L-Serine metabolism in rabbit, dog, and human livers was investigated, focusing on the relative contributions of the three pathways, one initiated by serine dehydratase, another by serine:pyruvate/alanine:glyoxylate aminotransferase (SPT/AGT), and the other involving serine hydroxymethyltransferase and the mitochondrial glycine cleavage enzyme system (GCS). Under quasi-physiological in vitro conditions (1 mM L-serine and 0.25 mM pyruvate), flux through serine dehydratase accounted for only traces, and that through SPT/AGT substantially contributed no matter whether the enzyme was located in peroxisomes (rabbit and human) or largely in mitochondria (dog). As for flux through serine hydroxymethyltransferase and GCS, the conversion of serine to glycine occurred fairly rapidly, followed by GCS-mediated slow decarboxylation of the accumulated glycine. The flux through GCS was relatively high in the dog and low in the rabbit, and only in the dog was it comparable with that through SPT/AGT. An in vivo experiment with L-[3-3H,14C]serine as the substrate indicated that in rabbit liver, gluconeogenesis from L-serine proceeds mainly via hydroxypyruvate. Because an important role in the conversion of glyoxylate to glycine has been assigned to peroxisomal SPT/AGT from the studies on primary hyperoxaluria type 1, these results suggest that SPT/AGT in this organelle plays dual roles in the metabolism of glyoxylate and serine.

Among the three major enzymes involved in the metabolism of L-serine in mammalian liver, L-serine dehydratase (SDH),1 serine:pyruvate/alanine:glyoxylate aminotransferase (SPT/AGT), and serine hydroxymethyltransferase (SHMT), the former two are thought to participate in gluconeogenesis from L-serine (1). Notable features of SPT/AGT are that this enzyme is located entirely in peroxisomes in herbivores and humans, largely in mitochondria in carnivores (2–4), and in both organelles in rodents such as rat, and in the rat only the mitochondrial enzyme is induced by glucagon (5). It has been generally accepted from the known overproduction of oxalate in primary hyperoxaluria type 1, an inborn error of glyoxylate metabolism caused by a functional deficiency of peroxisomal SPT/AGT (6), that the enzyme in this organelle plays an important role in the conversion of glyoxylate to glycine, but the role of mitochondrial and peroxisomal SPT/AGT in the serine metabolism has not been elucidated. Cytosolic and mitochondrial isoforms of SHMT (cSHMT and mSHMT, respectively) have been shown to catalyze the interconversion between serine and glycine in conjugation with mitochondrial glycine cleavage enzyme system (GCS). This interconversion occurs especially when there is need for C1-substituted tetrahydrofolate cofactors or when either one of these amino acids are used or supplied (1). In the preceding paper (7), we considered SDH, SPT/AGT, and GCS to be the metabolic exits of the serine–glycine pool and showed that SDH is the major enzyme in the metabolism of L-serine in rat liver. The flux through SPT/AGT was enhanced by glucagon administration, but even after the induction, its contribution was about ¼ of that through SDH both in vitro and in vivo. The flux through GCS was comparable with that through SPT/AGT in glucagon-treated rats (7). However, this pattern of L-serine metabolism in rat liver may not be extrapolated to other animals, because the SDH activity is known to decrease drastically as the body size of animals increases (8). Although the results obtained with glucagon-treated rats suggested that mitochondrial SPT/AGT is involved in the metabolism of L-serine, whether its contribution can be substantial and whether the enzyme in peroxisomes plays dual roles in the metabolism of glyoxylate and serine remain obscure. This paper deals with the L-serine metabolism in rabbit, dog, and human livers. Dog and rabbit were chosen as representatives of carnivores and herbivores, respectively.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sources of all reagents and male Wistar rats are described in the preceding paper (7). Japanese white male rabbits weighing ~2 kg were obtained from a local dealer. Small pieces of human livers were obtained at the time of surgical operation (hepatic left lobectomy) on three patients who suffered from biliary tract carcinoma or liver metastasis of a sarcoma. Use of the liver samples from human subjects in this study was permitted by the Ethical Committee of Hamamatsu University School of Medicine, and the patients consented to use of the resected specimens. Small pieces of human liver specimens were also obtained on autopsy ~5 h postmortem. Small pieces of dog livers were provided when a research group headed by Dr. Kazuya Suzuki (First Department of Surgery, Hamamatsu University School of Medicine) performed an animal experiment according to the Guidelines for Animal Experimentation of Hamamatsu University School of Medicine.
The SPT activity of SPT/AGT in rat, rabbit, and dog livers was determined using the cytoplasmic extract (650 × g supernatant). For determination of the SPT activity in human liver, small pieces of liver specimens obtained on autopsy 5 h postmortem were homogenized, and the soluble supernatants from sonicated homogenates were subjected to the assay. The activities of SDH and cSHMT were determined with the soluble fraction, and that of mSHMT was determined using the 105,000 × g supernatant from the sonicated mitochondrial fraction. The activities are expressed as μmol/min per g of liver and are averages ± S.D. Values in parentheses indicate the number of independent determinations.

### Table I

|                     | SPT SDH  | cSHMT mSHMT |
|---------------------|----------|-------------|
| 24-h starved rat    | 0.06 (2) | 3.50 ± 1.12 (6) | 0.54 (2) | 0.05 (2) |
| Glucagon-treated rat| 0.66 ± 0.37 (3) | 4.30 (2) | 0.52 (2) | 0.05 (2) |
| Rabbit              | 1.23 ± 0.79 (4) | 0.22 ± 0.08 (3) | 2.91 ± 0.53 (3) | 0.20 ± 0.10 (3) |
| Dog                 | 3.21 ± 1.13 (3) | 0.09 ± 0.003 (3) | 1.15 (2) | 0.16 (2) |
| Human               | 2.73 ± 0.85 (4) | 0.06 ± 0.003 (3) | 0.84 ± 0.30 (3) | 0.15 (2) |

Assay of Enzymes—The SDH activity was measured by either the direct spectrophotometric method used in the preceding paper (7) or the method described by Ishikawa et al. (9) with modifications. In the latter method, the reaction was carried out at a physiological pH, 7.5, in the presence of NADH and lactate dehydrogenase, and the amount of lactate produced was determined. The serine:pyruvate aminotransferase activity of SPT/AGT (10) and the SHMT activity (7, 11) were determined as described in the papers cited. For the assay of mSHMT, mitochondria were sonicated in 0.125M sucrose, pH 7.4, containing 1.5 mM imidazole and HCl and 0.05 mM EDTA, and after centrifugation at 105,000 × g for 60 min, the supernatant was used.

Procedures for in Vitro Experiments—Cytoplasmic extracts, Mit- Ps suspensions, soluble fractions, and reconstituted cytoplasmic extracts were prepared from rabbit, human, dog, and rat livers as described (7). To obtain a mitochondrial suspension, a portion of the cytoplasmic extract (650 × g supernatant) was centrifuged at 8200 × g for 10 min, and the pellet was washed once and suspended in the homogenizing sucrose solution (0.25 M sucrose, 3 mM imidazole and HCl, and 0.1 mM EDTA, pH 7.4) to give a 2.5 ml suspension/g of original liver.

The reactions for the l-serine metabolism and the decarboxylation from [1-14C]glycine in vitro were carried out under the same conditions as those described in the preceding paper (7). Glycine, hydroxypro- pyruvate, and lactate from nonradioactive l-serine and 14CO2 formed from l-[1-14C]serine or l-[1-14C]glycine were determined as described (7).

Procedures for the Infusion Experiment with Rabbits—Doubly labeled substrates, l-[3-3H,14C]glycine (H/D ratio, 11.41 μCi/mM; 14C, 1.17 μCi/mM), 0.1 mM l-[3-14C]lactate (2.5 μCi/mM; 14C, 1.19 μCi/mM), or 1 mM d-[3,4-3H,14C]glucerase (H/D ratio, 9.95 μCi/mM; 14C, 1.14 μCi/mM) was infused into the portal veins of separate rabbits continuously for 15 min at a rate of 4 ml/15 min, and after termination of the infusion, the rabbits were allowed to metabolize the infused substrates for another 5 min as described for the infusion into rats. Then the livers were isolated and immersed in liquid nitrogen immediately. Radioactive glucose was isolated from the frozen livers, and its H and 14C radioactivities were differentially counted as described (7).

### RESULTS

Activities of l-Serine Metabolizing Enzymes in Rabbit, Human, and Dog Livers—In Table I, the activities of SPT/AGT, SDH, and SHMT in rabbit, dog, and human livers are compared with those in 24-h starved and glucagon-treated rat livers. In confirmation of the results of Rowsell et al. (8), the SDH activity in the cytosol in rat liver was 1 order of magnitude lower than that in rat liver, and in dog and human livers it was a barely detectable level. As for SPT/AGT, on the other hand, its serine:pyruvate aminotransferase activity in rabbit liver was approximately twice the glucagon-induced level in the rat, and still higher activities were determined in human and dog livers. The SHMT activity was largely recovered in the soluble fraction, 8–15% of the total activity being...
detected in the mitochondrial fraction.

The evolution of $^{14}$CO$_2$ from [1-14C]glycine determined as a measure of the GCS activity (12, 13) was the highest in dog and rat livers, followed by that in human liver (Fig. 1). In rabbit liver containing the highest cSHMT and mSHMT activities, the activity to decarboxylate [1-14C]glycine was the lowest. These results altogether suggested that the flux of l-serine metabolism in rabbit, dog, and human livers is quite different from that in rat liver described in the preceding paper (7).

**Flux of the l-Serine Metabolism through SPT/AGT in a Mit-Ps Suspension**—It was not possible to deplete the SDH activity in rabbit liver soluble fraction with an anti-rat SDH rabbit antibody. The SDH activity was fairly low in rabbit, human, and dog livers (Table I), but to avoid possible in situ generation of a small amount of pyruvate from l-serine, we decided to use the Mit-Ps suspension (25,000 × g precipitate containing both mitochondria and peroxisomes) rather than the cytoplasmic extract (650 × g supernatant) for measurement of the flux through SPT/AGT. SPT/AGT is known to be located largely in mitochondria in dog liver and exclusively in peroxisomes in human and rabbit livers (2–4), although the peroxosomal SPT/AGT leaks out to some extent during the preparation of the Mit-Ps suspension.

To determine the effect of pyruvate concentrations on the flux through SPT/AGT in a Mit-Ps suspension, the reactions with 5 mM l-serine and those with 5 mM l-[1-14C]serine as the substrates were simultaneously carried out in the absence or presence of various concentrations of pyruvate, and the flux through SPT/AGT was determined as the sum of the hydroxypyruvate accumulated and the pyruvate-dependent increase in the $^{14}$CO$_2$ evolution, as in the experiment with rat liver (7). As shown in Fig. 2, the flux through SPT/AGT versus the pyruvate concentration curve was hyperbolic with rabbit and human liver Mit-Ps preparations containing SPT/AGT in peroxisomes. When dog liver Mit-Ps suspensions containing SPT/AGT in mitochondria were used, on the other hand, the curve was sigmoidal. This is probably because pyruvate enters mitochondria largely by diffusion at its high concentrations, but when the concentration is low, the entry occurs by a carrier-mediated process, which appears to be limited by the pyruvate use, as indicated in the case of rat liver mitochondria (14). As a result, the amount of hydroxypyruvate formed with dog liver Mit-Ps suspensions in the presence of quasi-physiological 0.25 mM pyruvate was only ~8% of that in the presence of 2 mM pyruvate and was less than that formed with human and rabbit liver Mit-Ps preparations under the same conditions, although dog liver contained a higher activity of SPT/AGT than human and rabbit livers (compare Table I).

**Relative Contributions of the Three Pathways to the Metabolism of l-Serine in Vitro in Rabbit, Dog, and Human Livers**—The flux through SPT/AGT was determined with a Mit-Ps suspension as described above, and that through SDH was evaluated by the amount of pyruvate formed from l-serine with a soluble fraction as in the case of rat liver (7). For determination of the amount of l-serine metabolized via glycine, a reconstituted cytoplasmic extract (a mixture of a Mit-Ps suspension and a soluble fraction) was incubated with l-[1-14C]serine in the presence of 0.1 mM tetrahydrofolate and 0.3 mM NADP$^+$ and in the absence of pyruvate, and the $^{14}$CO$_2$ evolved was determined. Pyruvate was omitted from the reaction mixture as in our previous observation (7) that the $^{14}$CO$_2$ evolution by way of glycine was independent of the presence of pyruvate, and in this experiment little pyruvate was accumulated even when l-serine was incubated with the soluble fraction, as described below. All reactions were carried out at 37 °C for 60 min with the subcellular preparations corresponding to 40 mg of liver.

It had been observed in other experiments that the reaction catalyzed by SHMT in the soluble fractions from rabbit, human, and dog livers proceeded linearly only for 5–6 min, and the activity was proportional to the enzyme concentration only when the soluble fractions corresponding to <10–15 mg of liver were used. Therefore, the amount of reconstituted cytoplasmic extracts and the incubation time used in this experiment were out of the linear range with respect to the cSHMT-catalyzed glycine formation from l-serine. Nevertheless, a large amount of glycine accumulation was observed, irrespective of rabbit, human, or dog liver, after the 60-min incubation (Table II). On the other hand, the $^{14}$CO$_2$ evolution from l-[1-14C]serine via glycine represented by the decarboxylation in the absence of pyruvate was very small in rabbit liver, and even in human and dog livers having higher activities of GCS, it accounted for only 4–15 and 20–30% of accumulated glycine, respectively. The in vitro conditions used for serine metabolism may be out of physiological range with respect to the glycine metabolism, because glycine accumulated in the reaction mixture after the 60-min incubation was at most 350 μg, whereas the glycine concentrations in animal livers have been believed to be on the order of several μmol/g (15, 16). However, the flux through GCS measured by the mitochondrial decarboxylation from 2.5 mM [1-14C]glycine was no more than 3–4-fold of those determined
as the flux through GCS in the metabolism of 1 mM L-serine (compare Fig. 1 and Table II). It appears that, as in the case of rat liver, cSHMT catalyzes fairly rapid conversion of serine to glycine, and the rate of the metabolism of L-serine to CO₂ by way of glycine is limited at the second step involving GCS under the in vitro conditions used. In fact, the flux through the GCS pathway in the three different animal species was in accordance with the activity of mitochondria to decarboxylate [1-14C]glycine, which was the highest in dog liver, followed by human and rabbit livers (Fig. 1).

It is noteworthy that the formation of hydroxypyruvate was evident no matter whether SPT/AGT is located largely in mitochondria (dog) or entirely in peroxisomes (human and rabbit). If one assumes that the physiological concentrations of L-serine and pyruvate in these animal livers were −1 and <0.25 mM, respectively, the relative contributions of the SPT/AGT pathway and that involving SHMT and GCS are calculated to be 96:4 in rabbit liver, 88:12 in human liver, and 57:43 in dog liver.

Unlike the case of rat liver, pyruvate accumulated by the 60-min incubation of the soluble fraction from rabbit liver with 0.5–5 mM L-serine was on a barely detectable level or sometimes too low to be determined with the lactate dehydrogenase method. In the case of dog and human livers, no accumulation of pyruvate was detectable, suggesting that the contribution of the SDH pathway is only traces in the livers of these animals. It is possible that when the SDH activity is very low, pyruvate accumulated during the 60-min incubation of L-serine with the soluble fraction does not necessarily represent the net pyruvate formation. For example, because the liver contains glutamate and a high activity of soluble alanine aminotransferase, a significant portion of the small amount of pyruvate formed could be converted to alanine. However, even when the expected amounts of pyruvate formation under the quasi-physiological conditions (1 mM L-serine) are calculated from the hepatic SDH activity (Table I) and its $K_m$ for L-serine according to the Michaelis-Menten equation, the flux through the SDH pathway is assessed to be less than that through GCS in human and dog livers, and only in rabbit liver could its calculated contribution be severalfold more than that through GCS.

Relative Contributions of SDH and SPT/AGT to Gluconeogenesis from L-Serine in Vivo in Rabbit Liver—To quantify the relative contributions of the SDH and SPT/AGT pathways to gluconeogenesis in rabbit liver in vivo, L-[3-3H,14C]serine, L-[3-3H,14C]glycerate, or D-[3-3H,14C]glycerate was infused into the livers of separate rabbits, and then the radioactive glucose formed was isolated, and its $^{3}H/^{14}C$ ratio was determined, according to the principle described in the preceding paper (7).

As in the case of rat liver, $^{3}H$ was almost lost on gluconeogenesis from L-[3-3H,14C]serine, and only in rabbit liver could its calculated contribution be severalfold more than that through GCS.
Such species-specific patterns of the L-serine metabolism approximately agree with the observed activities of serine-metabolizing enzymes in respective animals (Table I). Our results are compatible with the observation by Rowseil et al. (8) that in rabbit and dog livers the SPT/AGT activity is more than six times higher, and the SDH activity is much lower than the respective activities in rat liver.

It is noteworthy that SPT/AGT is involved in the L-serine metabolism, no matter whether the enzyme is largely located in mitochondria (dog liver) or entirely in peroxisomes (rabbit and human livers). It has been expected that mitochondrial SPT/AGT plays a role in L-serine metabolism, because in the rat, the flux through SPT/AGT in gluconeogenesis from L-serine was apparent only when liver mitochondrial SPT/AGT had been induced by glucagon (7). This was corroborated in the present study by demonstrating in in vitro experiments under quasi-physiological conditions that the flux through SPT/AGT accounts for a large portion of L-serine metabolized in dog liver. These results are in accordance with the observation of Revello and Freedland (17) that serine is mainly metabolized by transamination in hepatocytes isolated from the cat, another carnivore. As for the peroxisomal SPT/AGT, on the other hand, an important role in the conversion of glyoxylate to glycine has been indicated from the studies on primary hyperoxaluria type 1 (6). In the present study, the peroxisomal SPT/AGT was also shown to participate in L-serine metabolism in vitro (Table II). In addition, in the case of rabbit liver, an infusion experiment with L-[3-³H,¹⁴C]serine suggested that the flux through SPT/AGT contributes to gluconeogenesis from L-serine in vivo and accounts for as much as ~90% of it (Table III). It is evident that peroxisomal SPT/AGT plays dual roles in the metabolism of serine and glyoxylate. It is possible, although it has not yet been experimentally proved, that mitochondrial SPT/AGT also plays a role in the metabolism of glyoxylate. L-Hydroxyproline has been shown to be metabolized to glyoxylate in mitochondria (or peroxisomes, 18,000 x g precipitate; Ref. 18), and glyoxylate thus formed was proposed to be converted to glycine by mitochondrial SPT/AGT, although the quantities of the hydroxyproline-derived glyoxylate must be small (1, 19). SPT/AGT is thus a unique enzyme of dual organelle localization, and it is possible that the enzyme in either organelle plays dual roles, gluconeogenesis from serine and conversion of glyoxylate to glycine by transamination. Such dual functions probably come from the fact that serine:glyoxylate aminotransferase (EC 2.6.1.45), the plant counterpart of animal SPT/AGT (EC 2.6.1.44/2.6.1.51), catalyzes transamination between serine and glyoxylate in the photorespiratory nitrogen cycle (20), forming hydroxypyruvate and glycine, the latter being converted back to serine by a mitochondrial enzyme system. It is unlikely that glyoxylate is supplied in the animal livers as abundantly as in the photorespiration in plant leaves. Therefore, the hepatic serine metabolism catalyzed by SPT/AGT may not be necessarily coupled with the glyoxylate-to-glycine conversion, and more versatile pyruvate may also be used as an amino acceptor from serine, although the affinity of the enzyme for glyoxylate is much higher than that for pyruvate, as demonstrated by much lower Kₘ for glyoxylate (10 μM) than that for pyruvate (480 μM) of the rat liver enzyme at pH 7.4 (21).

Because plant tissues contain significant amounts of glycolate (22), and the conversion of glycolate to glyoxylate takes place in liver peroxisomes, the peroxisomal location of SPT/AGT may be favorable for herbivores to convert the glycolate-derived glyoxylate to glycine and to prevent harmful overproduction of oxalate. For carnivores, on the other hand, there may be less need of the peroxisomal location of SPT/AGT, because meats contain much less glycolate (22). With respect to its role in the disposal of serine demonstrated in this study, SPT/AGT in either of the two organelles appears to function with similar efficiency. Thus the necessity and advantages to carnivores of having SPT/AGT in mitochondria are not yet fully understood and need further studies.
As for the metabolism of L-serine via glycine, the overall flux observed with reconstituted cytoplasmic extracts from rabbit, human, and dog livers was similar to that in the case of rat liver. As proposed in the preceding paper (7), the SHMT-catalyzed interconversion between serine and glycine may contribute, at least in part, to the maintaining of their intracellular concentration balance, in response to supply or use of these amino acids and tetrahydrofolate coenzymes, and the degradation of glycine by GCS may be considered as a metabolic exit of not only glycine but also L-serine. This view was further supported in the present study by showing that, irrespective of the animal species tested, the conversion of serine to glycine catalyzed by cSHMT occurred fairly rapidly, followed by the GCS-mediated slow decarboxylation of the accumulated glycine. However, the flux through GCS as well as that through SDH and SPT/AGT varied considerably from animal to animal. In dog liver in which the activity of GCS was relatively small, consistent with the previous observation by Felig et al. (23) that serine, as well as glycine, is less effective than alanine as a precursor for hepatic gluconeogenesis in humans.

Summarizing all the data and discussions presented in this paper together with those in the preceding one (7), we believe that SDH, SPT/AGT, cSHMT, mSHMT, and GCS work as schematically shown in Fig. 3 in the hepatic metabolism of L-serine and glycine in rats, rabbits, humans, and dogs.

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