The kinetics of transport of l-lactate, pyruvate, ketone bodies, and other monocarboxylates into isolated hepatocytes from starved rats were measured at 25 °C using the intracellular pH-sensitive dye, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein, to detect the associated proton influx. Transport kinetics were similar, but not identical, to those determined using the same technique for the monocarboxylate transporter (MCT) of Ehrlich Lettré tumor cells (MCT1) (Carpenter, L., and Halestrap, A. P. (1994) Biochem. J. 304, 751-760). $K_m$ values for l-lactate (4.7 mM), d-lactate (27 mM), d-l-2-hydroxybutyrate (3.3 mM), l-3-hydroxybutyrate (12.7 mM), and acetocetate (6.1 mM) were very similar in both cell types, whereas in hepatocytes the $K_m$ values were higher than MCT1 for pyruvate (1.3 mM, cf. 0.72 mM), d-3-hydroxybutyrate (24.7 mM, cf. 10.1 mM), l-2-chloropropionate (1.3 mM, cf. 0.8 mM), 4-hydroxybutyrate (18.1 mM, cf. 7.7 mM), and acetate (5.4 mM, cf. 3.7 mM). In contrast, the hepatocyte carrier had lower $K_m$ values than MCT1 for glycolate, chloroacetate, dichloroacetate, and 2-hydroxy-2-methylpropionate. Differences in stereoselectivity were also detected; both carriers showed a lower $K_m$ for l-lactate than o-lactate, while hepatocyte MCT exhibited a lower $K_m$ for o-lactate than d-lactate and d-2-chloropropionate and for l-lactate than o-3-hydroxybutyrate; this is not the case for MCT1.

A range of inhibitors of MCT1, including α-cyanocinnamate derivatives, phloretin, and niflumic acid, inhibited hepatocyte MCT with $K_i$ values significantly higher than for tumor cell MCT1, while stilbene disulfonate derivatives and p-chloromercuribenzenesulfonate had similar $K_i$ values in both cell types. The branched chain ketoacids α-ketoisocaproate and α-ketovalerate were also potent inhibitors of hepatocyte MCT with $K_i$ values of 270 and 340 μM, respectively. The activation energy of l-lactate transport into hepatocytes was 58 kJ mol$^{-1}$, and measured rates of transport at 37 °C were considerably greater than those required for maximal rates of gluconeogenesis. The properties of the hepatocyte monocarboxylate transporter are consistent with the presence of a distinct isofrom of MCT in liver cells as suggested by the cloning and sequencing of MCT2 from hamster liver (Garcia, C. K., Brown, M. S., Pathak, R. K., and Goldstein, J. L. (1995) J. Biol. Chem. 270, 1843-1849).

Most mammalian cells transport lactic acid across their plasma membranes by means of a monocarboxylate/proton co-transporter (MCT),$^1$ the characteristics of which have been investigated in a variety of cell types (see Poole and Halestrap (1993)). The most extensively studied MCT is that found in the red blood cell (Deuticke, 1982; Poole and Halestrap, 1993), which has been identified and partially purified in this laboratory (Poole and Halestrap, 1992). N-terminal sequencing (Poole and Halestrap, 1994) has shown it to be the same as the transporter recently cloned and sequenced from Chinese hamster ovary cells and named MCT1 (Garcia et al., 1994). Detailed studies of the kinetics and substrate and inhibitor specificity of monocarboxylate transport into heart cells has indicated that this tissue contains two isoforms of MCT that each have properties distinct from MCT1 (Poole et al., 1989, 1990; Wang et al., 1993, 1994, 1995). Immunofluorescent studies using MCT1-specific antibodies have shown that heart cells also contain a small amount of MCT1, but this is confined to the intercalated disk region (Garcia et al., 1994). Data from other laboratories suggest that the transporter found in skeletal muscle is yet another isoform (see Roth (1991) and Poole and Halestrap (1993)), and immunofluorescent studies of white muscle support these conclusions (Garcia et al., 1994).

The hepatocyte requires a very rapid transport mechanism for the uptake of lactic acid for gluconeogenesis and lipogenesis while lactic acid efflux occurs under hypoxic conditions. In addition, the liver is the major producer of ketone bodies and exports acetooacetic acid and β-hydroxybutyric acid during fasting or endurance exercise (see Poole and Halestrap (1993)). Lactate transport into liver cells has been investigated in this and other laboratories using radioactive tracer techniques (see Poole and Halestrap (1993)). Although some studies have been performed in vivo (Lupo et al., 1990), in the perfused liver (Schwab et al., 1979; Bracht et al., 1981) and in isolated liver plasma membrane vesicles (Quintana et al., 1988), it is doubtful whether these systems allow accurate kinetic analysis of the transporter itself (see Poole and Halestrap (1993)). Isolated hepatocytes are more suitable and have been used in this and other laboratories (Monson et al., 1982; Faoufournoux et al., 1985a; Edlund and Halestrap, 1988; Metcalfe et al., 1986). However, to resolve the true initial rates of transport, we have found it necessary to work at low temperatures (0 °C) and to

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$^1$ The abbreviations used are: MCT, monocarboxylate transporter; BCECF, 2′,7′-bis(carboxyethyl)-5(6)-carboxyfluorescein; CHC, α-cyano-4-hydroxycinnamate; DBDS, 4,4′-dibenzamidostilbene-2,2′-disulfonate; DIDS, 4,4′-disothiocyanostilbene-2,2′-disulfonate; KIC, α-ketoisocaproate; KIV, α-ketoisovalerate; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonate; pCMBS, p-chloromercuribenzenesulfonate; MOPS, 4-morpholinepropanesulfonic acid.
Monocarboxylate Transport into Liver Cells

Monocarboxylate transport over very short time periods (Edlund and Halestrap, 1988). At more physiological temperatures, the rapid and extensive uptake of labeled lactate may well represent metabolism rather than transport. The limited studies on the kinetics and substrate and inhibitor specificity of the hepatocyte monocarboxylate transporter performed in this way have confirmed that its properties are similar to those of MCT1. However, using immunofluorescent microscopy, Garcia et al. (1994) have shown that Syrian hamster hepatocytes contain little or no MCT1. Very recently, these workers have cloned and sequenced an MCT-related cDNA derived from hamster liver and have shown that when expressed in insect S9 cells using baculovirus, it stimulated pyruvate transport activity. This new isoform of MCT, named MTC2, is 60% identical to MCT1, and its transport activity is inhibited by phloretin and α-cyano-4-hydroxycinnamate (CHC) but apparently not by organomercurials. However, only very limited functional studies of MCT2 were performed, and it is clearly important for the properties of the new isoform to be fully characterized.

In this paper, we have used the intracellular pH-sensitive fluorescent dye 2,7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF) to provide such a detailed analysis of the kinetics and substrate and inhibitor specificity of monocarboxylate transport in rat hepatocytes as we have done previously in Ehrlich Lettré tumor cells (Carpenter and Halestrap, 1994) and isolated cardiac myocytes (Wang et al., 1994, 1995). Since fluorescence can be measured continuously with a very rapid time resolution, the technique produces both more accurate measurements of transport rates and allows them to be made at higher, more physiological temperatures and with any substrate. Our results confirm that the kinetics and substrate and inhibitor specificity of MCT in liver cells are similar to those of MCT1 but with some significant differences that may relate to its biological function or regulation. We also demonstrate that the transporter is capable of transporting l-lactate at rates considerably faster than required for maximum rates of metabolism, implying that the carrier is unlikely to be a major site of regulation of lactate metabolism under physiological conditions.

**EXPERIMENTAL PROCEDURES**

Materials—Chemicals and biochemicals, including all substrates and inhibitors of the monocarboxylate transporter, were obtained from sources given previously (Poole and Halestrap, 1988, 1991; Poole et al., 1990; Carpenter and Halestrap, 1994). Hepatocytes were prepared as described previously (Edlund and Halestrap, 1988) using collagenase digestion of livers from 250-g male Wistar rats starved overnight. Cells were >95% viable (trypan blue exclusion) and were stored on ice at about 20 mg of protein/ml of bicarbonate-buffered saline medium containing 10 mM bovine serum albumin under an atmosphere of O2/CO2 (19:1).

Loading of Hepatocytes with BCECF for Fluorimetric Measurement—The hepatocytes were washed once in bicarbonate-buffered saline without albumin before resuspending at 20 mg of protein/ml in the same buffer supplemented with acetoxymethyl ester of BCECF (10 μM). Cells were incubated at 37°C for 10 min before centrifugation and washed three times in the HEPES/MOPS-buffered saline medium (150 mM Na+, 5 mM K+, 150 mM Cl-, 1 mM Ca2+, 1 mM sulfate, 1 mM phosphate, 3.3 mM MOPS, 10 mM HEPES brought to pH 7.4 at 25°C using NaOH). The loaded cells were stored on ice, protected from light, at about 1-106 cells/ml (approximately 20 mg of protein/ml) until use, and showed no detectable decrease in fluorescence over 3 h.

Measurement of Monocarboxylate Transport—A 0.5-ml aliquot of the cells loaded with BCECF (final concentration of approximately 1.5 x 106 cells/ml) was added to 3 ml of HEPES/MOPS-buffered saline medium contained in a 4-ml fluorescence cuvette. The cells were kept in suspension by constant stirring using an overhead paddle stirrer integral to the cuvette holder of the fluorimeter, which was maintained at the required temperature (usually 25°C) by water circulation. BCECF fluorescence was measured as described previously, using dual wavelength excitation at 450 and 500 nm (2-nm slit widths, oscillating frequency of 397 Hz) with emission (>520 nm using a cut-off filter) detected with a photomultiplier orientated at 90 ° to the incident light and in close proximity to the cuvette. The ratio of fluorescent light corresponding to the two excitation wavelengths (the 450/500 ratio) was determined electronically, and the resulting signal was averaged by computer to give 2–4 data points per second, which were displayed in real time and stored for further analysis using software. The 450/500 ratio provides a measure of pH, which minimizes errors due to light scattering of the sample and loss of BCECF from the cell by bleeding or diffusion (Eisner et al., 1989). Once the fluorescence signal from the cell suspension had reached a steady state, usually after 1 min, substrates (neutralized as the sodium salts) were added to the suspension through a light-sealed addition port, and the subsequent fluorescence change was monitored. The initial rate of the fluorescence change was determined by fitting the data to a first order progress curve using Fig P and Pfit software (Biosoft, Cambridge, United Kingdom). The first two data points were omitted since these incorporated errors associated with the mixing time and addition artifacts. Whenever additions of colored reagents were made to the standard assay buffer, it was confirmed that these had no effect on the 450/500 fluorescence ratio.

Calibration, Measurement of Rates of Proton Flux, and Correction for Autofluorescence—To calibrate the BCECF 450/500 fluorescence ratio in terms of pH, the null-point technique was used (Eisner et al., 1989). This avoids the use of nigericin and the associated risk of contaminating the sample with this enzyme. In this technique, defined mixtures of sodium butyrate (a weak permeant acid) and trimethylamine hydrochloride (a weak permeant base) were added to the cell suspension until a mixture was found that gave no change in fluorescence. The pH was then calculated using the formula derived by Eisner et al. (1989) and consistently gave a value for pH of 7.5 under our normal incubation conditions. This is the same value as determined by others in HEPES-buffered saline using 14C-labeled 5,5'-dimethylloxazolidine-2,4-dione (McGivan, 1979) and is higher than usually observed in bicarbonate buffered media. This probably reflects the absence of HCO3/CO2 acidifying pathways that compensate acid extrusion pathways in the homoeostatic regulation of pH (Frelin et al., 1988). The rate of carrier-mediated transport was calculated as a rate of proton uptake in nmol of intracellular volume from the initial rate of fluorescence change. Determined by first order curve fitting as above, using the change in fluorescence ratio at equilibrium following the addition of increasing concentrations of butyrate. This weak acid rapidly enters the cells as the uncharged acid and equilibrates according to the pH gradient as follows: [Butyrate]-[Butyrate]-<OH> . Thus, from the pH gradient, estimated as above, and the slope of the change in fluorescence ratio against the butyrate added against the change in fluorescence ratio observed at equilibrium (Fig. 1), changes in fluorescence ratio can be converted into uptakes of protons in nmol of H+/μl of intracellular volume (Carpenter and Halestrap, 1994). However, a problem we have encountered when applying this technique to liver cells is the variable contribution of endogenous, unmeasured fluorogenic proteins to the fluorescence. For this reason, we found autofluorescence to account for <10% of the signal and thus the calibration curve to be highly reproducible between experiments, in liver cells autofluorescence was much greater and represented 20–50% of the signal observed depending on the initial loading with BCECF and its subsequent bleaching or leakage from the cell. A major contributor to the autofluorescence was probably mitochondrial flavoproteins and cytochromes, which are present at high concentrations in liver cells. To circumvent this problem, detailed calibration curves were performed on three separate occasions and used to determine accurate rates of l-lactate transport into the same cells. From these values, a mean rate of transport of 5 mM l-lactate transport (in mmol of intracellular l-lactate) was used as a reference point. The rate of transport at 5 mM l-lactate was used as a reference point, and all other rates were corrected to absolute values by reference to this rate. It should be noted that the only parameter influenced by this correction is the VMAC of the transporter; Km and Ka values are independent of the correction value used. We are aware that our methodology also fails to take into account of intracellular accumulation of protons into mitochondria. However, this is unlikely to invalidate our results since we measured initial rates of the change in fluorescence ratio following addition of monocarboxylates, and the cytosolic dye will be the first to see the intracellular monocarboxylates and associated influx of protons.

Presentation of Results—Calculation of kinetic constants was performed using non-linear least squares regression analysis of the data to the relevant kinetic equation using Fig P and Pfit software (Biosoft). These analyses generally produced standard errors for each parameter...
RESULTS

Calibration of BCECF Fluorescence Measurements for Determination of Rates of Proton Flux—Fig. 1 shows the maximum change in 500/450 fluorescence ratio that occurred following addition of increasing concentrations of butyrate or lactate. Mean data from three separate experiments are presented. The initial slope of the curve for butyrate was calculated by interpolation to be 25 mV per mM butyrate added. Since the pH gradient across the plasma membrane was 0.1 alkaline inside, this represents 20 mM per ml intracellular butyrate or 20 mV per ml of intracellular volume (see Carpenter and Halestrap 1994). Thus, from an initial rate of change of fluorescence ratio per second (ΔF/F0), the initial rate of substrate transport (V) can be calculated in nmol min⁻¹ per ml of intracellular space as follows: V = ΔF/F0 × 60/20 or V = ΔF/F0 × 3ΔF.

This calculation assumes that the proton/lactate stoichiometry is 1:1, as we have shown previously for MCT in tumor cells (Carpenter and Halestrap, 1994) and heart cells (Wang et al., 1994). The assumption is further justified by the similarity of the maximum change in pH, induced by increasing concentrations of butyrate and L-lactate shown in Fig. 1. In the same three experiments, the initial rate of fluorescence ratio change upon addition of 5 mM L-lactate was 3.53 ± 0.07 s⁻¹ when calculated by first order regression analysis of the data as described in the legend to Fig. 2. This represents an initial rate of lactate transport of 0.2 ± 0.2 nmol min⁻¹ per ml of intracellular space. In all subsequent experiments, the initial rate of change of fluorescence ratio observed with 5 mM L-lactate (ΔFFlac) was used as a reference for the determination of the rates of transport of other substrates in nmol min⁻¹ per ml intracellular space (V) from their initial rate of change of fluorescence ratio (ΔF) as follows: V = 10.2 × ΔF/ΔFFlac.

The possibility that pH regulatory processes might lead to an underestimate of the true rates of transport using this technique was considered. However, the absence of bicarbonate in the buffer prevented bicarbonate-dependent processes operating, while interference by the Na⁺/H⁺ antiporter appeared minimal since addition of 0.1 mM dimethyl-amiloride was without effect on the initial rate of change fluorescence ratio, although the return of pH to normal levels following monocarboxylate-induced acidification was slowed by its presence (data not shown).

Substrate Specificity and Transport Kinetics—In Fig. 2a, we show the time courses of the change in 500/450 fluorescence ratio upon addition of increasing concentrations of L-lactate in the presence and absence of 5 mM CHC. These time courses can be analyzed by first order regression analysis as shown in Fig. 2b, and this allows calculation of true initial rates of transport. In Fig. 3, mean rates of transport from the three experiments used for the calibration in Fig. 1 are fitted by non-linear least squares analysis to the Michaelis-Menten equation, and it is apparent that the fit of the data is very good. In previous experiments involving the uptake of ¹⁴C substrates at 0°C, it was found necessary to subtract the rate of transport observed in the presence of 5 mM CHC to obtain a good fit of the data to
the Michaelis-Menten equation. The CHC-insensitive rate was taken to represent both the free diffusion of undissociated monocarboxylic acid and entry of the carboxylyate anion through an anion channel (Edlund and Halestrap, 1988). However, it is clear from the data presented in Fig. 3 that such a correction was unnecessary at 25°C, where CHC almost totally abolished l-lactate uptake. Indeed, the rate of transport in the presence of CHC still showed a hyperbolic relationship with substrate concentration, implying that residual transport was on the carrier and not by diffusion. This contrasts with the situation at 0°C, where the CHC-insensitive rate of transport increased linearly with substrate concentration as predicted for free diffusion. The temperature dependence of diffusion is expected to be less than that of carrier-mediated transport, and thus the greater relative contribution of diffusion to the overall transport rate at 0°C is not surprising.

Table I presents the $K_m$ and $V_{max}$ values for a variety of different monocarboxylates determined in the same manner as described for l-lactate. In each case, the initial rate of change of the 500/450 fluorescence ratio was determined by increasing concentrations of acetate (●) or l-lactate (●, ○) in the absence (●, ○) or presence (●, ○) of 5 mM CHC as described in Fig. 2. Data are presented as means ± S.E. (error bars) of three experiments, each performed on a separate hepatocyte preparation whose loading with BCECF was similar. In the case of l-lactate, these experiments were the same as those shown in Fig. 1. The data for l-lactate in the absence of CHC were fitted by non-linear least squares regression analysis to the Michaelis-Menten equation to give values for $K_m$ and $V_{max}$ (±S.E.) of 5.62 ± 0.41 mM and 7.55 ± 0.23 mM s$^{-1}$, respectively. For acetate, data in the presence of CHC were analyzed by linear regression, which gave a slope (±S.E.) of 0.090 ± 0.002 s$^{-1}$. The data in the absence of CHC were analyzed by least squares regression analysis to the equation $v = (V_{max}S)/(K_m + S) + k[S]$, where $k$ is the slope of the linear regression and is taken to represent the first order rate constant for transport by diffusion of the undissociated acid as described in the text. $K_m$ and $V_{max}$ values were (±S.E.) 5.45 ± 1.15 mM and 3.39 mM s$^{-1}$. Data from additional experiments with different BCECF loading are included in Table I.

Inhibitor Specificity—In Fig. 6, we present data showing the inhibition of the transport of 5 mM l-lactate into liver cells by increasing concentrations of some well-established inhibitors of MCT1. These are CHC and DIDS (Fig. 6a), DBDS and phloretin (Fig. 6b) and pCMBS (Fig. 6c). Data were fitted by least squares regression analysis to a non-competitive model of inhibition, and thus the derived $K_i$ values cannot strictly be regarded as a $K_i$, since it is likely that, in some cases (e.g. the stilbene disulfonates), inhibition is actually competitive (Poole and Halestrap, 1993). The rather large errors shown for pCMBS, especially at concentrations close to the $K_{0.5}$, reflect considerable variation in the inhibition observed from day to day. In two out of four experiments, a sigmoid inhibition curve was observed, while in two other experiments it was hyperbolic. Although we have no certain explanation for this observation, it is possible that the difference represents the presence of variable quantities of other reactive thiols in the cell suspension that are preferentially attacked by pCMBS. Table II summarizes $K_{0.5}$ values derived from several experiments on different hepatocyte preparations (expressed as means ± S.E.) and also presents values for MCT1 in Ehrlich Lettre tumor cells measured under similar conditions (Carpenter and Halestrap, 1994).

Temperature Dependence of Transport—In Fig. 7, we present data illustrating the effect of temperature on the rate of transport of 5 mM l-lactate into hepatocytes. The initial rates of l-lactate transport were calculated from the traces of Fig. 7a by first order analysis of the traces shown and plotted as an Arrhenius plot (lnV against 1/T), which is shown in Fig. 7b. The slope of the linear plot gave an activation energy of 58 kJ
Kinetic parameters for the transport of various monocarboxylates into isolated rat hepatocytes

Data are given either as the means ± S.E. of the number of experiments shown or where only two experiments were performed; both values are given. For 2-hydroxy-2-methyl-propionate, the derived parameter values ± S.E. are given for the mean data of three separate experiments, since the data from individual experiments could not be fitted reliably. In all but two cases, transport was assumed to be entirely carrier-mediated, since the CHC-insensitive rate was <30% even at the highest substrate concentration used. However, for acetate and 4-hydroxybutyrate, rates of transport were corrected by the rate observed in the presence of 5 mM CHC as explained in Fig. 3. Km and Vmax values for monocarboxylate transport into mouse Ehrlich Lettré tumor cells were taken from Carpenter and Halestrap (1994).

| Substrate                        | Km      | Vmax      |
|----------------------------------|---------|-----------|
|                                 | Liver   | Tumor cells |
| C1                               |         |           |
| Formate                          | 126/163 | >100      |
| C2                               |         |           |
| Acetate                          | 5.4 ± 0.6 | 3.7 |
| Glyoxylate                       | 26.6 ± 9.2 | 63.3 |
| Oxamate                          | 33.8 ± 6.7 | 49.2 |
| Glycolate                        | 6.5/7.7 | 14.6 |
| Chloroacetate                    | 0.46 ± 0.03 | 0.72 |
| Dichloroacetate                  | 0.26 ± 0.04 | 0.60 |
| C3                               |         |           |
| Pyruvate                         | 1.27 ± 0.37 | 0.72 |
| L-Lactate                        | 4.70 ± 0.73 | 4.54 |
| D-Lactate                        | 27.0 ± 3.08 | 27.5 |
| D-2-Cl-propionate                | 0.50 ± 0.08 | 0.74 |
| L-2-Cl-propionate                | 1.30 ± 0.15 | 0.76 |
| C4                               |         |           |
| 3-Oxobutyrate                    | 6.07 ± 0.77 | 5.48 |
| L-3-Hydroxybutyrate              | 3.29 ± 1.27 | 2.62 |
| D-3-Hydroxybutyrate              | 24.7 ± 2.17 | 10.1 |
| L-3-Hydroxybutyrate              | 12.7 ± 1.39 | 11.4 |
| 4-Hydroxybutyrate                | 12.3 ± 1.68 | 7.70 |
| 2-Hydroxy-2-methyl-propionate    | 32.6 ± 12.8 | >100      |

Comparison with the data in Table I suggest that in all cases, these values are about half those observed at 25 °C. A similar effect was seen on the Km values for DBDS, which in two experiments at 4 °C was found to be 131 and 78 μM compared to the mean value of 181 ± 20 μM at 25 °C. In Ehrlich Lettré tumor cells, it was also observed that Km values measured at temperatures below 15 °C were about half those measured at higher temperatures (Carpenter and Halestrap, 1994).

**DISCUSSION**

Comparison of Data Obtained Using BCECF Fluorescence and Radiotracer Techniques—The data we present in this paper provide a detailed characterization of monocarboxylate transport into rat hepatocytes. The Km values for L-lactate (2.5 mol-1), while in four separate experiments in which rates of transport of 5 mM L-lactate were measured at both 25 and 37 °C, the first order rate constant for uptake was 2.31 ± 0.28 greater at 37 °C than 25 °C. This corresponds to an activation energy of 53.4 ± 6.5 kJ mol-1, which is slightly less than obtained for the complete plot shown in Fig. 7b. For some substrates and inhibitors, we determined Km or K0.5 values at 4 °C using the BCECF technique. The mean Km values (±S.E. of the number experiments indicated) for L-lactate, D-2-chloro-propionate, and L-2-chloropropionate were 2.49 ± 0.18 (5), 0.35 ± 0.10 (3), and 0.78 ± 0.22 mM (3), respectively, while in two separate experiments the derived values for pyruvate were 0.9 and 0.4 mM and for dichloroacetate were 0.26 and 0.20 mM. Comparison with the data in Table I suggests that in all cases, these values are about half those observed at 25 °C. A similar effect was seen on the K0.5 for DBDS, which in two experiments...
Monocarboxylate Transport into Liver Cells

**Figure 6.** Inhibition of the initial rate of lactate transport into hepatocytes by increasing concentrations of various inhibitors. Hepatocytes were preincubated with the inhibitors indicated at the concentrations shown for 2 min before addition of 5 mM lactate and measurement of the initial rate of increase in the 500/450 fluorescence ratio. Data are presented as means ± S.E. (error bars) of three to five separate experiments. $K_{0.5}$ values (± S.E.), derived from these plots as described in Fig. 5, are given in Table II, where $K_{0.5}$ values derived from each separate experiment are also presented as means ± S.E.

In the present paper, the predicted $V_{max}$ at 0 °C would be 2.4 nmol min$^{-1}$ per μl compared with the measured value of 3.3 nmol min$^{-1}$ per μl (Edlund and Halestrap, 1988). Our data also confirm our previous conclusion (Edlund and Halestrap, 1988; Poole and Halestrap, 1993) that kinetic parameters derived by others using radioactive tracer techniques at higher temperatures (Fafournoux et al., 1985a; Monson et al., 1982) probably underestimated true initial rates of transport. In contrast to Fafournoux et al. (1985b), we find no evidence for the presence of a specific propionateg transporter in rat hepatocytes. The $K_{0.5}$ value for CHC reported here (373 μM) compares with a non-competitive $K_{i}$ value of 214 μM determined radioactively at 0 °C (Edlund and Halestrap, 1988). The difference between these values may be a consequence of both the different pH gradient and temperature used in the two studies, both of which would influence $K_{0.5}$ values in the observed direction (Poole and Halestrap, 1993; Carpenter and Halestrap, 1994). We showed previously that DIDS, PCMBs, and quercetin (a phloretin analogue) can inhibit lactate transport into rat hepatocytes, but no detailed studies were made (Edlund and Halestrap, 1988).

Comparison of the Properties of Rat Liver MCT with MCT1—The data presented in Table I demonstrate that the substrate specificity of the rat hepatocyte MCT is similar to that of MCT1 but also indicate some clear differences. Both carriers have a broad specificity for short chain monocarboxylates with side chains of 1–4 carbon atoms, which may be variously substituted on positions 2, 3, or 4. Chlorine is the preferred substitution, followed by the oxo and hydroxyl groups. Formate, oxamate, glyoxylate, and 2-hydroxy-2-methylpropionate are poor substrates for both isoforms, although the liver cell isoform appeared to have a slightly greater affinity. Both transporters exhibit a $K_m$ for the lactate about 5-fold less than that for the D-isomer while the $V_{max}$ values are similar for both isomers. In contrast, for both the hepatocyte and tumor cell MCT, the $V_{max}$ for transport of 3-ketoxybutyrate is about half that of the D-isomer. However, 3-ketoxybutyrate has a 2-fold lower $K_m$ for liver MCT than the D-isomer, while tumor cell MCT1 shows no difference in the $K_m$ values between the two stereoisomers. Tumor cell MCT1 also demonstrates no significant stereoselectivity for D-2-chloropropionate over the L-isomer, while hepatocyte MCT has a lower $K_m$ for the D than the L-isomer; both isomers have the same $V_{max}$. The $V_{max}$ values for 2-chloropropionate, chloroacetate, dichloroacetate, and pyruvate are all significantly less (50–70%) than for lactate for both the tumor cell and hepatocyte carriers.

The branched chain keto acids KIC and KIV show an initial rapid rate of transport followed by very slow uptake, which is associated with inhibition of the subsequent uptake of lactate (Fig. 4) for which $K_{0.5}$ values are similar to CHC (Fig. 5). This phenomenon was also observed in tumor cells (Carpenter and Halestrap, 1994). We have argued previously it may reflect an initial rapid carrier-mediated entry of these monocarboxylates into the cell where some substrate, rather than being released, forms an inactive carrier-substrate complex involving tight binding of the substrate to a hydrophobic pocket on the inner face of the carrier (Carpenter and Halestrap, 1994). Previous data from other laboratories have suggested the existence of both sodium-dependent and independent transport mechanisms for KIV and KIC in freshly isolated and cultured hepatocytes, both inhibitable by pyruvate (Kilberg and Gwynn, 1983; Nalecz et al., 1984). However, the relationship between these earlier studies using radiotracer techniques and the present ones is not obvious.

Of the other inhibitors tested, none was found that inhibited either isomerase specifically, but there were differences observed in some of the $K_{0.5}$ values. In particular, phloretin, niflumic
Inhibitory effects of a variety of reagents on lactate transport into rat hepatocytes

**TABLE II**

| Inhibitor                           | K_{0.5} (μM) | Rat hepatocytes | Ehrlich-Lettre’ cells |
|-------------------------------------|--------------|-----------------|-----------------------|
| α-Cyanocinnamate analogues          |              |                 |                       |
| α-{1-phenylindol-3-yl)-acrylate      |              |                 |                       |
| Phenylpyruvate                      | 4780         | 1               |                       |
| α-Ketosacaproate (KIC)              | 270 ± 28 (288 ± 21) | 3               |                       |
| α-Ketoisovalerate (KIV)             | 342 ± 40 (347 ± 24) | 3               |                       |
| Other Inhibitors                    |              |                 |                       |
| Phloretin                           | 23.3 ± 6.8 (27.0 ± 2.4) | 3               |                       |
| Niflumic acid                       | 20.0 ± 2.7   | 3               |                       |
| 3-Isobutyl-1-methylxanthine         | 178/195      | 2               |                       |
| pCMBS                               | 100 ± 28 (108 ± 7.1) | 4               |                       |

DIDS, SITS, and DBDS have similar K_{0.5} values for the two transporters as does pCMBS.

Taken together, our results suggest that the MCT present in rat liver cells is similar but distinct from MCT1. The recent cloning of MCT2 from hamster liver cells supports this conclusion (Garcia et al., 1995). However, the properties of MCT2 expressed in insect Sf9 cells using the baculovirus expression system are different from those observed in the present paper. In particular, MCT2 appeared to be insensitive to organomercurials unlike MCT1 and to have a higher affinity for pyruvate than MCT1. This is clearly inconsistent with the results presented here. Furthermore, the K_{0.5} values for pyruvate and the K_{0.5} values for CHC and phloretin were severalfold higher than reported here. These differences may reflect the different membrane environment of the transporter in the insect cells but may also be a consequence of the radioactive transport assay used by Garcia et al. (1995). They utilized the accumulation of 0.5 μM [14C]pyruvate after 1 min by transformed cells; this is likely to represent the combination of pyruvate transport and metabolism (see Poole and Halestrap, 1993). If significant metabolism of the labeled pyruvate were to occur over this time period, this technique would not provide a reliable measurement of initial rates of transport. Although we cannot be certain that the kinetics we observe with isolated rat hepatocytes represent rat MCT2, Garcia et al. (1995) were unable to demonstrate the presence of any MCT1 in hamster liver cells using an MCT1-specific antibody. Thus, it seems probable that the kinetics we describe represent those of MCT2 in its natural environment.

**Physiological Implications**—The differences we have observed in the properties of MCT1 and MCT2 using the BCECF technique are modest and give few clues as to what advantages there are in having a different MCT isoform in liver. Both MCT1 and MCT2 have similar affinities and activities for the major physiological substrates pyruvate, L-lactate, and the ketone bodies acetate, 3-oxobutyrate (acetoacetate), and D-3-hydroxybutyrate (β-hydroxybutyrate). Surprisingly, the liver isoform appears to have a lower affinity for D-3-hydroxybutyrate than MCT1 despite the liver being the major site of ketone body production. However the kinetics have only been measured for influx, while ketone bodies are exported from the liver, and the
K_s for efflux may be different from that for influx (Wang et al., 1994). An alternative reason for the presence of a different isoform of MCT in liver would be a need for tissue-specific regulation of MCT expression. Indeed, a stimulation of the liver monocarboxylate transporter has been reported following starvation or diabetes and by hormone treatment of isolated hepatocytes (Metcalfe et al., 1986).

From the temperature dependence of the transporter, the maximum rate of transport at 37 °C can be estimated to be about 50 nmol min⁻¹ per µl of intracellular volume. This translates into a transport rate of 100 nmol min⁻¹ per mg of protein, which can be compared with maximal rates of gluconeogenesis from L-lactate in starved hepatocytes of about 8 nmol glucose min⁻¹ per mg of protein (Quinlan and Halestrap, 1986; Groen et al., 1983). Although two L-lactic acid molecules are required to make a glucose, it would still appear that the carrier is unlikely to present a major limitation on the rate of gluconeogenesis despite suggestions to the contrary made by others (Metcalfe et al., 1986). This conclusion is in agreement with measurements made of intracellular lactate and pyruvate concentrations in hepatocytes under conditions of rapid gluconeogenesis (Groen et al., 1983). Our data also confirm the widely held view that lactate, pyruvate, and the ketone bodies equilibrate across the liver cell plasma membrane, allowing extracellular lactate/pyruvate and β-hydroxybutyrate/acetocacetae ratios to be used to estimate cytosolic and mitochondrial NADH/NAD⁺ ratios, respectively (see Tischler et al. (1977), Groen et al. (1983), and Poole and Halestrap (1993)).

Acknowledgments—We thank the Wellcome Trust and the University of Bristol for financial support and Dr R. C. Poole for helpful discussions.

REFERENCES
Bracht, A., Kelmer Bracht, A., Schwab, A. J., and Scholz, R. (1981) Eur. J. Biochem. 114, 471-479

Carpenter, L., and Halestrap, A. P. (1994) Biochem. J. 304, 751-760
Deuticke, B. (1982) J. Membr. Biol. 70, 89–103
Edlund G. L., and Halestrap, A. P. (1988) Biochem. J. 249, 117-126
Eisner, D. A., Kenning, S. C., O’Neil, S. C., Pocock, G., Richard, C. D., and Valdeomillos, A. (1989) Pflügers Arch. 413, 553–558
Fafournoux, P., Demigne, C., and Remesy, C. (1985a) J. Biol. Chem. 260, 292-299
Fafournoux, P., Remesy, C., and Demigne, C. (1985b) Biochim. Biophys. Acta 818, 73–80
Frélin, C., Vigne, P., Ladoux, A., and Lazardinski, M. (1988) Eur. J. Biochem. 174, 3-14
Garcia, C. K., Goldstein, J. L., Pathak, R. K., Anderson, R. G. W., and Brown, M. S. (1994) Cell 76, 865–873
García, C. K., Brown, M. S., Pathak, R. K., and Goldstein, J. L. (1995) J. Biol. Chem. 270, 1843-1849
Groen, A. K., Vervoorn, R. C., Van der Meer, R., and Tager, J. M. (1983) J. Biol. Chem. 258, 14346–14353
Kilberg, M. S., and Gwynn, M. B. (1983) J. Biol. Chem. 258, 11524–11527
Lupo, M. A., Cefalu, W. T., and Pardridge, W. M. (1990) Metab. Clin. Exp. 39, 374–377
McGivan, J. D. (1979) Biochem. J. 182, 697–705
Metcalfe, H. K., Monson, J. P., Welch, S. G., and Cohen, R. D. (1986) J. Clin. Invest. 78, 743–747
Monson, J. P., Smith, J. A., Cohen, R. D., and Iles, R. A. (1982) Clin. Sci. (Lond.) 62, 411–420
Nalecz, K. A., Wojtczak, A. B., and Wojtczak, L. (1984) Biochim. Biophys. Acta 805, 1–11
Poole, R. C., and Halestrap, A. P. (1988) Biochem. Soc. Trans. 16, 602–603
Poole, R. C., and Halestrap, A. P. (1991) Biochem. J. 275, 307–312
Poole, R. C., and Halestrap, A. P. (1992) Biochem. J. 283, 855–862
Poole, R. C., and Halestrap, A. P. (1993) Am. J. Physiol. 264, C761–C782
Poole, R. C., and Halestrap, A. P. (1994) Biochem. J. 303, 755–759
Poole, R. C., Halestrap, A. P., Price, S. J., and Levi, A. J. (1999) Biochem. J. 364, 409–418
Poole, R. C., Cranmer, S. L., Halestrap, A. P., and Levi, A. J. (1999) Biochem. J. 369, 827–829
Quintana, I., Felipe, A., Remesar, X., and Pastor-Anglada, M. (1988) FEBS Lett. 235, 224–228
Roth, D. A. (1991) Med. Sci. Sports Exercise 23, 925–934
Schwab, A. J., Bracht, A., and Scholz, R. (1979) Eur. J. Biochem. 102, 537-547
Tischler, M. E., Friedrichs, D., Coli, K., and Williamson, J. R. (1977) Arch. Biochem. Biophys. 184, 222–236
Wang, X. M., Poole, R. C., Halestrap, A. P., and Levi, A. J. (1993) Biochem. J. 290, 249–258
Wang, X. M., Levi, A. J., and Halestrap, A. P. (1994) Am. J. Physiol. 36, H1759–H1769
Wang, X. M., Levi, A. J., and Halestrap, A. P. (1966) Am. J. Physiol., in press