Flux of the L-Serine Metabolism in Rat Liver

THE PREDOMINANT CONTRIBUTION OF SERINE DEHYDRATASE*

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Hai-Hui Xue‡‡§§, Michio Fujie‡‡, Takanori Sakaguchi, Toshiaki Oda‡, Hirofumi Ogawa**,
Nancy M. Kneer‡‡§§, Henry A. Lardy‡‡, and Arata Ichiyama‡‡§§

From the §First Department of Biochemistry, ¶Equipment Center, and ¶¶Second Department of Surgery,
Hamamatsu University School of Medicine, Hamamatsu, Shizuoka 431-3122, Japan, **Department of Biochemistry,
Toyama Medical and Pharmaceutical University Faculty of Medicine, Toyama 930-0194, Japan, and ‡§§Institute for
Enzyme Research and the Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

L-Serine metabolism in rat liver was investigated, focusing on the relative contributions of the three pathways, one initiated by L-serine dehydratase (SDH), another by serine:pyruvate/alanine:glyoxylate aminotransferase (SPT/AGT), and the other involving serine hydroxymethyltransferase and the mitochondrial glycine cleavage enzyme system (GCS). Because serine hydroxymethyltransferase is responsible for the interconversion between serine and glycine, SDH, SPT/AGT, and GCS were considered to be the metabolic exits of the serine-glycine pool. In vitro, flux through SDH was predominant in both 24-h starved and glucagon-treated rats. Flux through SPT/AGT was enhanced by glucagon administration, but even after the induction, its contribution under quasi-physiological conditions (1 mM L-serine and 0.25 mM pyruvate) was about 1/6 of that through SDH. Flux through GCS accounted for only several percent of the amount of L-serine metabolized. Relative contributions of SDH and SPT/AGT to gluconeogenesis from L-serine were evaluated in vitro based on the principle that 3H at the 3 position of L-serine is mostly removed in the SDH pathway, whereas it is largely retained in the SPT/AGT pathway. The results showed that SPT/AGT contributed only 10–20% even after the enhancement of its activity by glucagon. These results suggested that SDH is the major metabolic exit of L-serine in rat liver.

L-Serine is known to be physiologically important as a substrate for hepatic gluconeogenesis (1), in addition to its role as a major donor of one-carbon units. It has been established that three pathways are involved in the metabolism of L-serine: 1) that catalyzed by serine hydroxymethyltransferase (SHMT), which is responsible for the reversible conversion of L-serine to glycine and tetrahydrofolate (THF) to glycine and 5,10-methylene-THF; 2) that catalyzed by serine:pyruvate/alanine:glyoxylate aminotransferase (SPT/AGT), leading to the formation of hydroxy-THF; and 3) that catalyzed by L-serine dehydratase (SDH), resulting in the formation of pyruvate (2). Glycine formed by the action of SHMT is thought to be metabolized mainly by the mitochondrial glycine cleavage enzyme system (GCS) (3), whereas hydroxy-THF and pyruvate are either catabolized or converted to glucose depending on the nutritive conditions of animals (Fig. 1).

SHMT exists in some eukaryotic cells, including hepatocytes, as distinct isoforms, one located in the cytosol and the other in mitochondria (referred to as cSHMT and mSHMT, respectively) (2). As for their physiological functions in the liver and kidney, mSHMT has been proposed to be coupled with GCS in the conversion of glycine to serine, using 5,10-methylene-THF formed by the latter enzyme and thus regenerating THF (2, 4). It was also proposed that cSHMT cooperates with mSHMT and GCS to transfer one-carbon units from mitochondria to the cytosol (5). Therefore, the degradation of glycine by GCS appears to be usually associated with synthesis of serine from another molecule of glycine. The SPT/AGT activity is essentially confined to the liver and shows a unique species-specific organelle distribution in mammals, based on their food habits. In carnivores such as dog, this enzyme is largely located in mitochondria, whereas in humans and herbivores such as rabbit, it is entirely peroxisomal (6–8). In rat liver, its activity is detected in both the organelles, but only the mitochondrial activity is induced by glucagon, as a result of alternative transcription initiation from two sites on a single gene and selective stimulation by cAMP of the transcription from the upstream start site (9, 10). One of the major physiological roles of peroxisomal SPT/AGT is catalysis of the conversion of glyoxylate to glycine. This view is supported by the overproduction of oxalate in primary hyperoxaluria type 1, an inborn error of glyoxylate metabolism caused by a functional deficiency of peroxisomal SPT/AGT (11). Mitochondrial SPT/AGT has been presumed, mainly because of its induction by glucagon (2, 12), to participate in gluconeogenesis, but supporting evidence is still insufficient, and whether peroxisomal SPT/AGT is involved in serine metabolism, in addition to its role in the conversion of glyoxylate to glycine, has not been studied so far. SDH is largely confined to the liver and located solely in the cytosol. The liver SDH activity is known to be inversely related to body size in higher animals (13). In the rat, therefore, its activity is fairly high and induced under some gluconeogenic conditions (2). However, its substantial contribution to the metabolism of L-serine has been questioned, despite the high activity in rat liver, mainly because of its very high K_m (50–70 mM) for L-
serine, and indeed the results of experiments involving 14C-serine (14) argued against this possibility.

Flux of the L-serine metabolism through the different pathways had been studied mainly in rat liver and in the 1970s, when the knowledge of SPT/AGT was not sufficient. It is possible from the considerations mentioned above that L-serine metabolism in different animal species shows distinct patterns, and the investigation of this subject is necessary and intriguing. Such work was initiated focusing on the relative contributions of the three pathways, the SDH and SPT/AGT pathways, and that involving SHMT and GCS (Fig. 1). This paper deals with the L-serine metabolism in rat liver in vitro and in vivo. The metabolism of L-serine in the livers of other animal species will be described in an accompanying paper.

**Experimental Procedures**

**Materials**—[1-14C]Glycine (54 mCi/mmol), l-[1-14C]serine (55 mCi/mmoll), and l-[3-14C]serine (55 mCi/mmoll) were purchased from American Radiolabeled Chemicals (St. Louis, MO), and l-[3-3H]serine (28 Ci/mmol) was from Amersham Pharmacia Biotech. Glucagon, THF, and glyoxylate reductase were from Sigma, and lactate dehydrogenase was from Roche Molecular Biochemicals. Analytical grade ion exchange resins, AG 1 × 8 (100–200 mesh) and AG 50W × 8 (100–200 mesh), were obtained from Muromachi Chemical Industries (Tokyo, Japan), and used after conversion to their acetate and H+ forms, respectively. An anti-SDH antibody was raised in rabbits using a rat liver SDH as an antigen (15), and purified by precipitation with 45% saturated ammonium sulfate, followed by negative adsorption to a DEAE column.

**Preparation of Subcellular Substrates**—Freshly killed rats were divided into two groups. One was starved for 24 h, and the other was subjected to glucagon induction as described previously (18). Briefly, the animals were fasted for 24 h, and then glucagon was injected intraperitoneally at a dose of 150 μg/100 g of body weight. The starvation was continued until killing another 24 h later.

Subcellular fractionation of rat liver was carried out essentially as described by de Duve et al. (19). The liver was homogenized in 2.5 vol of 0.25 M sucrose, pH 7.4, containing 3 mM imidazole and HCl and 0.1 mM EDTA using a loosely fitted Potter-Elvehjem homogenizer. The homogenate was centrifuged at 650 × g for 10 min, and the precipitate was homogenized and centrifuged again as above. The combined 650 × g supernatants (cytoplasmic extract) were further centrifuged at 25,000 × g for 20 min, and the resultant supernatants including mitochondria and peroxisomes were suspended in the sucrose solution to make a 2.5 ml suspension/g of liver (Mit-Ps suspension). The 25,000 × g supernatant was further centrifuged at 105,000 × g for 60 min, and the resultant supernatant was adjusted with the sucrose solution to 5 ml/g of liver (soluble fraction). A portion of the soluble fraction was treated with an anti-SDH IgG and a goat anti-rabbit IgG to immunoprecipitate the SHDH activity, according to the protocols used in our previous study (18). A reconstituted cytoplasmic extract was prepared by mixing the Mit-Ps suspension and the soluble fraction, both from a given wet weight of the starting liver, and an SDH-depleted cytoplasmic extract was prepared by combining the Mit-Ps suspension with the anti-SDH-treated soluble fraction. When a heavy mitochondrial fraction was to be prepared, the cytoplasmic extract was centrifuged at 33,000 × g for 10 min, and the precipitate was suspended in the sucrose solution to make a 2.5-ml suspension/g of liver. In preliminary experiments, the recovery of protein from 1 g of liver to the heavy mitochondrial and the Mit-Ps fractions was ~15 and 32 mg, respectively.

The reaction mixture (1 ml) for the L-serine metabolism in vitro comprised 25 mM potassium phosphate, pH 7.4, 40 mM Tricine-NaOH, pH 7.4, a suitable quantity of 1 m sucrose for adjustment of the osmolality, various additions, and the subcellular preparations equivalent to 40 mg of liver. After 5 min of preincubation, the reaction was initiated by the addition of the substrate, carried out at 37 °C for 60 min, and terminated by adding 200 μl of 2 N perchloric acid, followed by neutralization with 1 N KOH. Glycine was determined as a phenyl isothiocyanate derivative using a PICO-TAG amino acid analyzer (Waters, Milford, MA). Hydroxypyruvate, pyruvate, and lactate were measured enzymatically (20). To determine 14CO2 evolution, a parallel incubation with l-[1-14C]serine (0.05 mCi/mmoll) as the substrate was run simultaneously, and the 14CO2 evolved was trapped, and its radioactivity was counted as described previously (21), with a counting efficiency of ~71%. The reactions for determination of the 14CO2 evolution from 1-14Cglycine (0.05 mCi/mmoll) by mitochondrial suspensions were carried out under the same conditions as above.

**Preparation of Doubly Labeled Substrates**—l-[3-3H,14C]Serine was prepared by mixing l-[3-3H]serine (1 mCi) and l-[3-14C]serine (0.1 mCi), followed by passage through an AG 1 column (bed vol, 1 ml) to remove any possible acidic impurities. The effluent was lyophilized, and the dried residue was dissolved in Krebs-Ringer phosphate buffer, pH 7.4. Then nonradioactive L-serine was added to a final concentration of 0.5 m.

l-[3-3H,14C]Lactate was prepared from l-[3-3H,14C]serine through the sequential actions of rat liver SDH, partially purified (second acetone fraction) according to the method of Nakagawa et al. (22), and lactate dehydrogenase. The reaction mixture (8 ml) comprised 0.1 M potassium phosphate, pH 8.0, 0.1 mM pyridoxal phosphate, 1 mM EDTA, 22 units of lactate dehydrogenase, 41 units of SDH, 0.25 mM l-[3-3H,14C]serine (H, 233.6 μCi; 14C, 23.8 μCi), and 0.33 mM NADH. The reaction was initiated by the addition of l-[3-3H,14C]serine, and the decrease in the absorbance at 340 nm was monitored at 37 °C. H2SO4 was then added to 0.83 N, and after the denatured protein had been removed by centrifugation, the acidified reaction mixture was subjected to partitioning chromatography on a silicic acid column with CB8 (8% n-butanol in chloroform, equilibrated with 0.5 N H2SO4) as the eluting solvent, essentially according to the method of Varner (23), using a longer column (0.8 × 60 cm), l-[3-3H,14C]Lactic acid eluted was quantitatively transferred to the aqueous phase by neutralization with 1 mM NaOH to a phenol red end point and vigorous agitation to ensure intimate contact between the two phases. The pooled aqueous phase was treated with a steareate-deactivated activated charcoal, followed by filtration through a 0.45-μm polytetrafluoroethylene filter (Ekikorside 13CB; Gelman Sciences Japan, Tokyo, Japan) (24). The filtrate was
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Reactions were carried out at 37 °C for 60 min with a heavy mitochondrial suspension corresponding to 40 mg of liver, as described under "Experimental Procedures." The amounts of l-serine metabolized through the SPT/AGT and SHMT pathways are calculated as described in the text. Based on the observation that the amounts of endogenous l-serine, glycine, and hydroxypyruvate in the reaction mixture were far less than that added as the substrate, and that of glyoxylate was formed, the amount of 14CO2 formed was calculated from the specific activity of the substrate l-[1-14C]serine. The values represent nmol formed or metabolized/60 min, and those in parentheses represent percentages of contributions of individual pathways. ND, not detectable. The data are representative of three independent experiments with similar results.

| Pyruvate added initially (mM) | 0 | 0.25 | 0.5 | 1.0 | 2.0 |
|-------------------------------|---|------|-----|-----|-----|
| Glycine formation (%) | 70 | 75 | 68 | 68 | 70 |
| Glyoxylate reductase | ND | ND | 4 | 6 | 9 |
| CO2 evolution (%) | 15 | 13 | 14 | 12 | 13 |
| SPT/AGT pathway (%) | ND (~0) | ND (~0) | 4 (5) | 6 (7) | 9 (10) |
| SHMT pathway (%) | 85 (~100) | 89 (~100) | 83 (95) | 81 (93) | 83 (90) |
| Glucagon-treated rat | | | | | |
| Glycine formation (%) | 63 | 79 | 76 | 78 | 70 |
| Glyoxylate reductase | ND | 32 | 86 | 181 | 282 |
| CO2 evolution (%) | 16 | 17 | 29 | 27 | 25 |
| SPT/AGT pathway (%) | ND (~0) | 43 (31) | 98 (52) | 192 (67) | 290 (77) |
| SHMT pathway (%) | 79 (~100) | 95 (69) | 92 (48) | 94 (33) | 86 (23) |

The frozen livers were crushed in a stainless steel pan, which had been chilled in liquid nitrogen. Then, the frozen powder was homogenized with 2.67 vol of 0.6 N perchloric acid. After neutralization of the acid extract with KOH, nonionic components were separated from charged ones by sequential passage through an AG 1 column (bed vol, 1 ml) and an AG 50W column of the same size. Glycine in the nonionic fraction was identified as the major radioactive compound by chromatography on a high performance thin layer chromatography plate (Silica Gel 60; Merck) with n-propanol and 2 N NH4OH (65:35, vol/vol) as the solvent, followed by autoradiography. For determination of the 15N/14C ratio, the radioactive glucose in the nonionic fraction was converted with hexokinase and ATP/Mg2+ to l-[1-14C]glucose 6-phosphate, followed by isolation of the latter by chromatography on an AG 1 column (bed vol, 1 ml) with 6 N formic acid as the eluting solvent. The eluate was evaporated in a counting vial, and then 15N and 14C were separately counted. In the perfusion experiment, lactate and pyruvate were isolated from another portion of the liver extract by partitioning chromatography on a silicic acid column, and their 15N and 14C radioactivities were determined as above.

RESULTS

Relative Contributions of Mitochondrial SHMT and SPT/AGT to the Metabolism of l-Serine—First, we tried to compare the quantities of l-serine metabolized by mitochondrial SPT/AGT and the mitochondrial isozyme of SHMT. Heavy mitochondria corresponding to 40 mg wet weight of 24-h starved or glucagon-treated rat liver were incubated with 5 mM l-[1-14C]serine at 37 °C for 60 min in the absence or presence of 0.25, 0.5, 1, or 2 mM pyruvate to measure the 14CO2 evolution. To determine hydroxypyruvate and glycine, parallel incubations with nonradioactive l-serine were run simultaneously. Preliminary experiments showed that this amount of the mitochondrial suspension was the maximum quantity, and 60 min was the maximum incubation time with which the hydroxypyruvate formation and 14CO2 evolution from l-[1,14C]glycine, l-[1-14C]serine, and l-[3,14C]serine proceeded almost linearly with respect to both time and the amount of mitochondria added. As shown in Table I, the accumulation of glycine was essentially unaffected by the glucagon administration and was independent of the presence of pyruvate. In contrast, the hydroxypyruvate formation was highly dependent on the addition of pyruvate and was enhanced 20–30-fold by the glucagon treatment, in good agreement with the known induction of mitochondrial SPT/AGT by glucagon (10). In experiments with l-[1-14C]serine, 14CO2 evolution can be ascribed to two routes: one further metabolism of [1,14C]glycine by GCS (14) and the other enzymatic or nonenzymatic decarboxylation of [1,14C]hydroxypyruvate (28, 29). In 24-h starved rats, hydroxypyruvate formation was hardly detectable unless >0.5 mM pyruvate was added, and the 14CO2 evolution was independent of the addition of pyruvate (Table I), probably because of the low levels of the pyruvate-dependent hydroxy-
pyruvate formation, suggesting that the $^{14}$CO$_2$ evolved represents decarboxylation via [1-$^{14}$C]glycine. Therefore, the hydroxypyruvate formation and the sum of glycine accumulation and $^{14}$CO$_2$ evolution were taken to represent the flux of l-serine metabolism through SPT/AGT and that through SHMT, respectively. In glucagon-treated rats, on the other hand, no hydroxypyruvate formation was detectable in the absence of pyruvate, but in its presence, not only the hydroxypyruvate formation but also the $^{14}$CO$_2$ evolution increased significantly (Table I). Because it is unlikely that pyruvate stimulates the flux through glycine only in glucagon-treated rats, we assumed that the pyruvate-dependent increase in the $^{14}$CO$_2$ evolution represents decarboxylation by way of hydroxypyruvate. Therefore, the flux through SHMT was calculated from the sum of accumulated glycine and $^{14}$CO$_2$ evolved in the absence of pyruvate. Likewise, the flux through SPT/AGT was calculated from the sum of hydroxypyruvate and the pyruvate-dependent increase in the $^{14}$CO$_2$ evolution.

The concentration of pyruvate in the reaction mixture decreased considerably, being 50–70 μM after 60 min of incubation, when its initial concentration was 0.25 mM. Because the reported hepatic concentration of pyruvate in the rat is in the range of 0.04–0.25 mM (20), the incubation in the presence of 0.25 mM pyruvate can be considered to be quasi-physiological with respect to the pyruvate concentration. As summarized in Table I, the mitochondrial metabolism of l-serine under these conditions as much as 80 nmol of serine were converted to glycine or metabolized by way of glycine (compare Table I), although mitochondria corresponding to 40 mg of liver may have contained only 0.15 nmol of the THF cofactor (32).

This suggested that 5,10-methylene-THF formed in mitochondria is converted in situ to free THF and CO$_2$ through the sequential actions of 5,10-methylene-THF dehydrogenase, 5,10-methenyl-THF cyclohydrolase, and 10-formyl-THF dehydrogenase (14). In contrast, the SHMT activity in the soluble fraction required the addition of the THF cofactor, and the production of 5,10-methylene-THF proceeded linearly with time only for ~10 min, although it was proportional to the amount of the soluble fraction up to 8 mg of liver equivalent in 0.1 ml of the reaction mixture. The activity of cSHMT determined in the linear range was fairly high and ~0.5 μmol/min per g of liver. On the other hand, SDH in the cytosol catalyzed the reaction almost linearly for up to 60 min (compare Table II), and when the concentration of l-serine was 5 mM, it showed a comparable activity with that of cSHMT.

**Effect of ATP on the $^{14}$CO$_2$ Evolution from l-[1-$^{14}$C]Serine in a Cytoplasmic Extract**—In agreement with Yoshida and Kikuchi (3, 14, 33), $^{14}$CO$_2$ production from l-[3-$^{14}$C]serine exceeded that from l-[1-$^{14}$C]serine when the radioactive serines were added to a mitochondrial suspension or homogenate at physiological concentrations. In homogenate or cytoplasmic extract containing the SDH activity, the decarboxylation from [1-$^{14}$C]pyruvate contributes to the $^{14}$CO$_2$ formation from l-[1-$^{14}$C]serine, in addition to that via [1-$^{14}$C]glycine and [1-$^{14}$C]hydroxypyruvate. In the present study, the $^{14}$CO$_2$ evolution from l-[1-$^{14}$C]serine with a reconstituted cytoplasmic extract (a mixture of a soluble fraction and a Mit-Ps suspension) was augmented as much as 5–8-fold in the presence of 4 mM ATP and 10 mM Mg$^{2+}$. This phenomenon was regarded as indicating that ATP/Mg$^{2+}$ enhances the decarboxylation from l-[1-$^{14}$C]serine via [1-$^{14}$C]pyruvate, based on the following observations (Table II): 1) in experiments involving a homogenate or reconstituted cytoplasmic extract, the decarboxylation from [1-$^{14}$C]pyruvate was augmented approximately 10-fold by ATP/Mg$^{2+}$, but that from [3-$^{14}$C]pyruvate was minimal regardless of the presence or absence of ATP/Mg$^{2+}$; 2) the addition of 2 mM nonradioactive pyruvate caused ~87% reduction of the ATP/Mg$^{2+}$-dependent increase in the $^{14}$CO$_2$ evolution from l-[1-$^{14}$C]serine, whereas the effect on the decarboxylation in the absence of ATP/Mg$^{2+}$ was at most 20%; and 3) when 5 mM l-serine was incubated with a reconstituted cytoplasmic extract, as much as 1.2 mM pyruvate was accumulated in the reaction mixture in 60 min, and the pyruvate accumulation was reduced to 0.2 mM in the presence of ATP/Mg$^{2+}$; and 4) when a Mit-Ps suspension was used in place of a cytoplasmic extract, addition of ATP/Mg$^{2+}$ rather inhibited slightly the l-[1-$^{14}$C]serine-derived $^{14}$CO$_2$ evolution. It was thus suggested that the supply of serine-derived pyruvate to mitochondria by SDH is much higher than formerly believed. The $^{14}$CO$_2$ evolution from

| Subcellular fractions | Pyruvate formation | $^{14}$CO$_2$ evolution |
|-----------------------|--------------------|------------------------|
|                       | 30 min             | 60 min                 | 30 min      | 60 min      |
|                       | nmol               | nmol                   | ngol       | ngol        |
| Sup alone             | 793                | 1312                   | –          | –           |
| Mit-Ps alone          | –                  | –                      | 11.0       | 21.5        |
| Mit-Ps + Sup          | 768                | 1212                   | 21.6       | 40.2        |
| Mit-Ps + Sup + ATP/Mg$^{2+}$ | 117 | 190                   | 108        | 345         |
| Anti-SDH-treated Sup  | –                  | 1029                   | –          | –           |
| Preimmune IgG-treated Sup | –                  | –                      | 10.2       | 20.7        |
| Mit-Ps + Anti-SDH-treated Sup | – | –                     | –          | –           |
| Mit-Ps + Preimmune IgG-treated Sup | – | –                     | 21.0       | 38.2        |
TABLE III

L-Serine metabolism in vitro in 24-h starved and glucagon-treated rat livers

Reactions were carried out at 37 °C for 60 min with an SDH-depleted reconstituted cytoplasmic extract (mixture of an anti-SDH-treated soluble fraction and a Mit-Ps suspension) or a soluble fraction (105,000 × g supernatant) corresponding to 40 mg of liver, as described under “Experimental Procedures.” Flux through SDH, SPT/AGT, and GCS was calculated as described in the text. The values represent nmol formed or metabolized/60 min, and those in parentheses represent percentages of contributions of individual pathways. ND, not detectable. The data are the representative of three independent experiments with similar results.

| L-Serine added initially (mM) | 0.5 | 1.0 | 2.5 | 5.0 |
|-----------------------------|-----|-----|-----|-----|
| 24-h starved Rat             |     |     |     |     |
| Pyruvate formation           | 72  | 144 | 458 | 1038|
| Hydroxypyruvate formation    | ND  | ND  | ND  | 1.2 |
| Glycine accumulated          | 32  | 49  | 84  | 114 |
| CO₂ evolution                |     |     |     |     |
| With 0.25 mM pyruvate        | 1.4 | 2.8 | 8.2 | 15.4|
| Without pyruvate             | 1.2 | 2.6 | 8.4 | 16.1|
| Flux through SDH (%)         | 72 (98) | 144 (98) | 458 (98) | 1038 (98) |
| Flux through SPT/AGT (%)     | ND (–0) | ND (–0) | ND (–0) | 1.2 (0.1) |
| Flux through GCS (%)         | 1.2 (2) | 2.6 (2) | 8.4 (2) | 16.1 (2) |
| Glucagon-treated Rat         |     |     |     |     |
| Pyruvate formation           | 88  | 220 | 603 | 1175|
| Hydroxypyruvate formation    | 5   | 17  | 36  | 47  |
| Glycine accumulated          | 33  | 47  | 87  | 116 |
| CO₂ evolution                |     |     |     |     |
| With 0.25 mM pyruvate        | 3.8 | 7.8 | 20.7| 34.5 |
| Without pyruvate             | 3.5 | 6.1 | 13.3| 23.3 |
| Flux through SDH (%)         | 88 (91) | 220 (89) | 603 (91) | 1175 (93) |
| Flux through SPT/AGT (%)     | 6 (6) | 19 (8) | 43 (7) | 58 (5) |
| Flux through GCS (%)         | 0 (4) | 6 (3) | 13 (2) | 29 (2) |

L-[3-14C]serine was independent of ATP/Mg²⁺ and was increased severalfold on the addition of 0.1 mM THF and 0.3 mM NADP⁺, but not with either one alone, as reported previously (14).

Depletion of the SDH Activity in the Soluble Fraction with an Anti-SDH Antibody—To eliminate the 14CO₂ evolution from L-[1-14C]serine via [1-14C]pyruvate and the unfavorable accumulation of pyruvate formed from serine in the reaction mixture with a reconstituted cytoplasmic extract, we attempted to deplete the SDH activity in the soluble fraction with an anti-SDH antibody. The soluble fraction corresponding to 40 mg of liver from both 24-h starved rats and glucagon-treated rats was found to contain 20–25 milliunits of the SDH activity when liver from both 24-h starved rats and glucagon-treated rats was assayed, and 14CO₂ formation from L-[1-14C]serine was much higher.

As summarized in Table III, the flux through SDH was shown to be far higher than those through SPT/AGT and GCS under every condition examined. As for the flux through SHMT-GCS, the incubation time was out of the linear range with respect to the cSHMT-catalyzed glycine formation from serine, but a large amount of glycine accumulation was observed, and 14CO₂ formation from L-[1-14C]serine in the absence of pyruvate (decarboxylation via glycine) proceeded almost linearly for 60 min (compare Table II). Under the conditions used, therefore, the formation of glycine from serine by cSHMT may have occurred fairly rapidly, followed by GCS-catalyzed gradual degradation of glycine. The contribution of the flux through GCS was relatively small compared with the flux through SDH, and that of the SPT/AGT pathway was perceptible only in the glucagon-treated group. Under the quasi-physiological conditions with 1 mM l-serine and 0.25 mM pyruvate (initial concentrations), the calculated contributions of the SDH and SPT/AGT pathways and the pathway via glycine to the metabolism of l-serine are 98, ~0, and 2%, respectively, in 24-h starved rats and 89, 8, and 2%, respectively, in the glucagon-treated rats.

The flux through GCS may be underestimated in this experiment, because the concentration of accumulated glycine reached to only 0.07–0.12 mM, whereas its concentration in rat liver in vivo was thought to be maintained at ~2.5 μmol/g (34) by active transport and net uptake from the bloodstream, in addition to the supply from serine. However, when 2.5 mM [1-14C]glycine was incubated with a mitochondrial suspension (a suspension of 8200 × g precipitate) corresponding to 40 mg of tissue, the amounts of 14CO₂ evolved were ~55 nmol/60 min for both 24-h starved rats and glucagon-treated rats. These values are less than half of the flux through SDH at 1 mM l-serine, and the calculated contributions of the flux through SDH, SPT/AGT and GCS under the supposed quasi-physiolog-
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vas transferase, respectively; mMDH was the malate dehydrogenase; pGOT was the phosphoenolpyruvate carboxykinase; and mlMDH was the mitochondrial malate dehydrogenase, respectively; from those at the 3 position of L-serine, respectively. (LDH, SuccDH, and cGOT, attributable to randomization at the succinate dehydrogenase step. Hydrogen at the 3 position is expected to be largely removed in mitochondria to the cytosol via the fumarase-catalyzed shuttling between malate and fumarate. Because malate thus formed in mitochondria as an intermediate is in rapid equilibrium with fumarate through the fumarase reaction, hydrogen derived from the 3 position of L-serine is thought to be retained throughout the gluconeogenic reactions in either pathway. On the other hand, the hydrogen at the 3 position is expected to be largely removed in the gluconeogenesis via pyruvate, whereas it is mostly retained in that via hydroxypyruvate. It is known that in rat liver and kidney, in which phosphoenolpyruvate carboxykinase is located predominately in the cytosol (35), gluconeogenesis from three-carbon precursors such as pyruvate, lactate, and alanine is associated with the transfer of oxaloacetate from mitochondria to the cytosol in the form of malate or aspartate (36). Because malate thus formed in mitochondria as an intermediate is in rapid equilibrium with fumarate through the fumarase reaction, hydrogen derived from the 3 position of L-serine is expected to be largely removed through exchange with water during the shuttling between malate and fumarate. In addition, in preparation of L-\([3-3H,14C]\)lactate and L-\([3-3H,14C]\)pyruvate, the \(^{3}H/^{14}C\) ratio of glucose formed was a little lower than that of the substrate, but this was not surprising, because we found in the perfusion experiment that radioactive pyruvate and lactate were formed from D-\([3-3H,14C]\)glycerate, in addition to glucose, and their \(^{3}H/^{14}C\) ratios were much lower than that of glucose. It is therefore conceivable that even under the gluconeogenic conditions, a portion of the 2-phosphoglycerate formed from D-glycerate is metabolized to pyruvate and then converted back to glucose, lowering the \(^{3}H/^{14}C\) ratio of the latter.

As shown in Table IV, these assumptions were proved to be approximately the case, by showing that \(^{3}H\) was almost lost on gluconeogenesis from L-\([3-3H,14C]\)lactate or L-\([3-3H,14C]\)pyruvate in both infusion and perfusion experiments. In the case of gluconeogenesis from D-\([3-3H,14C]\)glycerate, the \(^{3}H/^{14}C\) ratio of glucose formed was a little lower than that of the substrate, but this was not surprising, because we found in the perfusion experiment that radioactive pyruvate and lactate were formed from D-\([3-3H,14C]\)glycerate, in addition to glucose, and their \(^{3}H/^{14}C\) ratios were much lower than that of glucose. It is therefore conceivable that even under the gluconeogenic conditions, a portion of the 2-phosphoglycerate formed from D-glycerate is metabolized to pyruvate and then converted back to glucose, lowering the \(^{3}H/^{14}C\) ratio of the latter. In both the infusion and perfusion experiments, \(^{3}H\) was also lost on gluconeogenesis from L-\([3-3H,14C]\)serine, and the \(^{3}H/^{14}C\) ratio of glucose formed was between those in the cases of L-\([3-3H,14C]\)lactate or L-\([3-3H,14C]\)pyruvate and D-\([3-3H,14C]\)glycerate, suggesting that both the SDH and SPT/AGT pathways participate in the gluconeogenesis from L-serine. From proportional allotment of the \(^{3}H/^{14}C\) ratios of glucose obtained with L-\([3-3H,14C]\)serine between the two control values (those obtained with L-\([3-3H,14C]\)lactate or L-\([3-3H,14C]\)pyruvate and D-\([3-3H,14C]\)glycerate), the SPT/AGT pathway was shown to account for only a trace in the liver of 48-h starved rat, and in the infusion experiment its contribution in glucagon-treated rat was estimated to be \(-10\%\). In the perfusion experiment, gluconeogenesis from L-\([3-3H,14C]\)serine as well as that from lactate, pyruvate, and L-alanine was augmented 1.6–1.8-fold by the *in situ* addition of 30 mM glucagon in both 48-h starved rats and glucagon-treated rats. It was also observed that the \(^{3}H/^{14}C\) ratio of glucose formed from L-\([3-3H,14C]\)serine was lower in the *in situ* presence of glucagon than in its absence, probably reflecting the known stimulation by glucagon of gluconeogenesis from or via pyruvate (37). In the presence and absence of *in situ* glucagon the contributions of the SPT/AGT pathway in glucagon-treated rats were also estimated to be \(-10\%\) and 20\%, respectively. All these results suggested the predominant contribution of the SDH pathway *in vivo* and in the perfused liver.

**DISCUSSION**

SHMT, SDH, and SPT/AGT are known to be the three major enzymes involved in the hepatic metabolism of L-serine, but the situation of SHMT appears to be different from those of the latter two enzymes. Because both serine and glycine are physiologically important amino acids that are used in a variety of ways in the cells, their intracellular concentration should be maintained in a given range, and cSHMT and mSHMT to-
Together with GCS are thought to play crucial roles in the interconversion between serine and glycine. The equilibrium of the cSHMT-catalyzed reaction appears to lie far on the side of the glycine formation in vivo, and when glycine is to be converted to serine such as in the case of gluconeogenesis from glycine, this conversion may be accomplished mainly by mSHMT in conjugation with GCS, which supplies 5,10-methylene-THF to mSHMT at the expense of another molecule of glycine. The intracellular partitioning of the serine and glycine interconversion in the liver was suggested by the observation by Yoshida and Kikuchi (38) that in a congenital hyperglycinemia patient deficient in GCS, the level of blood serine was in the normal range, whereas those of glycine in the blood and urine were elevated far above the normal. It was also reported that decarboxylation of [1-14C]glycine by liver mitochondrial suspensions or homogenate is accompanied by the formation of nearly an equimolar quantity of [14C]serine (3). Considering a role of GCS to be the disposal of glycine and serine in addition to its role in supplying C1 unit-substituted THF derivatives in mitochondria, the roles of SPT/AGT and SDH as the metabolic exits of serine and glycine are analogous to that of GCS rather than SHMT. Indeed, we observed in experiments with an SDH-depleted reconstituted cytoplasmic extract that the conversion of L-serine to glycine occurred fairly rapidly, in part owing to the low Km of cSHMT for L-serine, followed by the GCS-catalyzed slow decarboxylation of [1-14C]glycine formed.

When the flux of the serine and glycine metabolism through SDH, SPT/AGT, and GCS was compared in rat liver, the flux through SDH was shown to be the largest, contrary to the general understanding that SDH may not play a substantial role because of its very high Km for its substrate. In vitro experiments, the amount of L-serine metabolized through the quasi-physiological conditions with 1 mM L-serine and 0.25 mM glycine formation synthase in conjugation with the release of CoASH. The NAD+ dehydrogenase activity, allowing the efficient decarboxylation of pyruvate to acetyl-CoA, which in turn activates carboxylation of pyruvate to oxaloacetate in addition to its oxidation to acetyl-CoA. A portion of the oxaloacetate thus formed is converted to malate by malate dehydrogenase with the concomitant oxidation of NADH to NAD+, and another portion is converted to citrate by citrate synthase in conjugation with the release of CoASH. The NAD+ and CoASH thus formed may then enhance the pyruvate dehydrogenase activity, allowing the efficient decarboxylation of pyruvate to acetyl-CoA, which in turn activates carboxylation of pyruvate to oxaloacetate. In support of this view, Pande and Parvin (50) observed that the α-cyanoacinnamate-sensitive uptake of pyruvate into mitochondria requires concurrent oxidation of pyruvate, and they also believe that the CoASH and NAD+ present in mitochondria are able to support the oxidation of pyruvate.

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