 µmol of Mb. Extract I (4 ml) of rectal smooth muscle containing less than 0.05 µmol of Hb was applied to the column. The amount of Hb in the aliquot of Extract I was much less than the capacity of the affinity column, which can bind at least 0.3 µmol of Hb. Fig. 4 shows the HPLC pattern obtained for Extract I before (Fig. 4a) and after (Fig. 4b) passage through the column. The α and β chains of Hb (about half of the total protein) have clearly been removed by the column.

Quantitative correspondence of the HPLC absorbance with the spectrophotometric determination can be determined as follows with the use of hemin and protein extinction coefficients previously determined (17). The quantity of human Hb in the original solution, determined from the α and β chain absorbances in Fig. 4a, was found to be 41.8 nmol. The total heme (measured as hemin) in Fig. 4a corresponds to 73.4 nmol in the original solution. The difference (73.4 – 41.8) should give the heme attributed to Mb, 31.6 nmol, which corresponds to 174 µmol of Mb/kg tissue. Although this value is within 8% of the quantity of Mb, 188 µmol, determined by difference spectrophotometry of Extract I after affinity chromatography, SDS electrophoresis of fraction 4 in Fig. 4b showed that it is largely composed of a 66-kDa polypeptide, and only traces of 17-kDa protein are present (data not shown). Fraction 4 was electroblotted onto a polyvinylidene difluoride membrane for sequencing by the Microanalysis Facility of the University of Texas with a model 477A Applied Biosystems Sequencer. The 20-residue sequence obtained showed that the 66-kDa protein is serum albumin. Therefore, most of the apparent Mb in the spectrophotometric assay must be attributed to heme-binding serum albumin and not Mb.

Heme-binding by serum albumin was tested with the Schuder et al. (13) spectrophotometric assay for Mb content as follows. Hemin (Fluka, 1 µl of 12.4 mM stock in 0.1 N NaOH) was added to 4 ml of 10 mM phosphate buffer, pH 7, containing 8 nmol of BSA (Sigma) to give a 1.5 molar ratio of hemin to albumin. An apparent Mb content, 0.66 µmol, was determined spectrophotometrically as described by Schuder et al. (13) (see “Materials and Methods”).

In contrast to the smooth muscle analysis, Extract I of rectal skeletal muscle did not show a large quantity of serum albumin. Analysis of fraction 7 in Fig. 4c showed that it consists of Mb and that serum albumin was absent (data not shown).

Analysis of Extracts I and II of rectal smooth muscle tissue...
after SDS-PAGE showed that the two extracts have different protein band patterns (Fig. 5a). Additional cellular proteins were obtained in Extract II. When Extract I and Extract II were probed with anti-Mb antibody on a Western blot, a single band of about 17 kDa was detected in both Extracts I and II (Fig. 5b). We conclude that a low concentration of Mb is present in both Extracts I and II and that Extract I did not extract all the protein.

**Size Exclusion Chromatography**—Application of a mixture of BSA (66 kDa), horse Mb (17 kDa), and human Hb (65 kDa) to a Bio-Rad size exclusion column failed to isolate the Mb because of the partial dissociation of the human Hb tetramers to...
dimers, which caused the Mb and Hb peaks to overlap (data not shown). However, separation of horse Mb from BSA alone did occur (Fig. 6a). When Extract I, after removal of Hb with the affinity column (see above), was applied to the SEC column (Fig. 6b), a small shoulder on the human serum albumin peak corresponded in position to Mb. The amount of protein in this shoulder is roughly estimated to be about 10% of the serum albumin. Comparison of the quantity of serum albumin shown in Fig. 6 allows us to estimate the quantity of Mb to be 0.16 mg/g wet tissue, a rough estimate at best because we are at the limit of detectability for the Mb. This difficulty does not compromise the estimation of Mb in skeletal muscle tissue because the latter contains a much larger quantity of Mb.

**DISCUSSION**

Our results confirm the early report by Lankester (1870) that Mb occurs in human rectal smooth muscle (2, 3), but only at very low concentrations. We show by immunohistological techniques that Mb is also present in all other smooth muscle tissues examined: colon, small intestine, uterus, bladder, arteries, and veins. Table I summarizes the presence and content of Mb in various tissues. However, attempts to quantify the Mb in the rectal tissue with existing protocols failed because they depended on the assumption that the only interfering heme-containing protein is Hb (13). The procedure of de Duve (20), which relies entirely on determination of hemin as the pyridine hemochromogen derivative, would also fail to measure Mb accurately. The reason for these failures is that substantial quantities of heme-binding serum albumin can be present. The protocols do not distinguish between heme or hemin from Mb and from serum albumin. The procedure of Schuder et al. (13) adequately removes contaminating Hb by affinity chromatography but does not remove serum albumin. Therefore affinity separation followed by size exclusion chromatography (Fig. 6) should be the method of choice to separate both of these contaminants from the much smaller Mb. Size exclusion chromatography alone is less satisfactory because of the tetramer to dimer dissociation of Hb.
Southern hybridization with cDNA for human Mb gave a small but clear signal with tissue of the small intestine and uterus, a very weak signal from the colon, and none for the bladder, whereas the rectal smooth muscle gave a very strong signal. Several possible explanations for the large differences in mRNA may be suggested. Mb might be expressed only in special, as yet unspecified, metabolic circumstances. Alternatively, a small subset of cells in the rectal smooth muscle tissue might have a substantial content of Mb, but the analysis of a relatively large mass of tissue would provide only a diluted estimate of the Mb. The latter possibility is enhanced by studies of another tissue, the esophageal sphincter of the opossum, which has been extensively used as a model for the esophageal sphincter in man (21–24). This sphincter may be similar to the rectal smooth muscle sphincter of man. The esophageal sphincter tissue of the opossum has several properties that distinguish it from the smooth muscle of the nearby esophageal body: 1) The rate of oxygen consumption of the sphincter tissue is higher than the esophageal body (25). 2) The tonic contraction of the sphincter is entirely aerobic and cannot be maintained anaerobically. In contrast, the esophageal body contractions can be partially maintained anaerobically (22). 3) The apparent mitochondrial profile area is larger in the sphincter cells than in the esophageal body (26). 4) Lactic dehydrogenase type I isozyme is present in the sphincter but not in the esophageal body (27). These observations show that the aerobic metabolism of the sphincter tissue is unique. Under aerobic conditions the muscle is tonically contracted and is only briefly relaxed to allow swallowing. The aerobic demand would make the presence of Mb advantageous. No similar metabolic studies of rectal tissue appear to have been made. The presence of mRNA for Mb in small intestine but not colon may be correlated with the different physiological functions of the tissues. Perhaps even a small amount of Mb may be advantageous in small intestine tissue but not colon. Peristaltic waves in the small intestine move at 1–2 cm/s over long periods of time, and the villi, present at a density of 10–40/mm², have smooth muscle and contract independently almost continuously every 10 s (28). In contrast, the colon is devoid of villi, and muscular activity is infrequent.

Our finding of some Mb in smooth muscle of arteries and veins suggests that it may play a role in limiting the time of vasodilation induced by nitric oxide delivered by S-nitrosohemoglobin (11). NO reacts rapidly with MbO₂ to form NO⁻ and MetMb (29, 30), so that Mb should shorten the lifetime of NO action in vasodilation. The lifetime of NO in Mb-rich heart muscle is known to be short (31). The vasodilation produced by NO depends on activation via cGMP of calcium-dependent potassium channels that cause hyperpolarization (32). The presence of Mb should shorten the time of hyperpolarization. Lancaster (33) has suggested that the action of NO may be restricted by Mb to localized areas of heart and skeletal muscle, a newly identified function for Mb. Similar localization may also occur in smooth muscle tissue. Mb should protect against the toxic effects of high NO production associated with tissue damage by accelerating its destruction. The proposed role of Mb in NO metabolism requires that the MetMb product be recycled. Ascorbate-mediated redox cycling of Mb has been shown to be capable of serving this function (34). Although the ascorbate content of human smooth muscle is not known, it is accumulated in many tissues and can reach concentrations of 300–800 μM in heart muscle and ~200 μM in skeletal muscle (35).

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| Muscle Tissue          | Mb content                                      | Reference |
|------------------------|-------------------------------------------------|-----------|
| Human smooth           |                                                 |           |
| Rectal                 | + immunohistochemical, mRNA                     | Present results |
| Uterine smooth         | + immunohistochemical, mRNA                     | Present results |
| Uterine smooth         | 0.2–0.6                                         | 5         |
| Colon                  | 0                                               | 6,7       |
| Bladder                | + immunohistochemical                            | Present results |
| Colon                  | 0                                               | 7         |
| Small intestine        | + immunohistochemical, mRNA                     | Present results |
| Pigeon smooth          |                                                 |           |
| Gizzard                | 9.0                                             | 13        |
| Human striated         |                                                 |           |
| Rectal                 | 3.2                                             | Present results |
| Pectoral               | 1.7                                             | 5         |
| Pectoral               | 7.6                                             | 7         |
| Intercostal            | 4.9                                             | 6         |
| Cardiac                | 4.3                                             | 6         |
| Rectus abdominis       | 12.3                                            | 7         |
| Rat, cat, and beef     |                                                 |           |
| Cardiac                | 2.6–5.4                                         | 13        |
| Rat                    |                                                 |           |
| Pectoral               | 5.2                                             | 13        |
| Pigeon                 |                                                 |           |
| Breast                 | 4.9                                             | 13        |
| Cardiac                | 3.6                                             | 13        |
| Seal                   |                                                 |           |
| Pectoral               | 33–42*                                          | 18        |
| Diaphragm              | 20–21*                                          | 18        |
| Porpoise               |                                                 |           |
| Psoas                  | 48ᵇ                                             | 19        |
| Heart                  | 10 ± 1ᵇ                                         | 19        |

ᵃ Calculated on the basis of O₂ capacity with 1.32 cm³ O₂/g Mb, assuming a molecular weight of ~17,000.
ᵇ Spectrophotometric assay of extracted MbCO. The values given are for dried muscle. Approximate wet weight values were estimated with the assumption that the muscle is 70% water.
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1. Qiu, Y., Sutton, L., and Riggs, A. F. (1995) *FASEB J.* 9, 1353 (abstr.)
2. Lankester, E. R. (1871) *Pflügers Arch. Gesamte Physiol.* 4, 315–320
3. Lankester, E. R. (1872) *Proc. Roy. Soc. Lond. B Biol. Sci.* 1, 70–80
4. Biörck, G. (1949) *Acta Med. Scand.* 226, (suppl.) 1–216
5. Jaisle F., and Huber, K. (1966) *Klin. Wochenschr.* 44, 1182–1184
6. Kagen, L. J., and Gurevich, R. (1967) *Immunology* 12, 667–673
7. Fasold, H., Riedl, G., and Jaisle, F. (1970) *Eur. J. Biochem.* 15, 122–126
8. Wittenberg, J. B. (1965) *J. Gen. Physiol.* 49, 57–74
9. Wittenberg, J. B. (1970) *Physiol. Rev.* 50, 559–636
10. Wittenberg, B. A., and Wittenberg, J. B. (1990) *Annu. Rev. Biophys. Biophys. Chem.* 19, 217–241
11. Jia, L., Bonaventura, C., Bonaventura, J., and Stamler, J. S. (1996) *Nature* 380, 221–226
12. Weller, P., Jeffrey, A. J., Wilson, V., and Blanchetot, A. (1984) *EMBO J.* 3, 439–446
13. Schauder, S., Wittenberg, J. B., Haseltine, B., and Wittenberg, B. A. (1979) *Anal. Biochem.* 92, 473–486
14. Antonini, E. (1965) *Physiol. Rev.* 45, 123–170
15. Bollag, D. M., and Edelstein, S. J. (1991) *Protein Methods*, p 96–116, Wiley-Liss, Inc., New York
16. Zhu, H., Owahy, D. W., Riggs, C. K., Nolasco, N. J., Stoops, J. K., and Riggs, A. F. (1996) *J. Biol. Chem.* 271, 30007–30021
17. Ownby, D. W., Zhu, H., Schneider, K., Beavis, R. C., Chait, B. T., and Riggs, A. F. (1993) *J. Biol. Chem.* 268, 13539–13547
18. Andersen, H. T. (1986) *Physiol. Rev.* 66, 212–243
19. Blessing, M. H. (1971) *Comp. Biochem. Physiol.* 41A, 475–480
20. de Duve, C. (1948) *Acta Chem. Scand.* 2, 264–289
21. Christensen, J., Freeman, B. W., and Miller, J. K. (1973) *Gastroenterology* 64, 1119–1125
22. Christensen, J. (1982) *Proc. Soc. Exp. Biol. Med.* 170, 194–202
23. Christensen, J., Conklin, J. L., and Freeman, B. W. (1973) *Am. J. Physiol.* 225, 1265–1270
24. Percy, W. H., Sutherland, J., and Christensen, J. (1991) *Dig. Dis. Sci.* 36, 1057–1065
25. Schulze-Delrieu, K., and Crane, S. A. (1982) *Am. J. Physiol.* 242, G258–G262
26. Christensen, J., and Roberts, R. L. (1983) *Gastroenterology* 85, 650–656
27. Prasad, R., Mukhopadhyay, A., and Prasad, N. (1978) *Experientia* 34, 484–485
28. Bloom, W., and Fawcett, D. W. (1968) *Textbook of Histology*, 9th Ed., p. 574, W. B. Saunders, Philadelphia, PA
29. Doyle, M. P., and Hoekstra, J. W. (1981) *J. Inorg. Biochem.* 14, 351–358
30. Eich, R. F., Li, T., Lemon, D. D., Doherty, D. H., Curry, S. R., Atten, J. F., Mathews, A. J., Johnson, K. A., Smith, R. D., Phillips, G. N., and Olson, J. S. (1996) *Biochemistry* 35, 6976–6983
31. Kelm, M., and Schroder, J. (1996) *Circ. Res.* 66, 1561–1575
32. Cornfield, D. N., Reeve, H. L., Tolarova, S., Weir, E. K., and Archer, S. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 8089–8094
33. Lancaster, J. R., Jr. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 8137–8141
34. Galaris, D., Cadenas, E., and Hochstein, P. (1989) *Arch. Biochem. Biophys.* 273, 497–504
35. Hornig, D. (1975) *Ann. N. Y. Acad. Sci.* 258, 103–118