The newly cloned proton-linked monocarboxylate transporter MCT3 was shown by Western blotting and immunofluorescence confocal microscopy to be expressed in all muscle fibers. In contrast, MCT1 is expressed most abundantly in oxidative fibers but is almost totally absent in fast-twitch glycolytic fibers. Thus MCT3 appears to be the major MCT isoform responsible for efflux of glycolytically derived lactic acid from white skeletal muscle. MCT3 is also expressed in several other tissues requiring rapid lactic acid efflux. The expression of both MCT3 and MCT1 was decreased by 40–60% 3 weeks after denervation of rat hind limb muscles, whereas chronic stimulation of the muscles for 7 days increased expression of MCT1 2–3-fold but had no effect on MCT3 expression. The kinetics and substrate and inhibitor specificities of monocarboxylate transport into cell lines expressing only MCT3 or MCT1 have been determined. Differences in the properties of MCT1 and MCT3 are relatively modest, suggesting that the significance of the two isoforms may be related to their regulation rather than their intrinsic properties.

Lactic acid is both a major fuel for skeletal muscle (“red” oxidative fibers) and a major metabolic end product (“white” glycolytic muscles). Even oxidative fibers become net lactic acid exporters when oxygen supply cannot meet demand, and glycolysis is stimulated to maintain ATP supplies. Fatigue occurs when lactic acid builds up within the myocyte. This causes intracellular pH (pHi) to drop, inhibiting both glycolysis and contractile activity (1, 2). In the extreme case further muscle activity is totally prevented, a phenomenon used to advantage by anglers “playing” their fish to exhaustion. The transport of lactic acid out of skeletal muscle fibers is essential if such intracellular accumulation of lactic acid is to be prevented.

Better removal of lactic acid from the muscle fibers might improve athletic performance during intense exercise and enable better muscle function and subsequent recovery under pathological conditions such as inherited mitochondrial diseases, hypoxia, and reperfusion following a period of ischemia.

Transport of lactic acid into skeletal muscle fibers for oxidation is thought to be mediated by the proton-linked monocarboxylate transporter (MCT) isoform MCT1 whose expression correlates with the oxidative capacity of muscle fibers and is increased following chronic muscle stimulation (3, 4). However, sarcolemmal membranes of muscle fibers that are primarily glycolytic do not contain significant amounts of MCT1 yet transport lactic acid by means of a saturable carrier that is inhibited by known inhibitors of MCT1 (3, 5, 6). These data imply the presence of another MCT isoform in such glycolytic fibers. MCT kinetics in heart (7–9) and liver (10) cells also imply the existence of other MCT isoforms, and this conclusion has been confirmed by cloning and sequencing studies.

The first MCT isoform (MCT1) was cloned from Chinese hamster ovary cells (11) and has since been cloned and sequenced from human (12), rat (13), and mouse (14). Topology predictions suggest that the transporter contains 12 transmembrane helical domains in common with many other substrate transporters, and we have confirmed this prediction experimentally (15). Another MCT isoform (MCT2) has been cloned from hamster (16) and rat (17). This is the predominant MCT isoform in hamster liver, but is not widely distributed in rat tissues and is absent in skeletal muscle (17). Thus it seems probable that an additional MCT isoform is responsible for lactic acid efflux from those muscle fibers lacking MCT1. Recently we have cloned and sequenced four new members of the MCT family and investigated their tissue distribution by Northern blotting (18). We found that mRNA for one of these isoforms is present in large amounts in skeletal muscle, and we termed this isoform MCT3 because of its close homology to chicken MCT3, an MCT isoform found exclusively in the chicken retinal epithelium (19, 20). It remains to be established whether chicken MCT3 and mammalian MCT3 are produced by equivalent genes, perhaps as alternatively spliced forms (20), or are distinct gene products. Here we use antipeptide antibodies to confirm that mammalian MCT3 protein is also strongly expressed in skeletal muscle and in several other tissues.
glycolytic cells. We have characterized its kinetics and shown that it is down-regulated in rat hind limb skeletal muscle by chronic denervation.

**EXPERIMENTAL PROCEDURES**

**Materials**

Ehrlich Lettré tumour cells (ELT), the bovine kidney cell line NBL1, and COS cells were cultured as described elsewhere (21–23). Isolated rat cardiac myocytes, prepared as described previously (8), were kindly provided by Dr. Elinor Griffths and human cardiac ventricles muscle by Professor Giannini Angelini (Bristol Heart Institute). Human white cells were obtained from the local blood bank and human placenta from the local maternity hospital. Antipeptide antibodies to MCT1 and MCT3 were raised in rabbits against C-terminal peptides, conjugated to keyhole limpet hemocyanin as described previously (15). The peptides used, including an N-terminal cysteine for coupling to keyhole limpet hemocyanin, were CQKTEGGPKREEPSV for human MCT1 (cross-reacts with MCT1 from most species), CPQNSSGDPAEESPV for Chinese hamster MCT1 (cross-reacts with rat but not human MCT1), and CEPEKNGKEVHTPETSV for human MCT3. Antibodies were purified by affinity purification using the immobilized peptide as described previously (15). All reagents for immunofluorescence microscopy were from Sigma.

**Methods**

**Detection of MCT Isoforms in Cell and Tissue Membrane Fractions**—Preparation of crude plasma membranes from a variety of tissue homogenates was performed in the presence of a range of protease inhibitors (24) and samples separated by SDS-PAGE and analyzed by Western blotting with anti-MCT1 and -MCT3 antibodies using ECL detection (15). In a large preparation of plasma membranes from cell lines the initial cell disruption involved homogenization with a Potter-Elvehjem homogenizer (50 strokes) followed by 20 passes through a fine (25-gauge) needle.

**Measurement of MCT Isoforms in Individual Muscles**—Where required, denervation or chronic stimulation of rat hind limb muscles was performed as described previously (4, 5); the procedures had received ethical approval by the relevant committees. An untreated contralateral limb always acted as a control for the treated limb of the same animal. Muscles were dissected out, frozen in liquid nitrogen, and stored at −80 °C. Samples of muscle (40 mg) were homogenized (Polytron 2100, 2 × 30 s on maximum setting) in 1 ml of buffer (250 mM sucrose, 30 mM HEPES, 2 mM EGTA, 40 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). After centrifugation at 200,000 g for 90 min at 4 °C, the pellet was resuspended in 500 μl of Triton-SDS (10 mM Tris, 4% (w/v) SDS, 1 mM EDTA, pH 7.4) and homogenized (2 × 15 s with a Polytron). Following protein determination (Bio-Rad, detergent-compatible protein assay), samples were diluted to 2 mg of protein/ml and an equal volume of sample buffer added. Samples (20 μl) were then subjected to SDS-PAGE (8–18% gradient gel) and Western blotting as above. Staining was performed by scanning the film and analysis of band intensities with “Band Leader”, a shareware program. As described previously (3), by using increasing concentrations of homog enate it was confirmed that in all cases protein loading was such that the response of the film was linear with respect to the amount of MCT present. Samples of contralateral control muscle homogenates were analyzed on the same gel as samples (containing the same amount of protein) from denervated or chronically contracting muscles.

**Histochemistry and Immunofluorescence Confocal Microscopy**—Skeletal muscles were dissected from the hind limbs of freshly killed rats under a relaxing solution (127 mM KCH3SO4, 13 mM KCl, 5 mM HEPES, pH 7.4) before preparation of frozen sections (10 μm) were then made with a Nikon Diaphot inverted microscope. After allowing cells to fix for 30 s on maximum setting) in 1 ml of buffer (250 mM sucrose, 30 mM HEPES, 2 mM EGTA, 40 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). After centrifugation at 200,000 g for 90 min at 4 °C, the pellet was resuspended in 500 μl of Triton-SDS (10 mM Tris, 4% (w/v) SDS, 1 mM EDTA, pH 7.4) and homogenized (2 × 15 s with a Polytron). Following protein determination (Bio-Rad, detergent-compatible protein assay), samples were diluted to 2 mg of protein/ml and an equal volume of sample buffer added. Samples (20 μl) were then subjected to SDS-PAGE (8–18% gradient gel) and Western blotting as above. Staining was performed by scanning the film and analysis of band intensities with “Band Leader”, a shareware program. As described previously (3), by using increasing concentrations of homog enate it was confirmed that in all cases protein loading was such that the response of the film was linear with respect to the amount of MCT present. Samples of contralateral control muscle homogenates were analyzed on the same gel as samples (containing the same amount of protein) from denervated or chronically contracting muscles.

**Measurement of Monocarboxylate Transport into Cultured Cells**—After washing twice with PBS, cells were detached from the culture flasks by incubation with trypsin solution (Sigma) for 5 min (COS) or 30 min (NBL1), followed by inactivation of the trypsin with PBS containing 10% (w/v) fetal bovine serum. Cells were then washed twice in PBS and once in transport buffer (150 mM NaCl, 5 mM KCl, 1 mM KH2PO4, 1 mM MgSO4, 0.2 mM CaCl2, 3.3 mM MOPS, 10 mM HEPES, pH 7.4) before incubating in transport buffer containing 5 μM BCFE/FAM ( Biosurge, 500 strokes) for 20 min at room temperature. Loaded cells were collected by centrifugation and resuspended to about 5 × 106 cells/ml in transport buffer. A sample (10 μl) of these cells was then placed in a 50-μl perspex incubation chamber on polylsine-coated coverslips, held in a thermally controlled incubation vessel attached to the stage of a Nikon Diaphot inverted microscope. After allowing cells to fix for 30 s on maximum setting) in 1 ml of buffer (250 mM sucrose, 30 mM HEPES, 2 mM EGTA, 40 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). After centrifugation at 200,000 g for 90 min at 4 °C, the pellet was resuspended in 500 μl of Triton-SDS (10 mM Tris, 4% (w/v) SDS, 1 mM EDTA, pH 7.4) and homogenized (2 × 15 s with a Polytron). Following protein determination (Bio-Rad, detergent-compatible protein assay), samples were diluted to 2 mg of protein/ml and an equal volume of sample buffer added. Samples (20 μl) were then subjected to SDS-PAGE (8–18% gradient gel) and Western blotting as above. Staining was performed by scanning the film and analysis of band intensities with “Band Leader”, a shareware program. As described previously (3), by using increasing concentrations of homog enate it was confirmed that in all cases protein loading was such that the response of the film was linear with respect to the amount of MCT present. Samples of contralateral control muscle homogenates were analyzed on the same gel as samples (containing the same amount of protein) from denervated or chronically contracting muscles.

**RESULTS**

**Tissue Distribution of MCT3—Antipeptide antibodies to the C terminus of human MCT3 have been raised and used in Western blotting to determine the presence of MCT3 in plasma membranes from various rat and human tissues. Data are shown in Fig. 1. We subsequently cloned and sequenced rat MCT3 cDNA2 and confirmed that it exhibited 100% identity over the region against which the antibody was raised. MCT3 was detected in plasma membranes from rat hind limb muscle (primarily white muscle), which contained very little MCT1. In contrast, no MCT3 was detected in membranes from rat liver and heart or from brain, kidney, and testis (data not shown), all of which contained large amounts of MCT1. In available human tissues we found MCT3 in membrane preparations from heart, placenta, and white blood cells (Fig. 1), whereas MCT1 was detected only in membranes from heart. In all cases care was taken to inhibit proteases that might destroy the epitope detected by the antibody, and thus, the absence of a particular MCT in the membrane preparations appears to be real. The
Muscle homogenates were prepared from white muscle fiber types. WTA), white tibialis anterior (WTA), semimembranosis, extensor digitorum longus (EDL), plantaris (PL), red tibialis anterior (RTA), red gastrocnemius (RG), and soleus (SOL). In each case an equal amount of homogenate protein (about 40 μg) was analyzed by SDS-PAGE and MCT1 and MCT3 expression quantified by scanning Western blots developed by ECL as described under “Experimental Procedures.” MCT expression in each muscle type is expressed as a percentage of that expressed in the same amount of homogenate protein from WTA muscle. Data are given from two separate studies in different laboratories (●, ■) and are expressed as means ± S.E. (error bars) of three or six to eight muscle samples from separate rats respectively. The x axis represents the percentage of oxidative fibers (slow-twitch oxidative + fast-twitch oxidative glycolytic) in each muscle as reported elsewhere (32).

The relative expression of MCT1 and MCT3 in different muscle fiber types. Muscle homogenates were prepared from white gastrocnemius (WG), white tibialis anterior (WTA), semimembranosis, extensor digitorum longus (EDL), plantaris (PL), red tibialis anterior (RTA), red gastrocnemius (RG), and soleus (SOL). In each case an equal amount of homogenate protein (about 40 μg) was analyzed by SDS-PAGE and MCT1 and MCT3 expression quantified by scanning Western blots developed by ECL as described under “Experimental Procedures.” MCT expression in each muscle type is expressed as a percentage of that expressed in the same amount of homogenate protein from WTA muscle. Data are given from two separate studies in different laboratories (●, ■) and are expressed as means ± S.E. (error bars) of three or six to eight muscle samples from separate rats respectively. The x axis represents the percentage of oxidative fibers (slow-twitch oxidative + fast-twitch oxidative glycolytic) in each muscle as reported elsewhere (32).

The localization of MCT1 and MCT3 in different muscle fiber types. Immunofluorescence confocal microscopy with anti-MCT1 and -MCT3 antibodies was performed on frozen muscle sections as described under “Experimental Procedures.” In the top panel a lower magnification is used to allow display of a larger field of cells. This panel also includes sections stained with succinate dehydrogenase (SDH) to visualize the mitochondrial content of the different fibers. Some shrinkage is occurred during preparation of the soleus section.
lines do lack MCT activity (16), and we have confirmed this for *Spodoptera* f21 and *Drosophila* Schneider cells. However, we have been unsuccessful in establishing a stable MCT3-transfected insect cell line using a variety of expression vectors.

Thus we decided to look for a mammalian cell line that expressed MCT3 but no other MCT isoform. For this purpose we screened a range of cell lines using Western blotting with antipeptide antibodies that we have raised against C-terminal peptide sequences specific for each of the different MCT isoforms. As shown in Fig. 1 we have confirmed that mouse ELT tumor cells express only MCT1 as suggested by our previous studies (14), while the bovine kidney cell line NBL1 express only MCT3. Although not shown in Fig. 1 we have also been unable to detect any protein reacting with antipeptide antibodies we have raised to MCT2, MCT4, MCT5, MCT6, or MCT7 in either ELT or NBL1 cells. The COS cell line derived from monkey kidney also appeared to express mainly MCT3. How-
ever, the MCT1 antibody did give a faint band of about 43 kDa and also an additional band of about 85 kDa, both of which were abolished in the presence of MCT1 peptide. Thus it is likely that significant amounts of MCT1 are present in COS cells, the 85-kDa band probably reflecting dimeric MCT1, since this protein is known to aggregate in detergent (27).

For each of these three cell lines monocarboxylate transport was measured using the intracellular pH-sensitive fluorescent dye, BCECF, which detects the rapid decrease in pH, that accompanies proton-linked transport (8–10, 21). Data are shown in Fig. 5. Measurement of the true initial rate of fluorescence change was possible by fitting the curve to a first order uptake equation and subsequently converted into a rate of monomer uptake of monocarboxylate transport in nanomoles per μl intracellular space per minute (10, 21). The latter calculation required measurement of the pH gradient across the plasma membrane (0.1 pH units inside) determined using butyrate and trimethylamine and the total change in fluorescence ratio induced by increasing l-lactate concentrations once equilibrium had been reached. For each cell type, initial rates of monocarboxylate transport were measured on three or four different fields of cells at 1, 2, 5, 10, 20, and 30 mM l-lactate, d-lactate, and pyruvate. Results are summarized in Fig. 6 and the derived $K_m$ and $V_{max}$ values presented in Table I.

For three separate experiments with ELT cells, the $V_{max}$ values obtained for all three substrates were similar (about 30 nmol/min/μl intracellular volume), although slightly greater than the value of 20 nmol/min/μl measured previously using cells in suspension (21). The $K_m$ values obtained for l-lactate (6.4 mM), d-lactate (47 mM), and pyruvate (2.1 mM) are also higher than those measured previously (4.5, 27.5, and 0.7 mM, respectively). However, for pyruvate the value obtained must be treated with caution, since it was not possible to determine fluorescence changes accurately at concentrations <1 mM. In the case of NBL1 cells, which only contain MCT3, derived $K_m$ values for l-lactate (10.1 mM) and d-lactate (12.6 mM) were similar, while the $V_{max}$ values were different (14.8 and 7.6 nmol/min/μl intracellular volume, respectively). The derived $K_m$ value for pyruvate was 0.9 mM and the $V_{max}$ was 4.2 nmol/min/μl intracellular volume. Kinetic data for COS cells exhibit some features similar to ELT cells and others more like NBL1 cells. Bearing in mind that each cell line is from a different species, these data are consistent with the presence of both MCT1 and MCT3 in COS cells as implied by the data of Fig. 1.

Inhibition of lactate transport into heart, liver, and tumor cells by three MCT inhibitors (α-cyano-4-hydroxycinnamate derivatives, stilbene disulfonates, and phloretin) has indicated that different MCT isoforms may have different inhibitor specificities (1, 7–10). Thus we have determined $K_{0.5}$ values for these compounds as inhibitors of the transport of 4 mM l-lactate into each of the three cell types. Data are shown in Fig. 7 and Table I. For ELT cells $K_{0.5}$ values for α-cyano-4-hydroxycinnamate (0.45 mM), DIDS (0.64 mM), and phloretin (4.4 μM) were quite similar to the values (0.17 mM, 0.43 mM, and 5.1 μM, respectively) measured previously using cell suspensions (21). For NBL1 cells the $K_{0.5}$ values for α-cyano-4-hydroxycinnamate and phloretin were 1.0 and 3.2 μM, respectively, while for DIDS the pattern of inhibition was more complex. It appeared that there was a non-inhibitable component present (about 20% of the uninhibited rate) with the inhibitable component having a $K_{0.5}$ for DIDS of 28 μM. DIDS has also been shown to exhibit partial inhibition in rat heart cells, with a $K_{0.5}$ of about 79 μM (9). Data for the inhibition of l-lactate transport into COS cells are also given in Fig. 7 and Table I. $K_{0.5}$ values are more similar to the data for NBL1 cells than for ELT cells, and this is consistent with the large amount of MCT3 in both cell lines (Fig. 1).

**DISCUSSION**

**MCT3 Is a Major Route for Lactic Acid Efflux from White Skeletal Muscle and Other Cells**—The correlation of MCT1 expression with the oxidative capacity of skeletal muscle fibers and its low levels in fast-twitch glycolytic fibers has led us to propose that this MCT isofrom is important for the transport of lactic acid into muscle fibers for use as a respiratory fuel (3, 4). This implies that another MCT isofrom must be responsible for lactic acid efflux from fibers that are primarily glycolytic and express little MCT1. The data we present here suggest that MCT3 fulfils this role. It is present in all muscle fibers, but expressed least in totally oxidative fibers such as the soleus (Figs. 2 and 3). The abrupt decrease in MCT3 expression between red gastrocnemius (SO 30%, FOG 62%, FG 8%) and rat heart (62% SO, 30% FOG) suggests that it is the slow oxidative fibers that are especially low in MCT3, but more detailed analysis will be required to confirm this. There is likely to be an important role for MCT3 in lactic acid export in other cells; for example MCT3 is the only MCT expressed in human white blood cells, which are also highly glycolytic lactic acid exporters. Its presence in two kidney cell lines (COS and NBL1 cells) may also reflect a requirement of the cells from which they were derived (renal tubular epithelium) to export lactic acid taken up from the tubule lumen across the basolateral membrane and into the blood.

**TABLE I**

**Kinetic constants for substrates and inhibitors of MCT transport in different cell lines**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ELT</th>
<th>COS</th>
<th>NBLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Lactate</td>
<td>6.4 ± 0.7</td>
<td>6.6 ± 0.5</td>
<td>10.1 ± 2.8</td>
</tr>
<tr>
<td>d-Lactate</td>
<td>46.8 ± 17.4</td>
<td>12.8 ± 0.5</td>
<td>12.6 ± 3.4</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2.1 ± 0.5</td>
<td>1.3 ± 0.3</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>30.9 ± 1.2</td>
<td>18.4 ± 0.4</td>
<td>14.3 ± 1.6</td>
</tr>
<tr>
<td>$K_m$</td>
<td>33.5 ± 8.4</td>
<td>6.9 ± 0.5</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>28.5 ± 1.7</td>
<td>11.0 ± 1.1</td>
<td>4.2 ± 0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>ELT</th>
<th>COS</th>
<th>NBLI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHC</td>
<td>450 ± 12</td>
<td>1060 ± 110</td>
<td>1000 ± 110</td>
</tr>
<tr>
<td>DIDS</td>
<td>642 ± 47</td>
<td>56.3 ± 5.6</td>
<td>28.2 ± 4.8</td>
</tr>
<tr>
<td>Phloretin</td>
<td>44 ± 0.9</td>
<td>8.4 ± 2.5</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>$K_{0.5}$</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>% control rate</td>
<td>0.0</td>
<td>23.3 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

$V_{max}$ represents the S.E. of the derived parameter values for the fits shown in the two Figs. 6 and 7. The conversion of the 440/490 fluorescence ratio change into nanomoles of monocarboxylate transported per μl of intracellular space was performed as described in the text.
monocarboxylate transport into COS, NBL1, and ELT cells. The
ponent of the transport. Derived values (±S.E.) for
initiated 1 min after pre-exposure to the concentration of inhibitor [I]
experimental protocol was similar to that used in Fig. 6. Transport was
presented in Table I.

removal of lactate from the superfusion medium leads to a
MCT1 and MCT3 catalyze both lactic acid influx and efflux;
Refs. 1, 2, and 8). Furthermore, the data of Fig. 5 confirm that
values for influx and efflux obeying the Haldane equation (see
inhibition (4). However, the first preparation is incap-
able of giving accurate kinetic data for plasma membrane trans-
port, while sarcolemmal vesicle preparations may contain
vesicles of different sizes and are usually derived from more
than a single fiber type containing an unknown mixture of
MCT1 and MCT3. These factors complicate kinetic analysis,
although the problems are minimized by using giant vesicles
(2). \( K_m \) values for l-lactate derived using such vesicles are in
the range 12–40 mM and are similar whether vesicles are derived
mainly from white or red fibers (28). This is consistent with the
similar \( K_m \) values of MCT1 and MCT3 for l-lactate described here. Myotubules should be capable of giving accurate
transport data, and the derived \( K_m \) values for l-lactate in this
system was 12.5 mM, again similar to the values we have
derived. However, the profile of MCT isoforms in this cultured
myocyte cell line has not been established. In frog sartorius
muscle microelectrode measurements of single fibers gave a \( K_m \)
value for L-lactate of 10 mM (29). These data on a single white
muscle fiber probably most closely represent MCT3 kinetics.
Indeed, the \( K_m \) is the same as that reported here for NBL1
cells, which also contain only MCT3. \( K_m \) values for the trans-
port of pyruvate and D-lactate and the \( K_m \) values for CHC,
DIDS, and phloretin cannot easily be compared with data ob-
tained for sarcolemmal membrane vesicles or the perfused hind
limb, since there is a great deal of variation in the literature (2).
This may be due to the limitations discussed above.

Comparison of the Properties of MCT1 and MCT3 with the
Characteristics of Lactate Transport into Skeletal Muscle—A
comparison of the kinetics of MCT3 with the kinetics of mono-
carboxylate transport into skeletal muscle fibers is difficult for
two reasons. First, the properties of the transporter may vary
according to the cellular environment as a comparison of MCT1
kinetics in red blood cells and tumor cells illustrates (21). This
may partially reflect the ability of MCT1 to interact specifically
with other membrane proteins (27). Second, there are inherent
problems in measuring monocarboxylate transport across the
sarcolemmal membrane. Radiotracers have been used in the
perfused rat hind limb, a cultured myocyte cell line that can
form myotubules, and in isolated sarcolemmal vesicle prepara-
tions (see Refs. 1–4). However, the first preparation is incapa-
able of giving accurate kinetic data for plasma membrane trans-
port, while sarcolemmal vesicle preparations may contain
vesicles of different sizes and are usually derived from more
than a single fiber type containing an unknown mixture of
MCT1 and MCT3. These factors complicate kinetic analysis,
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tained for sarcolemmal membrane vesicles or the perfused hind
limb, since there is a great deal of variation in the literature (2).
This may be due to the limitations discussed above.

Regulation of MCT3 Expression in Skeletal Muscle—We
present data in this paper to show that denervation of rat hind
limb muscles down-regulates expression of both MCT1 and
MCT3 in white gastrocnemius, red gastrocnemius, and soleus
muscles. This parallels the decrease in rate of lactate efflux
measured in giant sarcolemmal vesicles prepared from the
same muscles and reflects the ability of denervation to dimin-
ish the activity of both oxidative and glycolytic fibers. The effect
is not due to a general decrease in muscle protein, since the
control and denervated samples used for SDS-PAGE contained
the same amount of protein. Furthermore, we have shown
previously that denervation increases GLUT-1 expression,
while decreasing GLUT-4 expression (30), and that the
decrease in succinate dehydrogenase and lactate dehydrogenase
activities is less than MCT1 and MCT3 (5). In contrast to
denervation, chronic stimulation of the rat hind limb up-regu-
lates lactate transport and MCT1 expression without affecting

![Fig. 7. The concentration dependence of some inhibitors of monocarboxylate transport into COS, NBL1, and ELT cells. The experimental protocol was similar to that used in Fig. 6. Transport was initiated 1 min after pre-exposure to the concentration of inhibitor [I] shown by superfusion with medium containing 4 mM L-lactate and inhibitor. Rates of transport (V) were expressed as a percentage of those in the absence of inhibitor and data fitted to the equation for partial inhibition (V = (V_{max}/(1 + [I]/K_{i}))) + V_{n} as described previously (7). This equation includes both an inhibitable (V_{i}) and noninhibitable (V_{n}) component of the transport. Derived values (±S.E.) for K_{i} and V_{n} are presented in Table I.](http://jbc.org/doi/fig)
MCT3 expression. The rate of stimulation used (10 Hz) induces a rate of contraction that mimics activation of oxidative fibers, and this is reflected in an increase in citrate synthase expression in both SO and FOG fibers (4). Whatever the mechanisms involved, it is clear that the expression of MCT1 and MCT3 can be separately regulated, perhaps in response to increased oxidative and glycolytic metabolism, respectively.

MCT3 and the Heart—It might be expected that heart myocytes should also contain both MCT1 and MCT3, since like skeletal muscle fibers, they can both oxidize lactic acid and, when hypoxic, produce large quantities of lactic acid by glycolysis. The presence of MCT1 in heart cells is well established, but it is located primarily in the intercalated disk and T-tubule regions (11, 26, 31), whereas transport occurs more rapidly in the center of the cell where MCT1 expression is less (31). This implies the presence of another MCT isoform, and we have provided extensive kinetic evidence for the presence of two distinct MCT isoforms in both rat and guinea pig cardiac myocytes (7–9, 31). The properties of MCT1 and MCT3 described here are consistent with these being the two isoforms present in the heart. Thus in heart cells stibene disulfonates such as DIDS also give only partial inhibition, and this inhibition appears to be of an isoform with a lower $K_m$ for pyruvate and D-lactate than the stibene disulfonate-insensitive isoform but with a similar $K_m$ for l-lactate. Both isoforms have a similar $K_{0.5}$ for phloretin. However, although we were able to detect large amounts of both MCT1 and MCT3 in sarcolemmal membrane from human heart, we were barely able to detect MCT3 in an equivalent membrane preparation from rat hearts (Fig. 1). We have also been unable to detect MCT3 mRNA in rat heart by either Northern blots or reverse transcription-polymerase chain reaction analysis. Neither did immunofluorescent confocal microscopy reveal any MCT3 in isolated rat heart cells (data not shown). Thus the absence of MCT3 in rat muscle, but its presence in human heart muscle, represents a species difference and implies that another MCT isoform with similar properties to MCT3 may be present in these cells.

In summary, MCT3 appears to be the MCT isoform expressed most in muscle fibers whose energy metabolism is mainly glycolytic, whereas MCT1 predominates in muscles that rely more on oxidative metabolism. Denervation of rat hind limb muscles decreases the expression of both MCT3 and MCT1, whereas chronic stimulation of the muscle increases expression of MCT1 but not MCT3. Differences in the kinetics of MCT1 and MCT3 are relatively modest and suggest that the significance of the two isoforms may be related more to their regulation than to any minor differences in their functional properties.

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Note Added in Proof—Very recently Philp et al. (Philp, N. J., Yoon, H., and Grollman, E. F. (1998) Am. J. Physiol. 274, R1824–R1828) have identified an additional rat MCT isoform (GenBank™ accession number AF092558), which has a similar degree of identity to chicken MCT3 as does our MCT isoform referred to as MCT3 in this paper and Ref. 18. However, the new rat MCT isoform of Philp et al. has the same exclusive retinal pigment epithelial distribution as chicken MCT3. Thus the MCT isoform expressed in mammalian muscle that is referred to as MCT3 in the present paper will in future be termed MCT4. Three further MCT isoforms identified by searching dbEST and subsequently cloned and sequenced (this paper and Ref. 18) will in future be referred to as MCT5, MCT6, and MCT7.

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Lactic Acid Efflux from White Skeletal Muscle Is Catalyzed by the Monocarboxylate Transporter Isoform MCT3

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