CHARACTERISTICS OF TAURINE TRANSPORT IN FRESHLY ISOLATED RAT HEPATOCYTES

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Abstract—Characteristics of taurine (2-aminoethanesulfonic acid) transport were studied in freshly isolated rat hepatocytes prepared by a collagenase perfusion technique. The uptake of taurine at 37°C was linear up to 30 min of incubation, but gradually decreased thereafter and reached a plateau at 90 min after initiation of the incubation. Taurine uptake at 4°C by isolated hepatocytes was not saturable, whereas that at 37°C was saturable with the following parameters: Km, 37 μM; Vmax, 0.043 nmoles/mg prot./min; and EA, 5.6 Kcal/mol. The taurine uptake at 37°C was found to be sodium dependent, and this was inhibited competitively by guanidinoethyl sulfonate and β-alanine with the Ki values of 1.75 mM and 285 μM, respectively. Conjugated cholate, conjugated chenodeoxycholate, alanine, isethionate and leucine had no effect on the taurine uptake. The present results indicate that taurine uptake by isolated hepatocytes consists of unsaturable and energy independent, and carrier-mediated and energy dependent transport processes.

It is well known that taurine and its metabolic pathways are widely distributed in various mammalian organs. In addition, various physiological roles of taurine have been demonstrated such as the regulation of cardiac excitability (1), neuromodulating action in neuronal tissues (2), and conjugate formation with bile acids in the liver. Furthermore, it has been also shown that taurine increases mitochondrial calcium binding (3) and stimulates glycogen synthesis, as well as suppresses the lactic acid production in the liver (4).

In the liver, taurine is not only synthesized, but also is accumulated in this organ by the transprot from circulating blood. The accumulated taurine is also known to be excreted from the liver after conjugating with bile acids. Oral administration of taurine to rats and humans has been found to decrease the ratio of glycine-conjugated to taurine-conjugated bile acid (G/T ratio) (5, 6), and a hypothesis, that one of the main factors regulating the change of G/T ratio may be taurine content in the liver, has been proposed from the experimental results obtained in perfused rat liver (7). These facts suggest that studies on taurine transport in the liver may be important not only to elucidate the physiological regulatory mechanisms of hepatic taurine, but also to examine alteration of the function of taurine associated with various hepatic disorders.

Because of the anatomical complexity of the liver, it seems difficult to analyze the kinetics of taurine transport in hepatocytes using in vivo or liver perfusion systems. A procedure for isolating the bulk of hepatocytes...
using collagenase has been developed recently (8), and these cells have been considered to retain normal metabolic activities.

In the present study, we have examined the kinetic properties of taurine transport in freshly isolated rat hepatocytes prepared by the collagenase perfusion technique.

MATERIALS AND METHODS

Animals: Male Wistar rats weighing 200–300 g and fed with a standard diet ad libitum were used in all experiments.

Preparation of isolated rat hepatocytes: Isolated rat hepatocytes were prepared by the collagenase perfusion technique according to the procedure of Berry and Friend (8) employing the slight modifications described by Ui and Tokumitsu (9). After anesthetizing the rats with pentobarbital (50 mg/kg of body weight) injected into the peritoneal cavity, the abdomen was opened by a U-shaped transverse incision and the intestine was displaced to the left side of the abdominal cavity. Immediately after inserting a Teflon cannula (19 gauge) into the portal vein, the vena cava inferior was cut off and the liver was perfused with Ca²⁺-free improved Krebs-Ringer (K-RI) phosphate buffer fully oxygenated with 95% O₂ and 5% CO₂ (37°C, pH 7.4) through the inserted cannula at the flow rate of 20 ml/min. Following a 5 min perfusion, the liver was removed from the carcass, and then the isolated liver was transferred to a perfusion apparatus in which the temperature was maintained at 37°C. The liver was immediately perfused with Ca²⁺-free K-RI buffer (pH 7.4) containing 2% dextran, which was fully oxygenated with 95% O₂ and 5% CO₂ and recirculated, at a flow rate of 100 ml/min. Twelve minutes after the initiation of the second perfusion, 0.05% collagenase was added to the perfusate, and the perfusion was continued for an additional 12 min at the same flow rate. The liver perfused with collagenase was minced with a scissors in ice-cold Ca²⁺-free Krebs-Henseleit (K-H) buffer, fully oxygenated and containing 2% bovine serum albumin, and was dispersed with gentle shaking in a plastic beaker. After the dispersion, hepatocytes were filtered twice through a Nylon stocking and centrifuged at 50×g at 4°C for 3 min. The pellet thus obtained was resuspended in Ca²⁺-free ice-cold K-H buffer, fully oxygenated with 95% O₂ and 5% CO₂, containing 2% bovine serum albumin. This suspension was recentrifuged at 50×g at 4°C, and the pellet obtained was washed three times using the same centrifuging and resuspending procedures. Finally, the pellet was resuspended in ice-cold K-H buffer containing penicillin G (20 mg/100 ml K-H buffer) and streptomycin (20 mg/100 ml K-H buffer). After calculating the number of isolated hepatocytes using a Neubauer hemocytometer, the final suspension was diluted with ice-cold K-H buffer containing antibiotics to adjust the cell number to 3–4×10⁶ per ml.

In the present study, it has been found that the average yield of hepatocytes is 6×10⁸ cells per liver, consistent with other reports (10, 11). The batch of isolated hepatocytes with a viability of more than 90%, which was confirmed by trypan blue exclusion test, was finally used for the uptake study.

Observation of viability changes of isolated hepatocytes during incubation: The alteration in the viability of isolated hepatocytes during uptake studies was examined in terms of the trypan blue exclusion, electron micrographic features, retention of intracellular lactic dehydrogenase (LDH) and the production of glucose.

For determinations of the trypan blue exclusion, the cell suspension was placed in a plastic vial aerated with 95% O₂ and 5% CO₂, and incubated at 37°C with reciprocal shaking at the rate of 90 rpm. Before and
after the incubation for 1 and 2 hr, respectively. 0.2% trypan blue solution (trypan blue was dissolved in 0.9% NaCl solution) was added to the cell suspension and trypan blue was adjusted to a final concentration of 0.01–0.02%. The percentage of isolated cells which were able to exclude trypan blue was then calculated.

In order to observe electron microscopic features of the isolated rat hepatocytes, the hepatocytes were pre-fixed with 0.1 M cacodylate buffer containing 2.5% glutaraldehyde, followed by the fixation with 1% OsO₄. The fixed cells were dehydrated quickly in graded ethanol concentrations, immersed in propylene oxide, and finally embedded in Epon. The ultrathin sections were stained with uranyl acetate and post-stained with lead nitrate, and examined by a Hitachi HS-11 electron microscope.

For the determination of intracellular and extracellular LDH activities, the method of Cabaud and Wroblewski (12) was used. Before and after the incubation of cell suspension for 1 and 2 hr, respectively, each suspension was centrifuged immediately. LDH activity was measured in both the supernatant and in the hepatocytes which were homogenized by a Polytron for 15 sec.

For the measurements of glucose production, an aliquot of the cell suspension was incubated and centrifuged at 0, 15, 30, 45, 60, 90 and 120 min after initiation of the incubation. Since glucose retaining in the cell after the incubation was negligible, glucose content in the supernatant was measured according to the procedure of Raabo and Terkildsen (13).

Measurement of taurine uptake by isolated hepatocytes: Two ml of the cell suspension was placed in a small plastic vial and incubated at 37°C with reciprocal shaking at the rate of 90 rpm. After the preincubation for 5 min at 37°C, the measurement of the uptake was initiated by adding 200 nmoles taurine containing 11–15 nCi of [3H]-taurine into 2 ml of the suspension. For terminating the uptake of taurine, quick separation of hepatocytes from the incubation medium was achieved by the centrifugal filtration technique as previously described (14). Fifty µl of 3 N KOH was placed in the bottom of a small centrifuge tube (Total volume: 400 µl) and 100 µl Silicone oil (SH 550 : SH 200=3:1) was layered on the top of the 3 N KOH layer, and finally the incubated cell suspension was layered on the Silicon layer. Following centrifugation for a few seconds in a microfuge, the hepatocytes had passed through the Silicone layer and had precipitated into the layer of 3N KOH. Radioactivities found in the 3N KOH layer were used to determine the uptake of [3H]-taurine. To determine the amounts of adherent to the hepatocytes and entrapped radioactivities of the incubation medium, [3H]-inulin was subjected to the identical uptake procedure, and the percent of total radioactivity associated with hepatocytes was calculated. The same calculation was applied to experiments using [3H]-taurine, and this value minus that obtained using [3H]-inulin was used in subtracting the adherent and entrained radioactivities.

For analyzing the radioactivity which sedimented with the hepatocytes, the centrifuge tube was cut off at the interphase between the Silicon oil and the 3N KOH layers, and the 3N KOH layer which contained sedimented hepatocytes was transferred to a glass scintillation vial. After adding 10 ml of Triton-toluene scintillator (Triton X-100: toluene containing 5 g PPO and 0.3 g POPOP per liter=1:2) and allowing the mixture to stand overnight, the radioactivity was measured in a liquid scintillation spectrometer.

Analytical methods for protein and taurine:
Protein content was determined by the method of Lowry et al. (15). Taurine content in hepatocytes was measured by the fluorometric method of Yoneda et al. (16).

**Chemicals and radioisotopes used:** [\(^3\)H]-Taurine (Spec. Act: 17.6 Ci/mmol) and [\(^3\)H]-inulin (Spec. Act: 0.19 mCi/mg) were obtained from the Radiochemical Centre (Amersham, England) and New England Nuclear (Boston, U.S.A.), respectively. Collagenase (type I) was purchased from the Sigma Chemical Co. (St. Louis, U.S.A.). Silicone oil was obtained from the Toray Silicone Co. (Tokyo, Japan).

Bovine serum albumin (fraction V, powder) was purchased from the Miles laboratories (Elkhart, U.S.A.). Guanidinoethyl sulfonate was a kind gift from Dr. Rayan J. Huxtable, University of Arizona Health Sciences Center, Arizona, U.S.A.

Other chemicals used were of analytical grade and were purchased locally.

**RESULTS**

**Viability of isolated hepatocytes:** The percentage of the isolated hepatocytes which were able to exclude trypan blue was approximately 93% as measured immediately after their isolation. This percentage decreased to 84% and 75% after maintaining them at 37°C for 1 and 2 hr, respectively (Fig. 1). These data are similar to those reported by van Bezooijen et al. (17).

Electron micrographs of the isolated hepatocytes indicated that the structure of the cells, including nuclei, mitochondria and other cytoplasmic organelles, were well preserved as reported in other papers (8, 17). A few microvilli were, however, recognized on the cell membrane (Fig. 2).

The ratio of LDH activity in the media to that in the freshly isolated hepatocytes was

![Fig. 1. Viability of isolated rat hepatocytes.](image-url)

The isolated hepatocytes were incubated in Krebs-Henseleit buffer at 37°C with reciprocal shaking at the rate of 80 rpm. The final concentration of trypan blue solution used for determining the viability of hepatocytes was 0.01−0.02%. Each value represents the mean±S.E. from four to twelve separate experiments.
from 0.15 to 0.20, and this value was in essential agreement with the data by Berry and Friend (8). The average percentages of LDH activity leaked from the cells after incubation for 1 hr and 2 hr were 5% and 4%, respectively, which are less than the values reported by Berry and Friend (8). These results suggest that the cell membranes of the hepatocytes may be well preserved, both functionally and structurally, during the incubation for 2 hr in K-H buffer at 37°C.

The glucose production by isolated hepatocytes incubated in K-H buffer at 37°C for 1 hr was 0.66 μmoles/mg protein and that for 2 hr was found to be 0.87 μmoles/mg protein. The amount of glucose secreted by the hepatocytes found in this study was slightly higher than that reported previously (10). These results also suggest that the isolated hepatocytes may have the metabolic pathways to produce glucose from endogenous glycogen through glycogenolysis, and these pathways may be operating actively.

Characteristics of taurine uptake by isolated hepatocytes: Figure 3 shows the time course of taurine uptake at 0 and 37°C by isolated hepatocytes in the presence of 100 μM taurine. Taurine uptake at 0°C for the 2 hr incubation was small, while uptake at 37°C was to a much larger extent and this was linear up to 30 min of incubation. The rate of uptake at 37°C was decreased thereafter and attained a plateau at 90 min after the initiation of incubation. Based on these data, the rate of the initial uptake observed during the first 15 min was selected for further studies. The taurine content in hepatocytes incubated for 15 min at 37°C in K-H buffer containing 100 μM taurine was found to be apparently higher than that observed in hepatocytes before the incubation, suggesting that the increase of net taurine content occurs after the incubation.

The initial rate of taurine uptake at 0°C was almost constant up to a concentration of 200 μM, which was presumed to represent the unsaturable process. On the other hand, the taurine uptake at 37°C showed a biphasic pattern and the presence of a saturation process.
Fig. 4. Lineweaver-Burk plot of taurine uptake by isolated hepatocytes. Kinetic constants were calculated from the Lineweaver-Burk plot. This plot reveals the presence of a saturable process which consists of two components: high affinity and low affinity systems. The apparent $K_m$ and $V_{max}$ of the high affinity transport system are $37 \mu M$ and $0.043$ nmoles/mg protein/min, respectively. Each value represents the mean of three to six separate experiments.

Fig. 5. Effects of metabolic inhibitors and withdrawal of sodium on taurine uptake by isolated hepatocytes. Sodium was completely replaced by choline when the effect of withdrawal of sodium on taurine uptake was determined. Each value represents the mean±S.E. from three to six separate experiments. *$P<0.05$, **$P<0.001$, compared with the control value (by the $t$-test).

The temperature dependency of the taurine uptake was further examined by incubating hepatocytes with six different concentrations of taurine (200, 150, 100, 50, 25 and 10 \mu M) as well as at five different temperatures (0, 10, 20, 30 and 37°C, respectively). The data obtained were analyzed by the Arrhenius plot which was linear for the five temperatures and corresponded to an average activation energy of 5.6 Kcal/mol. This value for the activation energy is comparable to that suggested for carrier-mediated transport (18, 18).

Kinetic parameters for the saturable process of taurine uptake were determined using Lineweaver-Burk plots (Fig. 4) in which a straight line was obtained. The plot revealed the existence of two components which were classified as low and high affinity transport systems. The apparent $K_m$ and $V_{max}$ values for the high affinity component were calculated to be $37 \mu M$ and $0.043$ nmoles/mg protein/min, respectively. Effects of some metabolic inhibitors and of sodium withdrawal are shown in Fig. 5. Ouabain (1 \mM), monooiodoacetate (1 \mM), and 2,4-dinitrophenol (100 \mu M) added from the period of preincubation significantly inhibited the taurine uptake. For examining the effect of $Na^+$ on the uptake of taurine, $Na^+$ was completely replaced by an equimolar concentration of choline chloride plus choline bicarbonate. Under these experimental conditions, the taurine uptake decreased remarkably. These results indicate that the uptake of taurine by hepatocytes is basically an energy- and sodium-dependent process.

The addition of several amino acids including isethionic acid, glycine, alanine and leucine at a concentration up to 1 \mM, respectively, showed no inhibition on the taurine uptake, whereas addition of guanidino-
ethyl sulfonate, β-alanine and hypotaurine at a concentration of 1 mM significantly inhibited the taurine uptake (Fig. 6). Guanidinoethyl sulfonate and β-alanine competitively inhibited the taurine uptake as demonstrated by the lineweaver-Burk plot in Fig. 7. The inhibition constants (Ki) were calculated from the intercept on the baseline of the Dixon plot, and the Ki values for guanidinoethyl sulfonate and β-alanine were found to be 1.75 mM and 285 μM, respectively.

Various conjugated bile acids such as taurocholate, taurochenodeoxycholate, glycocholate and glycochenodeoxycholate at a concentration up to 100 μM did not inhibit the taurine uptake (Fig. 8).

**DISCUSSION**

The viability of the isolated hepatocytes prepared in the present study has been found to be higher than that of the cells incubated in the medium containing amino acids and glucose (17), not only immediately after their isolation, but also after an incubation for 1 and 2 hr. These data are similar to those reported by Edwards (11). Electron micrographs of the isolated hepatocytes also showed that these cells were almost intact with the exception of minor changes at the
cell membranes. The leakage of LDH from the cells was very little, indicating that the changes of the cellular membranes observed in electron micrographs might not affect the cellular functions including the uptake of various substances. The glucose production by the isolated hepatocytes was sufficiently high. This fact also suggests that the metabolic pathway to produce glucose from endogenous glycogen through glycogenolysis may be well preserved in the isolated hepatocytes. From these results, we have decided that the isolated hepatocytes used in the present study are suitable for the analysis of characteristics of taurine transport in these cells.

Taurine has been thought to be actively accumulated by Hela cells (20) and Ehrlich ascites tumor cells (21). Moreover in mammals, the presence of active taurine transport was reported in brain (22), heart (23) and kidney (24). It has been also reported that hepatic taurine content is maintained by both local biosynthesis and the active uptake from blood plasma (25). The characteristics of taurine transport in the liver, however, have not been well analyzed because of the anatomical complexity of this organ.

One of the important findings obtained in this study is that the uptake of taurine by hepatocytes is constituted of two processes: an unsaturable and energy-independent process, and a saturable and energy-dependent process. The latter system seems carrier-mediated, and is competitively inhibited by \( \beta \)-amino acids such as \( \beta \)-alanine as well as by structurally related amino acids such as hypotaurine and guanidinoethyl sulfonate. These characteristics of the taurine uptake are similar to those found in Ehrlich ascites tumor cells (21) and in cerebral synaptosomes (26). Although the functional significance of the unsaturable and energy-independent uptake system for taurine is not clear at present, it is likely that this system may be consist of a simple diffusion process as suggested by Christensen and Liang (27), and involve the binding of taurine to the membrane of hepatocytes.

It has been reported that the hepatocytes in primary culture have a tendency for despecialization with functional changes such as decrease in activities of the glycogenolytic enzymes and in the ability of glycogen synthesis, and the reduction of drug-binding microsomal hemoprotein and cytochrome P-450. These evidences suggest that the hepatocytes in primary culture may have no original functions which are maintained in normal liver cells and that alterations in the characteristics of various transport systems may also occur.

Recently the presence of a taurine transport mechanism in the hepatocytes in primary culture has been reported (28). The apparent \( V_{\text{max}} \) value found in this study using the freshly isolated rat hepatocytes differs from that observed in the rat hepatocytes in primary culture (28). This fact suggests that the characteristics of the taurine transport system are also altered following the primary culture of isolated hepatocytes.

Huxtable et al. reported that taurine transport in the heart was competitively inhibited by hypotaurine, \( \beta \)-alanine and guanidinoethyl sulfonate (29). In the present study, we have also found that the taurine transport in isolated hepatocytes is competitively inhibited by \( \beta \)-alanine, hypotaurine and guanidinoethyl sulfonate. It is noteworthy, however, that guanidinoethyl sulfonate inhibits more strongly the taurine transport in cardiac tissues than that in isolated hepatocytes as judged from the difference in \( K_i \) values found in cardiac tissue (29) and that found in the present study using isolated hepatocytes. This difference may be explained
by the difference in the sensitivity of taurine transport systems to this agent in both organs, but the exact mechanisms underlying this difference remains to be determined.

In summary, in rat hepatocytes, taurine appears to be transported mainly by a saturable, sodium- and energy-dependent, and carrier-mediated process which is similar to that described in Ehrlich ascites tumor cells (21) and rat brain synaptosomes (26).

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REFERENCES
1) Welty, J.D. and Read, W.O.: Studies on some cardiac effect on taurine. J. Pharmacol. exp. Ther. 114, 110–115 (1964)
2) Kuriyama, K.: Taurine as a neuromodulator. Fedn Proc. 39, 2680–2684 (1980)
3) Dolara, P., Marino, P. and Buffoni, F.: Effect of 2-aminoethanesulfonic acid (taurine) and 2-hydroxyethane sulfonic acid (isethionic acid) on calcium transport by rat liver mitochondria. Biochem. Pharmacol. 22, 2085–2094 (1973)
4) Ohshima, T. and Fujihira, E.: Effect of taurine on metabolic function of isolated, perfused rat liver. Chem. Pharm. Bull. 19, 2020–2025 (1971)
5) Portman, O.W. and Mann, G.V.: The disposition of taurine-\(^{35}\)S and taurocholate-\(^{35}\)S in the rat: Dietary influences. J. biol. Chem. 213, 733–743 (1955)
6) Sjövall, J.: Dietary glycine and taurine on bile acid conjugation in man. Bile acids and steroids 75. Proc. Soc. exp. Biol. Med. 100, 676–678 (1959)
7) Hardison, W.G.M. and Proffitt, J.H.: Influence of hepatic taurine concentration on bile acid conjugation with taurine. Am. J. Physiol. 232, E75–E79 (1977)
8) Berry, M.N. and Friend, D.S.: High-yield preparation of isolated rat liver parenchymal cells. J. Cell Biol. 43, 506–520 (1969)
9) Ui, M. and Tokumitsu, S.: Isolated hepatocytes as an in vitro system useful for study of metabolic regulations. Protein. Nucleic Acid and Enzymes. 24, 3–13 (1979) (in Japanese)
10) Garrison, J.C. and Haynes, R.C., Jr.: Hormonal control of glycogenolysis and gluconeogenesis in isolated rat liver cells. J. biol. Chem. 248, 5333–5343 (1973)
11) Edwards, P.A.: The influence of catecholamines and cyclic AMP on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and lipid biosynthesis in isolated rat hepatocytes. Arch. Biochem. Biophys. 170, 188–203 (1975)
12) Cabaud, P.G. and Wróblewski, F.: Colorimetric measurement of lactic dehydrogenase activity of body fluid. Am. J. clin. Pathol. 30, 234–236 (1958)
13) Rasbo, E. and Terkildsen, T.C.: On the enzymatic determination of blood glucose. Scand. J. clin. Lab. Invest. 12, 402–407 (1960)
14) Baur, H., Kasperek, S. and Pfaff, E.: Criteria of viability of isolated liver cells. Hoppe-Seyler's Z. physiol. Chem. 356, 827–838 (1975)
15) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. 193, 285–275 (1951)
16) Yoneda, Y., Takashima, S., Hirai, K., Kurihara, E., Yukawa, Y., Tokunaga, H. and Kuriyama, K.: Microassay methods for taurine and cysteine sulfinate decarboxylase activity. Japan. J. Pharmacol. 27, 881–889 (1977)
17) van Bezooijen, C.F.A., van Noord, M.J. and Knook, D.L.: The viability of parenchymal liver cells isolated from young and old rats. Mech. Ageing Dev. 3, 107–119 (1974)
18) Plagemann, P.G.W. and Richey, D.P.: Transport of nucleotides, nucleic acid bases, choline and glucose by animals cells in culture. Biochim. Biophys. Acta. 344, 263–305 (1974)
19) Zylka, J.M. and Plagemann, P.G.W.: Purine and pyrimidine transport by cultured Novikoff cells. J. biol. Chem. 250, 5756–5767 (1975)
20) Piez, K.A. and Eagle, H.: The free amino acid pool of cultured human cells. J. biol. Chem. 231, 533–546 (1958)
21) Christensen, H.N., Hess, B. and Riggs, T.R.: Concentration of taurine, \(\beta\)-alanine, and triiodothyronine by ascites carcinoma cells. Cancer Res. 14, 124–127 (1954)
22) Lädesmäki, P. and Oja, S.S.: On the mechanisms of taurine transport at brain cell membranes. J.
23) Awapara, J.: The taurine concentration of organs from fed and fasted rats. J. biol. Chem. 281, 571-576 (1956)

24) Chesney, R.W., Scriver, C.R. and Mohyuddin, F.: Localization of the membrane defect in transepithelial transport of taurine by parallel studies in vivo and in vitro in hypertaurinuric mice. J. clin. Invest. 57, 183-193 (1976)

25) Sturman, J.A.: Taurine pool sizes in the rats: Effects of vitamin B-6 deficiency and high taurine diet. J. Nutr. 103, 1566-1580 (1974)

26) Meiners, B.A., Speth, R.C., Bresolin, N., Huxtable, R.J. and Yamamura, H.I.: Sodium-dependent, high-affinity taurine transport into rat brain synaptosomes. Fedn Proc. 39, 2695-2700 (1980)

27) Christensen, H.N. and Liang, M.: On the nature of the "non-saturable" migration of amino acids into Ehrlich cells and into rat jejunum. Biochim. Biophys. Acta. 112, 524-531 (1966)

28) Hardison, W.G.M. and Weiner, R.: Taurine transport by rat hepatocytes in primary culture. Biochim. Biophys. Acta. 598, 145-152 (1980)

29) Huxtable, R.J., Chubb, J. and Azari, J.: Physiological and experimental regulation of taurine content in the heart. Fedn Proc. 39, 2685-2690 (1980)