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CARNITINE-INDUCED UPTAKE OF L-CARNITINE INTO CELLS FROM AN ESTABLISHED CELL LINE FROM HUMAN HEART (CCL 27)

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Summary

L-Carnitine is actively transported into Girardi human heart cells, an established cell line from human heart. The present study was undertaken to investigate the effect of different concentrations of L-carnitine in the growth medium on the rate of uptake of L-[^{3}H]carnitine.

Increasing the concentration of L-carnitine from 2 to 100 μmol/l in the growth medium of the cells, increased the rate of uptake of L-[^{3}H]carnitine by about 50%. The maximal effect was reached after approx. 72 h incubation. The increase in rate seemed to be caused by synthesis of increased number of carriers, as judged by the increase in V with unchanged apparent K_m for the transport process. This effect of L-carnitine could be inhibited by cycloheximide, indicating the dependence on intact protein synthesis. The morphology of the cells was studied by electron microscopy. No myofilaments were found, thus the cells are dedifferentiated and no longer typical muscular cells.

Introduction

The concentration of L-carnitine is about 60 times higher in heart and skeletal muscle cells than in plasma [1—3]. This is most likely due to a concentrative transport mechanism, which has been described both in Girardi human heart cells (CCL 27), an established cell line from human heart [4,5], and in rat skeletal muscle [6]. The mechanism described provides the cells with a possible way to vary the intracellular concentration of L-carnitine, and thereby influence the rate of fatty acid oxidation. It is also likely that a defect in this mechanism is responsible for the pathogenesis in certain muscular diseases [7—10].

We have previously described the characteristics and the specificity of the transport of L-carnitine into Girardi human heart cells [5]. In the same system,

the present investigation was undertaken to study the effect of different concentrations of L-carnitine in the growth medium of the cells on the rate of uptake of L-[^{3}H]carnitine.

Materials and Methods

Materials. L-[*Me*-^{3}H]carnitine, L- and D-carnitine, L-acetylcarnitine and the Girardi human heart cells (CCL 27) were all obtained as previously described [4,5]. Other reagents were commercially available products of analytical grade.

Methods. The cells were cultured and the uptake of L-[*Me*-^{3}H]carnitine measured as reported [4,5], with one modification. The monolayers were more extensively washed prior to the uptake studies: 5 times with 3 ml medium devoid of L-carnitine and serum. This was done to remove unlabeled L-carnitine which would inhibit the uptake of the radiolabeled isotope. All experiments were carried out at 37° C.

The rate of uptake showed a considerable intraexperimental variation (range 2—10 pmol · μg^{-1} DNA · h^{-1}) in 96 controls from 24 separate experiments. Samples were therefore always compared to the mean of four controls in the same experiment. There was no correlation between the rate of uptake and amount of DNA in each dish. The monolayers were non-confluent during all experiments, and the growth medium was changed every third day.

L-carnitine in the calf serum was measured as described [11].

For scanning and transmission electron microscopy the cells were removed from the monolayers by trypsination. They were then fixed in 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.3) for 2 h and postfixed in 1% osmium tetroxide in Tyrode's solution (pH 7.4) for 1 h. The cells were dehydrated in graded ethanols and propylenoxide, embedded in Epon 812 and sectioned by a LKB ultramicrotome. They were stained with uranyl acetate and lead citrate, and were examined in a Siemens Elmiskop I and a Jeol 100 B electron microscope. Specimens were prepared for scanning electron microscopy by critical point drying and coating with gold-palladium. Examination was performed in a Jeol 50 A scanning electron microscope.

Results

L-Carnitine in increasing concentrations in the growth medium increased the rate of uptake of L-[^{3}H]carnitine to about 150% of controls, with maximal effect obtained at 100 μmol/l (Fig. 1). When 100 μmol/l L-carnitine was present in the growth medium the rate of uptake increased with time up to 72 h, with greatest change occuring between 24 and 48 h (Fig. 2). There was no difference in amount of DNA between controls and samples containing 100 μmol/l L-carnitine (mean ± S.E. was 102 ± 7 μg DNA in controls, 95 ± 7 μg DNA in incubations with 100 μmol/l L-carnitine).

This increase in rate of uptake could be due to either increased affinity of the carriers to L-carnitine or increase in number of carriers per μg DNA. In cells grown in presence of 100 μmol/l L-carnitine for 96 h there was an increase in the V of the transport with no change in the apparent K_m (Fig. 3). This implies an increased number of carriers per μg DNA in the cells grown in presence of

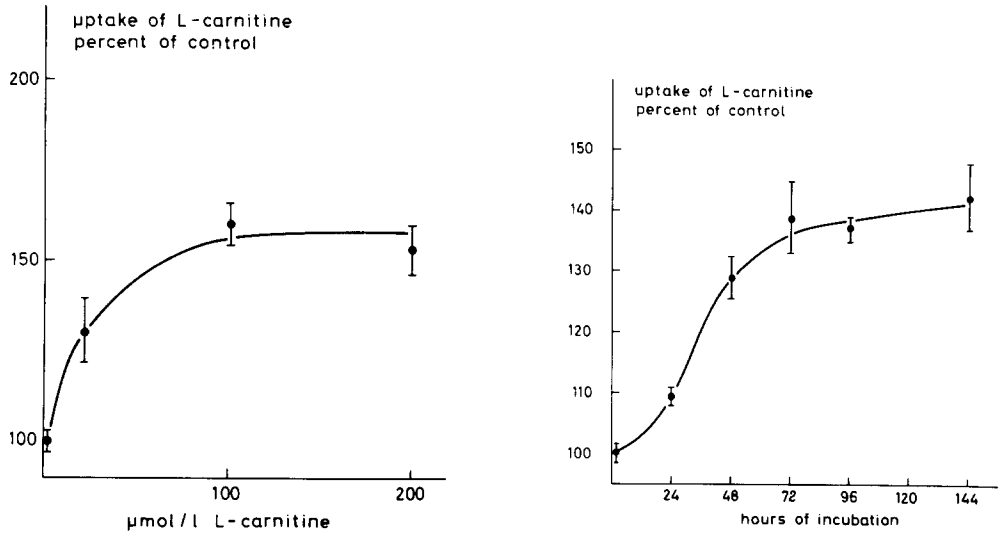

Fig. 1. The effect of increasing concentrations of L-carnitine in the growth medium of the cells on the uptake of L-[^{3}H]carnitine (2 µmol/l). The cells were grown for 144 h with different concentrations of L-carnitine. The uptake of L-[^{3}H]carnitine was measured during a 2 h incubation at 37°C. The results are from separate experiments. The rate of uptake in controls ranged from 2.9 to 4.7 pmol · µg^{-1} DNA · h^{-1}. The incubations contained 60—315 µg DNA. The values are the means, and vertical bars represent S.E. (22 control samples and 6—9 observations on each carnitine concentration indicated).

Fig. 2. The effect of L-carnitine (100 µmol/l) on the growth medium of the cells for varying time on the uptake of L-[^{3}H]carnitine (2 µmol/l). The results are from 10 separate experiments. The values are means of 8 observations, and vertical bars represent S.E. The range in the rate of uptake in controls was 2.2 to 8.1 pmol · µg^{-1} DNA · h^{-1}. The incubations contained 50—200 µg DNA.

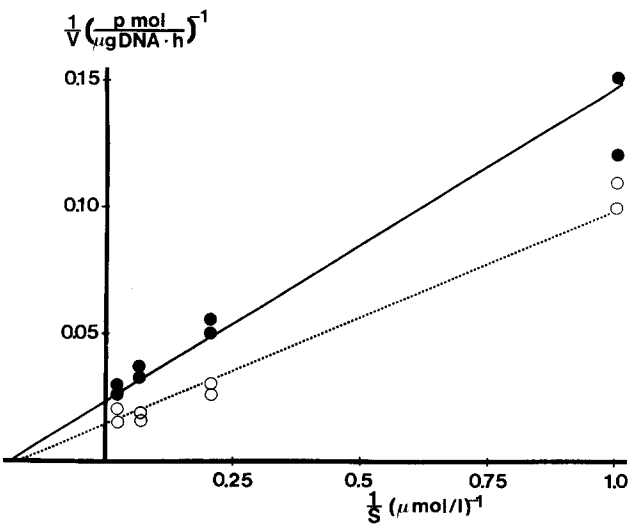

Fig. 3. Lineweaver-Burk plot of the uptake of L-[^{3}H]carnitine in cells grown in control medium (approx. 2 µmol/l L-carnitine) (●———●) and in presence of 100 µmol/l L-carnitine (○- - - - -○) for 96 h. The kinetic parameters were calculated accordinging to Neame and Richards [11], and lines drawn to fit the calculated results. The V increased from 39 ± 4 to 72 ± 17 pmol · µg^{-1} DNA · h^{-1}, while the apparent K_m was unchanged: 4.8 ± 1.2 and 5.9 ± 3.0 µmol/l in cells grown in control medium and in presence of 100 µmol/l L-carnitine, respectively. The incubations contained 60—80 µg DNA.

TABLE I

PERCENT UPTAKE OF L-[^{3}H]CARNITINE AFTER VARYING CONCENTRATIONS OF CYCLOHEXIMIDE AND L-CARNITINE IN THE GROWTH MEDIUM

The cells were grown for 72 h with or without 100 μmol/l L-carnitine. 48 h prior to the uptake studies, cycloheximide was added in the indicated concentrations. The uptake of L-[^{3}H]carnitine was measured during a 2 h incubation at 37°C with 2 μmol/l L-carnitine. Samples were compared to the mean of four control incubations containing 2 μmol/l L-carnitine in the growth medium. The results are from three separate experiments, and are given as mean of four observations ± S.E. The range in the rate of uptake in controls was 4.7—7.8 pmol · μg^{-1} DNA · h^{-1}. The incubations contained 45—185 μg DNA. The amount of cells planted in each incubation containing cycloheximide was four times the amount in incubations without the inhibitor. This was done to minimize the final intraexperimental variation of amount of DNA between different samples, and also serving as a control of the inhibition of growth by cycloheximide.

L-Carnitine (μmol/l)	Cycloheximide (μmol/l)			
	0	0.125	0.75	2.5
2	100 ± 2	115 ± 8	71 ± 7	51 ± 3
100	140 ± 4	149 ± 8	126 ± 9	50 ± 4

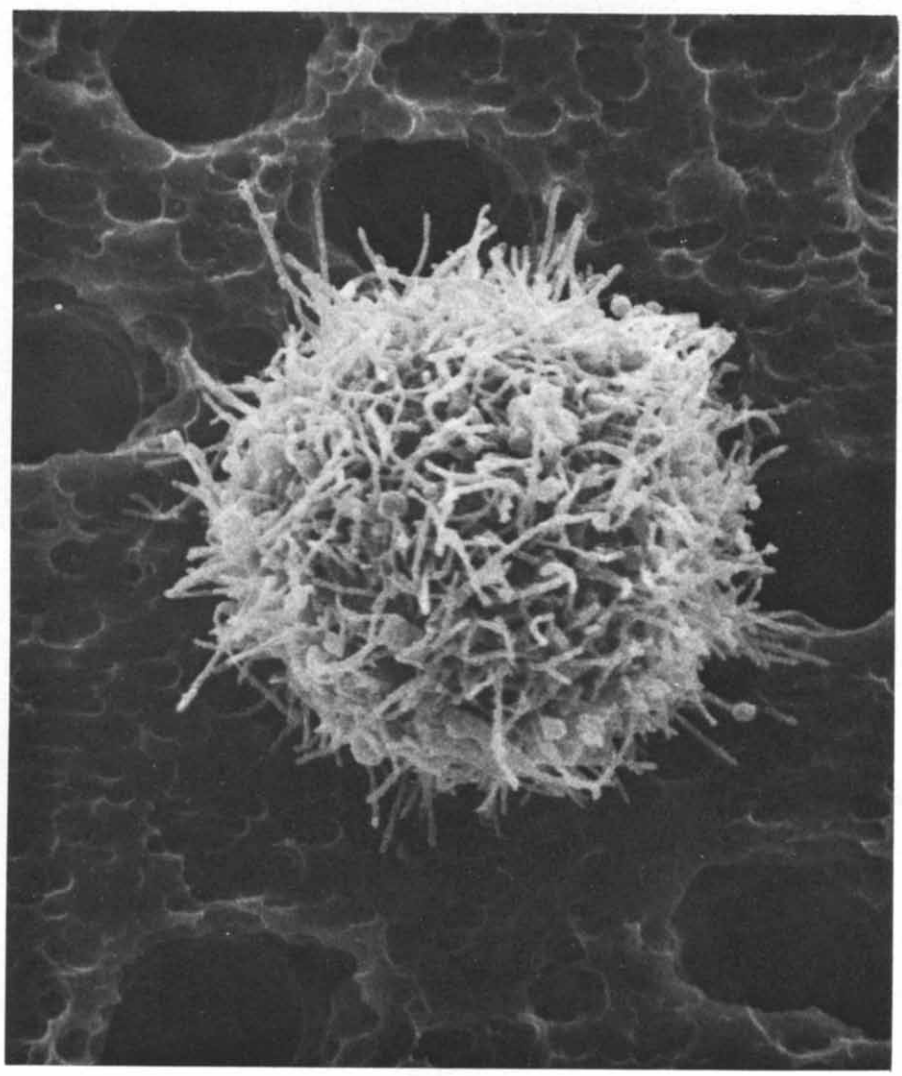

Fig. 4. Scanning electron micrograph of a typical cell, demonstrating numerous microvilli and filopodia (magnification X 4500).

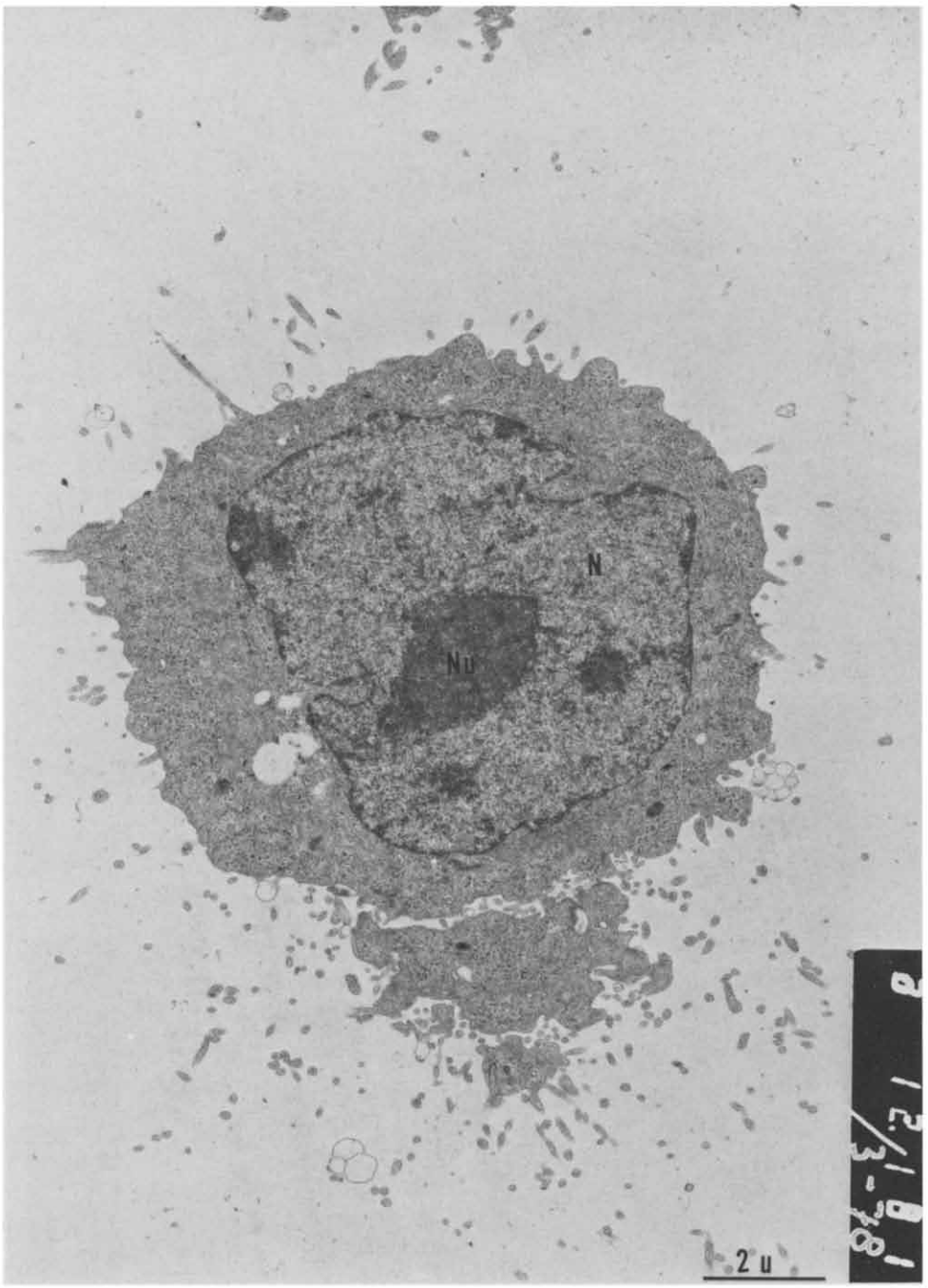

Fig. 5. Transmission electron micrograph of a typical cell. N, nucleus; NU, nucleolus. Bar = 2 μm.

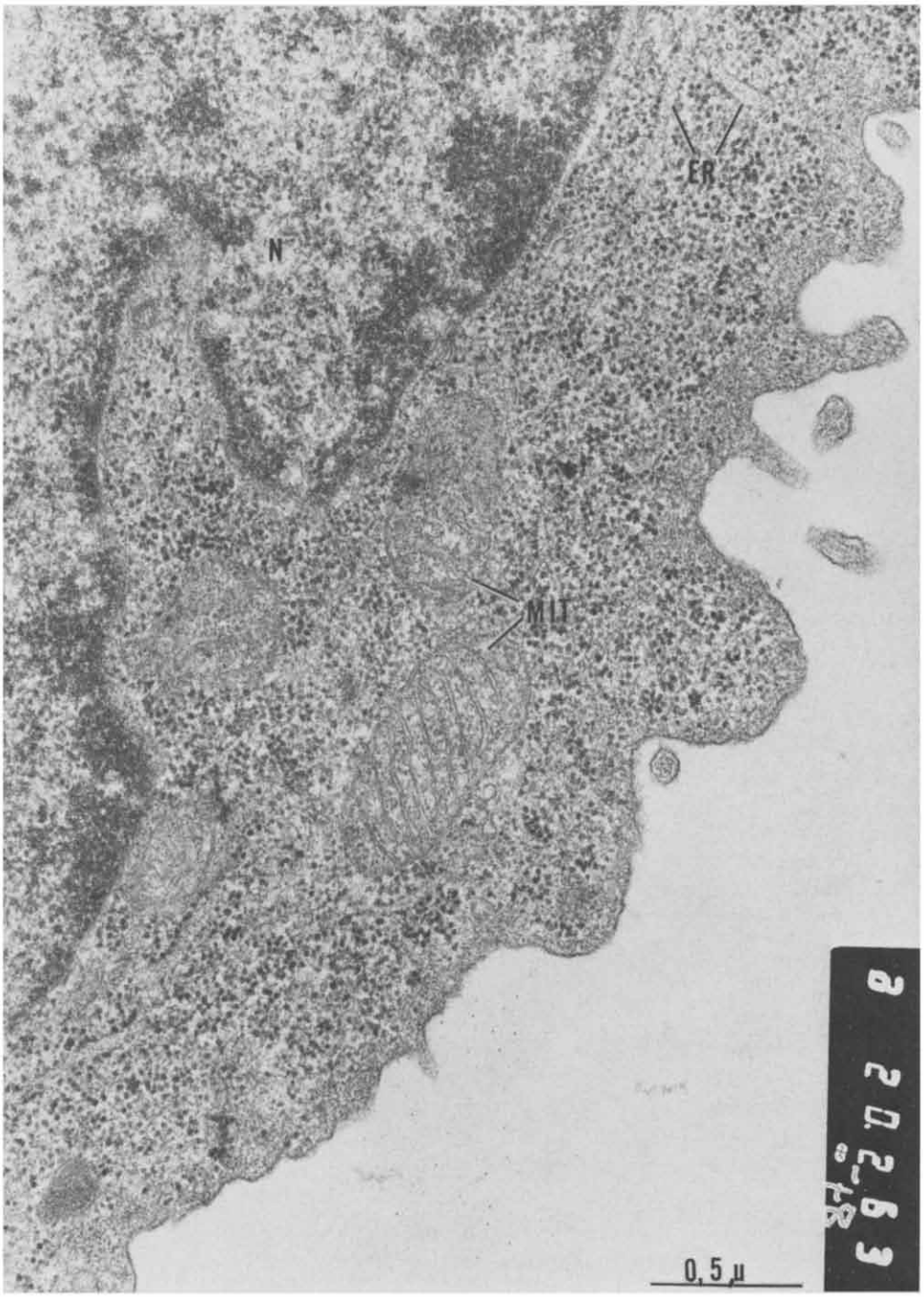

Fig. 6. Detail of a cell with rough endoplasmic reticulum (ER), mitochondria (MIT) and nucleus (N). Bar = 0.5 μm.

100 μmol/l L-carnitine. Whether this was due to activation of preformed carriers or synthesis of new carriers was studied by use of cycloheximide. Increasing concentrations of cycloheximide reduced both the basic and the stimulated rate of uptake of L-carnitine to 50% of controls at a concentration of 2.5 μmol/l (Table I). This indicated that intact protein synthesis was necessary both to maintain the basic rate of uptake, and to induce an increase in rate of uptake.

The specificity of the inducive process was tested using compounds structurally related to carnitine which were known to inhibit the uptake. No effect on the rate of uptake was noted when the cells were grown in presence of 100 μmol/l deoxycarnitine, D-carnitine and L-acetylcarnitine (rate of uptake: mean ± S.E., 107 ± 5%, 98 ± 5% and 108 ± 3%, respectively).

The concentrative transport mechanism for L-carnitine is especially active in this cell line [4]. It was therefore of interest to examine the morphology of the cell line. By scanning and transmission electron microscopy the cells were spherical (examined in suspension) with microvilli, filopodia, and some zeiotic blebs (Figs. 4 and 5). The cytoplasm did not contain myofilaments. Scattered rough endoplasmic reticulum, several mitochondria, free ribosomes and glycogen were noted (Fig. 6). In some of the cells lipid droplets were present. Thus, no characteristic trait of the origin of the cells had been preserved.

Discussion

The addition of L-carnitine to the growth medium resulted in an increase in the rate of uptake of L-[^{3}H]carnitine. This seemes to be caused by synthesis of an increased number of carriers. The observed increase is modest, giving a maximal rate of 150% of controls. However, the basic, 'unstimulated' rate of uptake is unknown. The control incubations too, did contain some L-carnitine in the calf serum employed (range of concentration 1.5—2 μmol/l in the final medium). It is also possible that the rate of uptake is influenced by hormonal factors present in the calf serum. Thus, the increase observed might not be the total variational span of the rate of transport.

The conditions commonly employed in cell culture is according to these results, not optimal for the uptake of L-carnitine. This might explain the lower concentration of L-carnitine found in this cell line as compared to the heart in vivo [4], though the reduction could also be a result of the dedifferentiation of the cells.

Cycloheximide (2.5 μmol/l) present in the growth medium for 48 h reduced both the basic and the stimulated rate of uptake to 50% of controls. The effect on the basic rate of uptake probably reflects the turnover of the carriers with impaired synthesis of new ones. The same concentration inhibited the stimulative effect of 100 μmol/l L-carnitine on the synthesis of new carriers. These results however, do not completely rule out the possibility that the addition of L-carnitine exerts its effect by decreasing the degradation rate of the carriers. The lack of stimulation of L-acetylcarnitine on the rate of uptake of L-carnitine is somewhat puzzling. We have previously found that L-acetylcarnitine is transported into the cells by the same mechanism as L-carnitine, and is rapidly mixed with the intracellular carnitine pool [5]. The addition of 100 μmol/l

L-acetylcarnitine to the growth medium would thus increase the intracellular concentration of L-carnitine. This seems to indicate that the stimulative effect of L-carnitine on the carrier synthesis is not solely mediated by an increase in the intracellular concentration.

The transport mechanism in this cell line is very similar to that described for isolated rat muscle [5,6], and also shares properties with the uptake mechanism for L-carnitine in isolated rat liver cells [13]. The morphology of the cell line (see Results) indicates a considerable dedifferentiation from the original heart cell. The described transport mechanism therefore cannot be considered specific for heart cells, but might be a more general cellular property, present also in cells devoid of myofilaments.

Endurance exercise induces an increase in the concentration of a number of enzymes involved in the energy metabolism of skeletal muscle cells [14]. Thus it seems possible that differentiated muscle cells have the ability to respond to a stimulus in the same way as the hereby reported carrier induction. In rats, similar excercise give rise to an increase in the intramuscular concentration of carnitine [15]. This might be effected through induction of extra carriers.

The stimulative effect of L-carnitine on the rate of transport reported here is obtained within physiological concentrations of L-carnitine [11,16,17]. Serum concentration of carnitine in a patient with systemic carnitine deficiency was of the same magnitude as the concentration of L-carnitine in our control incubations [18]. Oral replacement therapy with DL-carnitine has been reported to give clinical improvement in patients with carnitine-deficiency syndromes [8, 18,19]. Part of this effect might be due to the stimulus to an increase in carrier synthesis. It is therefore possible that the induction of carriers found in this dedifferentiated cell line is a mechanism operating in vivo.

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