Roles of Endogenous and Exogenous Taurine and Glycine in the Formation of Conjugated Bile Acids: Analyses Using Freshly Isolated and Primary Cultured Rat Hepatocytes

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Abstract—The regulatory roles of intracellular taurine and glycine, transported and biosynthesized in hepatocytes, on the formation of conjugated bile acids were studied using freshly isolated hepatocytes (fresh hepatocytes) and hepatocytes in primary culture (cultured hepatocytes). Transported taurine significantly increased the rate of taurocholic acid formation both in fresh hepatocytes and cultured hepatocytes. Similarly, the addition of cysteine and hypotaurine, which were metabolically converted to taurine in hepatocytes, facilitated the formation of taurocholic acid in these cells. On the other hand, exogenous glycine into the incubation medium had no effect on the formation of glycocholic acid both in fresh and cultured hepatocytes. In contrast, the addition of serine and threonine, which are metabolically converted to glycine in hepatocytes, significantly increased the formation of glycocholic acid in fresh hepatocytes, although little effect of the additions of serine and threonine on the formation of glycocholic acid was noted in the case of cultured hepatocytes. The present results indicate that the formation of taurine-conjugated bile acids in hepatocytes is maintained by both transported and intracellularly formed taurine in hepatocytes, while that of glycine-conjugated bile acids is regulated by glycine formed within hepatocytes, but not by transported glycine.

It has been well established that bile acids in hepatocytes are conjugated with taurine or glycine by two independent enzymatic reactions in hepatocytes (1, 2). The first reaction is the formation of a coenzyme thioester intermediate, bile acid-Co A, via bile acid: CoA ligase (EC 6.2.1.7) (3). The second reaction is catalyzed by bile acid-CoA: amino acid N-acyltransferase (EC 2.3.1.), and the formation of taurine- or glycine-conjugated bile acids (1, 4, 5) is completed by these precesses. It has also been reported that in the mammalian liver a single enzyme, bile acid-Co A: amino acid N-acyltransferase, catalyzes the conjugation of bile acids both with taurine and glycine, and this enzyme has a higher affinity for taurine than glycine in the rat liver (6, 7).

Taurine administrated orally increased the formation of taurine-conjugated bile acid (8–10), although dietary glycine had no effect on the formation of glycine-conjugated bile acids (8). In addition, the experiments using perfused rat liver revealed that the hepatocellular concentration of taurine was a major factor to determine the proportion of bile acids conjugated with taurine, while the formation of glycine-conjugated bile acids was observed only under the conditions of limited taurine supply (11). These results indicate that the formation of conjugated bile acids is dependent on the concentrations of taurine and glycine in hepatocytes. It is not known, however, if exogenously administrated taurine and glycine are preferentially utilized for the conjugation of bile acids to those biosynthesized in hepatocytes.

In the present study, we have therefore
investigated the origin of taurine and glycine conjugated with bile acids using freshly isolated hepatocytes (fresh hepatocytes) and hepatocytes in primary culture (cultured hepatocytes).

**Materials and Methods**

**Chemicals:** \[^{[3H]}\text{Cholic acid (16 Ci/mmol)}\] and \[^{[3H]}\text{inulin (0.19 mCi/mg)}\] were obtained from New England Nuclear (Boston, U.S.A.). \[^{[3H]}\text{Glycine (20 Ci/mmol)}\] was purchased from the Radiochemical Centre (Amersham, England). Collagenase (Type IV), N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), insulin and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Fetal calf serum, penicillin-G (K-salt) and streptomycin, carbenicillin, and Eagle's minimal essential medium (MEM) (powder) were purchased from Granite Diagnostics (Burlington, U.S.A.), Meiji Seika (Tokyo, Japan), Taito Pfizer (Tokyo, Japan) and Nissui Seiyaku (Tokyo, Japan), respectively. Silica gel G 60 was obtained from Merck (Darmstadt, F.R.G.). Guanidinoethyl sulfonate (GES) and glucagon were kind gifts of Dr. Ryan J. Huxtable, Dept. of Pharmacology, University of Arizona Health Sciences Center, U.S.A. and Kodama Co. (Tokyo, Japan), respectively. All other chemicals used were analytical grade and were obtained locally from commercial sources.

**Isolation and culture of hepatocytes:** Male Wistar rats weighing 200–300 g and having free access to laboratory chow and water were used for the isolation and primary culture of hepatocytes throughout this study. The procedures for preparing hepatocytes and maintaining hepatocytes in primary culture were carried out under sterile conditions, and all buffers and instruments were also sterilized before each use.

Isolated hepatocytes were prepared according to the method of Berry and Friend (12) with a minor modification (13). In brief, the rat liver was perfused with warm (37°C) Ca\(^{2+}\)-free improved Krebs-Henseleit buffer containing 0.05% collagenase, and it was then minced with scissors to separate the hepatocytes. Hepatocytes were suspended in ice-cold Ca\(^{2+}\)-free Krebs-Henseleit buffer and centrifuged at a low speed. The pellet was resuspended with ice-cold Ca\(^{2+}\)-free Krebs-Henseleit buffer. After washing the hepatocytes five times by repeating the procedure described above, the pellet was finally suspended with ice-cold improved Krebs-Ringer buffer containing penicillin-G (0.2 mg/ml) and streptomycin (0.2 mg/ml) and used as fresh hepatocytes.

For maintaining hepatocytes in primary culture, the fresh hepatocytes obtained after washing with ice-cold Ca\(^{2+}\)-free Krebs-Henseleit buffer were resuspended with MEM supplemented with 10% fetal calf serum, 10 mM HEPES, 10 mM TES, 10 \(\mu\)M dexamethasone, insulin (10 mU/ml), carbenicillin (0.1 mg/ml) and streptomycin (0.1 mg/ml), and the cell number was adjusted to 1.0×10\(^6\) cells per ml of the suspension. Three ml of this suspension were added into a Corning culture dish (60 mm in diameter) and incubated under humidified 5% CO\(_2\) in air at 37°C for 24 hr according to the method of Bonney et al. (14). The planting efficiency at 24 hr after inoculation was estimated by the method of Morse and Potter (15) and was found to be approximately 60%, which was comparable with the value reported previously (14, 16).

Hepatocytes used as fresh hepatocytes and applied to the primary culture had a viability of more than 90% when estimated by the exclusion of 0.2% trypan blue.

**Analysis of conjugated bile acids formed by fresh hepatocytes:** Hepatocytes were suspended in improved Krebs-Ringer buffer, and the number was adjusted to 6.0×10\(^6\) cells per ml of the suspension. Following preincubation of the cell suspension at 37°C under 95% O\(_2\)-5% CO\(_2\) for 15 min, 50 \(\mu\)M \[^{[3H]}\text{cholic acid was added into the incubation medium, and incubation was carried out for 60 min under the same conditions as the preincubation was performed. The reaction was terminated by separating cells from the incubation medium by gentle centrifugation (50×g) at 4°C for 3 min. After removing hepatocytes from the incubation medium, 4 volumes of methanol-acetone (1:1; v/v) were added to each
fraction of the pellet and supernatant. The mixture of the pellet and methanol-acetone was centrifuged at 800×g for 10 min, and the supernatant obtained was stored. The pellet obtained by this procedure was also mixed well with methanol-acetone and re-centrifuged under the same conditions described above. The supernatants prepared from the 1st and 2nd centrifugations were combined and lyophilized at 80°C (17). The extract containing bile acids was also prepared from the incubation medium by the same procedure for preparing the bile acids extract from hepatocytes and lyophilized at 80°C. Each lyophilized sample obtained from hepatocytes and incubation medium was then dissolved into methanol for the analysis of bile acids by thin-layer chromatography (18). Following the separation of each bile acid by thin-layer chromatography, spots corresponding to those of authentic cholic acid, taurocholic acid and glycocholic acid were scrapped off and transferred to scintillation vials containing methanol for extracting bile acid from silica gel. Ten ml of Triton-toluene scintillator [TritonX-100 : toluene (containing 5 g PPO and 0.3 g POPOP per liter)=2:1] was added into each vial, and the radioactivities of free and conjugated cholic acids were measured by a liquid scintillation spectrometer. Recoveries of free and conjugated cholic acids were more than 85%.

The total amounts of taurocholic acid and glycocholic acid formed by hepatocytes were calculated respectively by adding the amount of each conjugated bile acid detected in hepatocytes and that found in the incubation medium.

For examining the effects of exogenously added taurine, glycine, their metabolic precursors, and several drugs on the formation of conjugated cholic acids, these substances were added into the incubation medium at the start of preincubation.

**Analysis of conjugated bile acids formed by cultured hepatocytes:** Following aspiration of MEM and rinsing a culture dish three times with 5 ml warm (37°C) Krebs-Henseleit buffer, 2 ml of warm improved Krebs-Ringer buffer containing 0.2 mg/ml of penicillin-G and 0.2 mg/ml of streptomycin were added into the culture dish, and then cultured hepatocytes were preincubated at 37°C under humidified 5% CO₂ in air for 15 min. For examining the influences of exogenously added taurine, glycine, their precursors and several drugs, these substances were added into the incubation medium immediately before starting the preincubation. After the preincubation, the incubation of cultured hepatocytes was performed in the presence of 50 μM [³H]cholic acid under the same conditions used for the preincubation for 60 min. At 75 min after the initiation of preincubation, the incubation medium was removed from the culture dish by aspiration, and 4 volumes of methanol-acetone (1:1; v/v) was added to each culture dish. Cultured hepatocytes were then scrapped off, and the mixtures of methanol-acetone with hepatocytes and with culture medium were subjected to the same procedures as those used for the fresh hepatocytes, and the extracts obtained from each fraction were lyophilized at 80°C. Conjugated bile acids were analyzed by thin-layer chromatographically as described above.

**Uptake of glycine by fresh and cultured hepatocytes:** Fresh hepatocytes were suspended with Krebs-Henseleit buffer containing penicillin-G (0.2 mg/ml) and streptomycin (0.2 mg/ml), and the cell number was adjusted to 3.0×10⁶ cells per ml of the suspension. This suspension was incubated with 100 μM [³H]glycine at 37°C under 95% O₂-5% CO₂ for 60 min after preincubating at 37°C for 5 min. For terminating the reaction, cells were rapidly separated from the incubation medium by centrifugal filtration (13). The radioactivity accumulated in hepatocytes was measured by a liquid scintillation spectrometer after dissolving hepatocytes with Triton-toluene scintillator (13).

For the determination of glycine uptake by cultured hepatocytes, cells were incubated in Krebs-Henseleit buffer containing penicillin-G (0.2 mg/ml) and streptomycin (0.2 mg/ml) in the presence of 100 μM [³H]glycine at 37°C under humidified 5% CO₂ in air for 60 min after discarding MEM and rinsing hepatocytes three times with warm Krebs-Henseleit buffer. The reaction was terminated by the aspiration of incubation medium followed by rinsing five times with a total of
about 15 ml of ice-cold Krebs-Henseleit buffer within 45 sec. After solubilizing hepatocytes with 0.1 N NaOH, an aliquot of the digested cells was measured to determine the radioactivity accumulated into the hepatocytes (19, 20).

The net amounts of transported glycine by both fresh and cultured hepatocytes were calculated using [3H]inulin as described in previous reports (13, 19).

**Results**

**Formation of conjugated [3H]cholic acid by fresh hepatocytes:** The formation of conjugated [3H]cholic acid by fresh hepatocytes increased linearly up to 60 min after the addition of [3H]cholic acid and gradually decreased thereafter. A plateau was attained at approximately 90 min. On the other hand, the amount of [3H]cholic acid in the incubation medium decreased in parallel with the increase of the formation of conjugated [3H]-cholic acid (Fig. 1). Taking into account the influence of a decreased viability of cells during incubation, we have used a 60 min incubation to determine the formation of conjugated [3H]cholic acid.

The effects of taurine and glycine added exogenously into the incubation medium on the formation of conjugated bile acids are shown in Fig. 2. The formation of [3H]-glycocholic acid was not affected by glycine, although the addition of 1 mM taurine remarkably decreased the formation of [3H]-glycocholic acid. In contrast, the formation of [3H]taurocholic acid was significantly facilitated by the addition of more than 0.5 mM taurine. Furthermore, the addition of glycine had no effect on the formation of [3H]taurocholic acid in fresh hepatocytes. These results suggest that exogenous taurine, which is transported into hepatocytes, may be utilized in the formation of taurine-conjugated bile acids, but the glycine-conjugation of bile acids may not be influenced by exogenously added glycine.

These results are in agreement with the findings that freshly isolated hepatocytes accumulate taurine (13) but not glycine (Fig. 5).

It has been well established that cysteine and hypotaurine are converted to taurine in hepatocytes (21), whereas glycine is metabolized from its precursors, serine and

![Fig. 1.](image-url)

*Fig. 1.* Time course of formation of conjugated [3H]cholic acids by freshly isolated hepatocytes. Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 μM [3H]cholic acid at 37°C following the preincubation at 37°C for 15 min. An aliquot of the cell suspension was taken at various periods as indicated in the figure and centrifuged at 50xg for 3 min. Each value represents the mean±S.E.M. of three to seven separate experiments.*
Taurine and Glycine in Bile Acid Conjugation

Moreover, the precursor of taurine used in this study has been reported to be accumulated and converted to taurine in fresh and cultured hepatocytes (22). In preliminary experiments, we have determined the uptake of serine and threonine by high-performance liquid chromatographically (23) and found that these amino acids were indeed accumulated by freshly isolated hepatocytes. Based on these findings, we examined the effects of taurine and glycine which were metabolically converted from their precursors within hepatocytes on the formation of conjugated [3H]cholic acid (Table 1). Both serine and threonine at the concentration of 0.1 mM significantly increased the formation of [3H]glycocholic acid. Similarly, the additions of 1.0 mM cysteine and hypotaurine showed stimulatory effects on the conjugation of taurine with [3H]cholic acid. These results clearly indicate that the formations of glycine- and taurine-conjugated bile acids are also facilitated in the presence of metabolic precursors converted to glycine and taurine in fresh hepatocytes.

Guanidinoethyl sulfonate (GES) is known as an inhibitor for taurine transport in fresh hepatocytes (13) as well as in the heart (24). The presence of GES with taurine in the incubation medium reduced the formation of taurine-conjugated [3H]cholic acid in fresh hepatocytes (Table 2), indicating that exogenously added taurine certainly participated in the formation of taurine-conjugated bile acids.

Lombardini (25) has reported that isethionic acid, a metabolite of taurine formed in hepatocytes, inhibits the conjugation of taurine with cholic acid in liver homogenate, although the mechanism of inhibition remains obscure. In the present, we have also found that isethionic acid added into the incubation medium with taurine inhibits the formation of [3H]taurocholic acid in fresh hepatocytes. The inhibitory effect of isethionic acid on the bile acid conjugation with taurine was not due to the reduction of taurine.

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Fig. 2. Effect of exogenous taurine and glycine on formation of conjugated [3H]cholic acids by freshly isolated hepatocytes. Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 µM [3H]cholic acid at 37°C for 60 min following the preincubation in the presence of taurine or glycine at 37°C for 15 min. Numbers in parentheses are numbers of experiments. Each value represents the mean±S.E.M. *P<0.001, **P<0.02, compared with each control value.
uptake by fresh hepatocytes since isethionic acid had no effect on the transport of taurine in fresh hepatocytes (13).

Formation of conjugated $[^3]$H-cholic acid by cultured hepatocytes: The time course of the formation of conjugated $[^3]$H-cholic acid in cultured hepatocytes is shown in Fig. 3. A linear increase of the formation was observed up to 60 min after the addition of $[^3]$H-cholic acid into the incubation medium. The rate of the formation gradually decreased thereafter and reached to a plateau at 90 min after the initiation of incubation. In order to compare the rate of the formation of $[^3]$H-cholic acid conjugates by cultured hepatocytes with that by fresh hepatocytes, we measured the formation of conjugated $[^3]$H-cholic acid during a 60 min incubation with $[^3]$H-cholic acid.

Exogenous taurine (1 mM) significantly increased the formation of $[^3]$H-taurocholic acid in cultured hepatocytes in parallel with the reduction of $[^3]$H-glycocholic acid formation (Fig. 4). No change in the formation of $[^3]$H-taurocholic acid was observed, however, in the presence of 0.5 mM taurine, in spite of the remarkable increase of the formation of $[^3]$H-taurocholic acid by 0.5 mM taurine in fresh hepatocytes (Figs. 2 and 4.) On the other hand, no alteration in the

| Added precursors (mM) | No. of experiments | $[^3]$H-Cholic acid conjugates formed (% of conversion) |
|----------------------|-------------------|------------------------------------------------------|
|                      |                   | $[^3]$Glycocholic acid | $[^3]$Taurocholic acid |
| Control              |                   | 15.88± 3.75          | 16.41± 3.16          |
| Glycine (1.0)        | (7)               | 23.50±10.00          | 43.75±11.55          |
| Serine (0.1)         | (5)               | 30.58± 5.00*         | 14.70± 1.56          |
| Threonine (0.1)      | (5)               | 31.94± 3.70**        | 15.98± 1.90          |
| Taurine (0.1)        | (7)               | 14.23± 2.18          | 27.80± 5.04          |
| Taurine (1.0)        | (6)               | 3.02± 0.62**         | 78.62± 1.05****      |
| Cysteine (0.1)       | (5)               | 20.90± 1.70          | 13.54± 1.36          |
| Cysteine (1.0)       | (5)               | 9.63± 4.61           | 55.28± 4.64***       |
| Hypotaurine (0.1)    | (4)               | 14.70± 2.01          | 38.18± 9.10          |
| Hypotaurine (1.0)    | (4)               | 5.80± 1.56*          | 70.60± 3.31****      |

Table 1. Effect of metabolic precursors of taurine and glycine on formation of $[^3]$H-cholic acid conjugated by freshly isolated hepatocytes

Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 μM $[^3]$H-cholic acid at 37°C for 60 min following the preincubation in the presence of various concentrations of taurine, glycine and their precursors at 37°C for 15 min. Each value represents the mean±S.E.M. *P<0.05, **P<0.02, ***P<0.01, ****P<0.001, compared with each control value.

| Added substances      | (mM)  | No. of experiments | $[^3]$H-Cholic acid conjugates formed (% of conversion) |
|----------------------|-------|-------------------|------------------------------------------------------|
|                      |       |                   | $[^3]$Glycocholic acid | $[^3]$Taurocholic acid |
| Control [+ Taurine]  | (1)   | (6)               | 3.02±0.62            | 78.62±1.05            |
| +GES                 | (1)   | (4)               | 8.35±0.72*           | 40.65±9.53**          |
| +GES                 | (10)  | (4)               | 15.10±1.86*          | 29.48±7.07*           |
| +Isethionic acid     | (1)   | (4)               | 5.60±1.17            | 70.35±2.51**          |
| +Isethionic acid     | (10)  | (4)               | 3.90±0.67            | 70.20±2.06**          |

Table 2. Effect of guanidinioethyl sulfonate (GES) and isethionic acid on formation of $[^3]$H-cholic acid conjugates by freshly isolated hepatocytes

Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 μM $[^3]$H-cholic acid at 37°C for 60 min following the preincubation in the presence of 1 mM taurine plus various concentrations of GES or isethionic acid at 37°C for 15 min. Each value represents the mean±S.E.M. *P<0.01, **P<0.05, compared with the control value.
formations of both [3H]taurocholic acid and [3H]glycocholic acid was observed during the incubation with 1 mM glycine added into the incubation medium in cultured hepatocytes.

**Fig. 3.** Time course of formation of conjugated [3H]cholic acids by hepatocytes in primary culture. Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 μM [3H]cholic acid at 37°C following the preincubation at 37°C for 15 min. Each value represents the mean±S.E.M. of four to twelve dishes.

**Fig. 4.** Effects of exogenous taurine and glycine on formation of conjugated [3H]cholic acids by hepatocytes in primary culture. Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 μM [3H]cholic acid at 37°C for 60 min following the preincubation in the presence of various concentrations of taurine and glycine at 37°C for 15 min. Numbers in parentheses are numbers of dishes used for each experiment. Each value represents the mean±S.E.M. *P<0.01, **P<0.02, ***P<0.05, compared with each control value.
cytes as well as in fresh hepatocytes (Fig. 3). Based on these results, it may be concluded that exogenous taurine is involved in the formation of taurine-conjugated bile acids, but glycine added exogenously does not participate in the conjugation of bile acids in hepatocytes.

The effects of endogenous taurine and glycine, which were converted metabolically from their precursors within hepatocytes, on the formation of conjugated [³H]cholic acid in cultured hepatocytes were examined using the same methods as described in the case of fresh hepatocytes (Table 3). Cysteine and hypotaurine added into the incubation medium significantly facilitated the formation of [³H]taurocholic acid in cultured hepatocytes. On the other hand, the precursors of glycine such as serine and threonine had no effect on the formation of [³H]glycocholic acid. These results indicate that in cultured hepatocytes, endogenous taurine participates in the formation of taurine-conjugated bile acids, but endogenously formed glycine may not be involved in the formation of glycocholic acids.

The effects of GES and isethionic acid on the formation of [³H]taurocholic acid in cultured hepatocytes were found to be similar to those found in fresh hepatocytes (Table 4).

**Uptake of glycine by fresh and cultured hepatocytes**

Table 3. Effect of metabolic precursors of taurine and glycine on formation of [³H]cholic acid conjugates by hepatocytes in primary culture

| Added precursors | [³H]Cholic acid conjugates formed (%) of conversion | No. of dishes | [³H]Glycocholic acid | [³H]Taurocholic acid |
|------------------|-----------------------------------------------|-------------|-------------------|-------------------|
| Control          |                                               | (12)        | 1.78±0.26         | 30.14±1.63        |
| Glycine (1.0)    |                                               | (4)         | 4.92±1.01         | 35.03±1.86        |
| Serine (0.1)     |                                               | (6)         | 2.35±0.53         | 32.77±2.45        |
| Serine (1.0)     |                                               | (8)         | 1.79±0.29         | 26.39±2.27        |
| Threonine (0.1)  |                                               | (6)         | 2.00±0.46         | 32.07±0.50        |
| Threonine (1.0)  |                                               | (8)         | 1.32±0.10         | 25.88±3.31        |
| Taurine (1.0)    |                                               | (4)         | 0.65±0.05*        | 37.45±2.15**      |
| Cysteine (0.1)   |                                               | (4)         | 1.28±0.28         | 35.18±1.53**      |
| Hypotaurine (0.1)|                                               | (4)         | 1.25±0.22         | 37.23±2.34**      |

Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 µM [³H]cholic acid at 37°C for 60 min following the preincubation in the presence of various concentrations of metabolic precursors of taurine and glycine at 37°C for 15 min. Each value represents the mean±S.E.M. *P<0.001, **P<0.05, compared with the control value.

Table 4. Effect of guanidinoethyl sulfonate (GES) and isethionic acid on formation of [³H]cholic acid conjugates by hepatocytes in primary culture

| Added substances | [³H]Cholic acid conjugates formed (%) of conversion | No. of dishes | [³H]Glycocholic acid | [³H]Taurocholic acid |
|------------------|---------------------------------------------------|-------------|-------------------|-------------------|
| Control [+Taurine (1.0)] |                                               | (4)         | 0.65±0.05         | 37.45±2.15        |
| +GES (0.1)       |                                               | (3)         | 0.63±0.09         | 29.00±0.79**      |
| +GES (1.0)       |                                               | (4)         | 1.17±0.09*        | 29.88±0.84**      |
| +Isethionic acid (0.1) |                                               | (4)         | 0.70±0.04         | 31.20±0.87        |
| +Isethionic acid (1.0) |                                               | (3)         | 0.70±0.10         | 28.47±2.63**      |

Hepatocytes were incubated with improved Krebs-Ringer buffer containing 50 µM [³H]cholic acid at 37°C for 60 min following the preincubation in the presence of 1 mM taurine plus various concentrations of GES or isethionic acid at 37°C for 15 min. Each value represents the mean±S.E.M. *P<0.01, **P<0.05, compared with the control value.
hepatocytes: The uptake of glycine in both fresh and cultured hepatocytes was examined in this study since little information is available on the characteristics of glycine transport in these cells.

As shown in Fig. 5, the amount of $[^3]$H-glycine detected in fresh hepatocytes was almost identical to the value of the intercellular space estimated by $[^3]$H-inulin, which suggests that fresh hepatocytes may not accumulate glycine from the incubation medium. In contrast, it was found that cultured hepatocytes also lost the ability to accumulate glycine (Data not shown).

Discussion

Several diseases such as liver cirrhosis and malfunctions of the small intestine are known to produce the alteration of the ratio of glycine-conjugated bile acids to taurine-conjugated bile acids (G/T ratio) in bile. However, the effects of taurine and glycine which are biosynthesized within hepatocytes and transported from circulating blood by hepatocytes on the formation of conjugated bile acids and on the alteration of G/T ratio under physiological and pathological conditions have not been well clarified (8–11). In the present study, we have examined pharmacologically the effects of exogenous and endogenous taurine and glycine on the formation of conjugated bile acids using both fresh and cultured hepatocytes incubated with taurine, glycine and their metabolic precursors in the presence of unconjugated cholic acid.

We have reported previously that both fresh and cultured hepatocytes have the capacity to accumulate taurine and cholic acid mainly by saturable carrier-mediated transport systems (13, 19, 20, 26). In contrast, this study demonstrated that glycine is not accumulated in these cells. The results obtained in the present study also show that the capacities to conjugate bile acids with taurine and glycine are well preserved in both preparations of hepatocytes.

The G/T ratio in rat bile has been reported to be less than 0.5 (27–29). The G/T ratio of bile acids secreted from fresh hepatocytes has also been found to be within the range of 0.25 to 1.25 (28–30). Concerning the G/T ratio for conjugates of cholic acid, it has been reported that the values in rat bile and bile acids secreted from fresh hepatocytes and cultured hepatocytes from rat liver are 0.1–0.3 (28, 29), 0.3–2.0 (28, 29) and 0.01 (30), respectively. In this study, it has been found that the G/T ratio for conjugates of cholic acid formed by fresh and cultured hepatocytes is 1.0 and 0.06, respectively. Thus these values are in good agreement with those reported previously (28–30). The remarkable difference between the G/T ratio of conjugated cholic acids formed in fresh hepatocytes and that in cultured hepatocytes was noticed in this study as well as in several previous studies (28–30). The following facts may explain this difference. The ratio of taurine to glycine content in fresh hepatocytes and that in cultured hepatocytes has been found, in preliminary studies, to be 1.0 and 2.0, respectively. In addition, the amount of taurine detected in cultured hepatocytes is five times as large as that in fresh hepatocytes (22). Furthermore, it has been found that the affinity of the enzyme catalyzing conjugation of bile acids with taurine and glycine, bile acid-Co A: amino acid Nacyltransferase, for taurine is one hundred-fold higher than that for glycine in rat liver.
These results lead to the conclusion that the formation of taurine-conjugated bile acids in cultured hepatocytes should be superior to that of glycine-conjugated bile acids (yielded a low G/T ratio).

The effects of exogenously added taurine and glycine on the formation of conjugated bile acids were examined following the incubation of hepatocytes with taurine and glycine, respectively. We have previously reported that taurine is mainly accumulated by a carrier-mediated transport system both in fresh and cultured hepatocytes (13, 19). In addition, it was confirmed in the preliminary study that labeled taurine added into the incubation medium was conjugated with cholic acid in both fresh and cultured hepatocytes, which was analyzed by thin layer chromatography (18). The addition of 0.5 and 1.0 mM taurine increased the formation of taurocholic acid in fresh hepatocytes. In cultured hepatocytes, the taurocholic acid formation was facilitated in the presence of 1 mM taurine, but not by 0.5 mM taurine. These differences may be due to the difference in the concentration of endogenous taurine in both types of hepatocytes. Since cultured hepatocytes were incubated for 24 hr with MEM containing a high concentration of a precursor of taurine, cysteine, prior to use, the endogenous level of taurine in cultured hepatocytes might be higher than that in fresh hepatocytes (22). Therefore, taurine accumulated in the presence of 0.5 mM taurine may not be enough to show a significant effect on the formation of taurine-conjugated bile acids in cultured hepatocytes.

Glycine added into the incubation medium had no effect on the formation of glycine-conjugated bile acids in both freshly isolated and cultured hepatocytes. These results can be reasonably explained by the fact that these cells have no capacity to accumulate glycine. This view is further supported by previous reports that glycine administrated orally had no effect on the formation of glycine-conjugated bile acids (8, 9).

It has been demonstrated that cysteine is converted to hypotaurine via cysteine sulfenic acid and/or cysteic acid and finally to taurine in fresh and cultured hepatocytes (22) as well as in liver homogenate (21). Further-
cultured hepatocytes is unknown. It has been reported, however, that the formation of [3H]glycocholic acid in perfused liver is minimal when a large excess of [3H]-taurocholic acid is formed (11). The formation of taurocholic acid as well as taurine content in cultured hepatocytes is probably very high, thus the newly synthesized glycine from these metabolic precursors is only minimally utilized for the formation of glycocholic acid. GES, a competitive inhibitor for taurine transport, remarkably decreased the formation of taurocholic acid in the presence of taurine, indicating that the formation of taurine-conjugated bile acids is certainly maintained, at least in part, by taurine transported into hepatocytes. In both fresh and cultured hepatocytes, isethionic acid inhibited the conjugation of bile acids with taurine. The similar result was reported in an experiment using liver homogenate (25). Since isethionic acid has been found to be not converted to taurine in hepatocytes (21) and have no inhibitory effect on the taurine transport in hepatocytes (13), the effect of isethionic acid to reduce the formation of taurine-conjugated bile acids is unlikely to be due to the reduction of taurine transport by hepatocytes. Mechanisms underlying the inhibitory action of isethionic acid, however, remain to be studied.

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