The 7- to 10-fold increase in the rat liver serine:pyruvate aminotransferase activity after glucagon administration was shown to occur mainly in the mitochondrial matrix of parenchymal cells. The enzyme was purified from glucagon-treated rat liver mitochondria to apparent homogeneity as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A specific rabbit antibody was prepared against the purified enzyme. Upon Ouchterlony double diffusion analysis, the mitochondrial extracts of glucagon-treated rat liver produced a single and fused precipitin line between the purified enzyme against the antibody. The supernatant fraction of glucagon-treated rat liver and the mitochondrial extracts of normal liver were also shown to make a single and fused precipitin line with the purified enzyme, when applied in large amounts. The quantitative immunotitration demonstrated that the glucagon-induced increase in the activity of liver serine:pyruvate aminotransferase were accompanied by the parallel increase in the amount of the enzyme antigen. Isotopic leucine incorporation studies showed that the relative rate of synthesis of the enzyme was increased approximately 10-fold by glucagon administration under the conditions employed. The rate of the degradation of the aminotransferase in the normal rat liver was a relatively slow process with a half-life of approximately 30 h. Thus the accumulation of serine:pyruvate aminotransferase in rat liver mitochondria by glucagon treatment can be ascribed mainly to the rise in the rate of enzyme synthesis.

It has been generally accepted that serine dehydratase (EC 4.2.1.13), which catalyzes the formation of pyruvate from serine, is involved in initiating gluconeogenesis from serine in fasted adult rat liver (1-4). In 1969 to 1970, however, the operation of an alternative pathway has been suggested in which serine is first converted to hydroxypyruvate by the action of serine:pyruvate aminotransferase (EC 2.6.1.51) and then to 2-phosphoglycerate by way of d-glycerate. This enzyme sequence was proposed by Lardy et al. (5) and Vonesh et al. (6) to account for gluconeogenesis from serine in perfused livers of fasted adult rat in which the normal pathway via pyruvate was blocked by the use of quinolinate, an inhibitor of phosphoenolpyruvate carboxykinase (7). Rowsell et al. (8, 9) observed high levels of serine:pyruvate aminotransferase activity in livers of suckling neonatal rat and adult rat injected with glucagon suggesting that the alternative pathway may play a significant role under those conditions. Sallach et al. (10) and Hoshino et al. (11) reported that dibutyryl cAMP mimicks the effect of glucagon in increasing the serine:pyruvate aminotransferase activity in rat liver. The cAMP-mediated increase of the enzyme activity was shown to be prevented by treatments with cycloheximide or actinomycin D (11), suggesting the participation of protein synthesis. A predominantly mitochondrial location of the aminotransferase has been suggested by Rowsell et al. (12), Snell (13), and Noguchi et al. (14).

A great deal more information may be needed, however, before the role of this enzyme in serine gluconeogenesis is appreciated since the involvement of the alternative pathway via hydroxypyruvate has been the subject of controversy. The role of this pathway in fasted adult rat liver has been supported by Metz et al. (15) but questioned by Chan and Freedland (16), Sandoval and Sols (17), and Bhatia et al. (18).

The present study was undertaken to determine whether the enzyme exists in mitochondria of parenchymal cells, whether the increased level of this enzyme activity after glucagon administration is actually brought about by changing quantities of enzyme protein, and, if so, whether the increase is due to variation in the rate of enzyme synthesis.

**EXPERIMENTAL PROCEDURES**

**Materials** — Commercial sources of substrates, reagents, and materials were as follows: spinach glyoxylate reductase (EC 1.1.1.26), hog kidney d-amino acid oxidase (EC 1.4.3.3), yeast alcohol dehydrogenase (EC 1.1.1.1), soy bean trypsin inhibitor, NAD, NADH, ADP, glucose-6-phosphate, fructose-1,6-diphosphate, phosphoenolpyruvate, and pyridoxal 5-phosphate from Boehringer; bovine testis hyaluronidase (EC 3.2.1.35), glucagon, bovine serum albumin, ATP, NNN, hydroxypyruvate, and p-isocitratetrazolium violet from Sigma; Clostridium collagenase (EC 3.4.4.19) from Worthington, pronase of Streptomyces griseus from Kaken Chemical Co. Ltd., (Tokyo); DEAE-cellulose from Brown; Sephadex G-150 from Pharmacia; Freund's complete adjuvant and noble agar from DIFCO; L-14,5-3H]leucine (specific activity, 55 Ci/mmol) from the Radiochemical Centre (Amersham, England); Wistar rats from Shizuoka Labo-
Induction of Serine:Pyruvate Aminotransferase

1188

Treatment of Animals—Male Wistar rats (150 to 200 g) maintained at diet and water ad libitum were fasted except for the isotope incorporation studies and for the measurements of time course of the induction in which rats of approximately 100 g were used. Rats were injected intraperitoneally with 0.25 mg/100 g body weight of glucagon in 5 mM NaOH and then immediately subjected to fasting. Unless otherwise stated, the glucagon-treated rats were killed 24 h after the single injection. Control rats were treated in the same manner except that 5 mM NaOH was injected instead of glucagon solution.

Isolation of Liver Cells—Parenchymal and nonparenchymal cells were prepared from normal and glucagon-treated rat livers by the method of Brem and Friend (20) with modifications according to Claus et al. (21) and that of Mills and Zucker-Franklin (22), respectively. Microscopic observations revealed that the preparations of parenchymal and nonparenchymal cells were only very slightly contaminated by each other, red blood cells and cell debris. The absence of cytoplasmic staining by trypan-blue indicated that more than 90 to 95% of both types of cells were intact. Cells were finally suspended in 10 volumes of 0.25 M sucrose containing 5 mM Tris/Cl (pH 7.5) at 0° and homogenized by the use of a Potter-Elvehjem type Teflon-glass homogenizer until no intact cells were seen by light microscopy. These homogenates were centrifuged with 0.85% NaCl solution. The biochemical purity of both types of isolated cells was checked by the activities of peroxidase (23), catalase (23), and pyruvate kinase in the presence and absence of fructose 1,6-diphosphate (24).

Subcellular Fractionation of Rat Liver—Rat livers removed immediately after decapitation were minced into small pieces and homogenized in mannitol/sucrose solution (0.21 mM mannitol, 0.07 M sucrose, 0.1 mM EDTA, and 5 mM Tris/Cl, pH 7.5) or 0.24 mM sucrose (pH 7.0) in a Potter-Elvehjem type Teflon-glass homogenizer. Subsequent fractionation procedures were carried out at 0° essentially according to the method of du Veugel and Kervina (27).

The mitochondrial fraction obtained by the method of Fleischer and Kervina was suspended in mannitol/sucrose solution, incubated at 0° overnight and subjected to a sucrose density gradient centrifugation. The mitochondrial and nonmitochondrial fractions were combined and subjected to ammonium sulfate fractionation, and the protein precipitated between 0 and 42% saturation was dialyzed against 0.85% NaCl for 24 h and centrifuged.

Ouchterlony double diffusion analysis, immunoelectrophoresis, and equilibrium dialysis were performed by the methods of Ouchterlony (39), Scheidegger (40), and Kebat and Mayer (41), respectively.

Isotope Incorporation Studies—[3H]-labeled L-leucine (1 mM, 18.2 nmol) was diluted to 1.0 ml in 0.85% NaCl and a 0.25-ml aliquot was administered intraperitoneally to each rat. Rats were killed and the livers were removed, homogenized, and fractionated as described above. Soluble proteins were extracted from homogenate or mitochondria by freeze-thawing followed by sonication for 5 periods of 15 s. After centrifugation at 105,000 x g for 1 h, pellets were resuspended in 10 mM Tris/Cl (pH 7.5) and subjected to the second sonication extraction. The combined supernatants were used for measurements of radioactivity incorporated into serine:pyruvate aminotransferase protein. An aliquot of the supernatant was incubated with an excess amount of the specific antibody for 1 h at room temperature and then overnight at 4°. Immunoprecipitates were collected by centrifugation at 3,000 x g for 10 min and washed three times with cold 0.85% NaCl. The precipitates were solubilized with 1 ml of 0.5% sodium dodecyl sulfate and the radioactivity was measured by the use of an Aloka liquid scintillation spectrophotometer (model LSC-651) with a toluene-based scintillator containing 33% Trilon X-100, 0.5% 2,5-diphenyloxazole (PPO), and 0.03% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl-POPOP). Control incubations were carried out with the same amount of cold γ-globulin and the precipitate formed was manipulated in an exactly same manner as above. The experimental data were obtained in triplicate for the standard values and the radioactivity was determined as above.

RESULTS

Effect of Glucagon on Serine:Pyruvate Aminotransferase Activity of Rat Liver

Fig. 1 shows the time course in the increase of serine:pyruvate aminotransferase activity produced by glucagon administration. The basal levels of serine:pyruvate aminotransferase activity in the liver of adult rats were as low as...
transferase activity produced by glucagon administration. Rats were injected intraperitoneally with 0.25 mg of glucagon per 100 g body weight as described under "Experimental Procedures." Control rats received 5 mM NaOH instead of glucagon solution. At the time indicated rats were killed and livers and kidneys were removed and immediately homogenized with 9 volumes of mannitol/sucrose solution. Serine:pyruvate aminotransferase activity was determined as described under "Experimental Procedures" using an aliquot of whole homogenate of liver (●) and kidney (▲) of glucagon-treated rats and liver (○) and kidney (△) of control rats.

Upon subcellular fractionation of control and glucagon-treated rat livers by the method of de Duve et al. (26), 55 to 70% of the total aminotransferase activity was recovered in mitochondrial fraction in both cases in agreement with other workers (12-14). The nuclear, lysosomal, microsomal, and supernatant fractions contained approximately 10 to 25, 6, 9, and 5% of the total activity, respectively, and the distribution pattern of serine:pyruvate aminotransferase among various subcellular fractions was quite similar to that of glutamate dehydrogenase used as a marker enzyme of mitochondria. The aminotransferase activity in the nuclear fraction was almost totally removed when nuclei were purified by the method described earlier (43). Although the mitochondrial fraction contained high activities of catalase and acid phosphatase, marker enzymes of peroxisome and lysosome, respectively, serine:pyruvate aminotransferase behaved differently from these enzymes and coincided with the activity of succinate dehydrogenase on sucrose density gradient centrifugation of the mitochondrial fraction.

Upon submitochondrial fractionation, serine:pyruvate aminotransferase was recovered in the mitoplast fraction which was composed of inner membrane and matrix as shown in Table II. Monoamine oxidase was recovered in the outer membrane fraction in agreement with other past studies (44). Catalase was almost quantitatively solubilized by the digitonin treatment probably due to rupture of peroxisomes during the procedure. The serine:pyruvate aminotransferase activity in mitoplast and mitochondria was easily solubilized by freeze-thawing or sonic treatment. These results indicated that serine:pyruvate aminotransferase activity in mitochondria of parenchymal cell is increased by glucagon administration and is localized in soluble matrix or loosely attached to inner membrane of mitochondria.

**Localization of Serine:Pyruvate Aminotransferase in Liver**

Table I shows serine:pyruvate aminotransferase activity in the isolated parenchymal and nonparenchymal cell preparations. In accordance with earlier reports (23, 24), the parenchymal cell preparation contained L-type pyruvate kinase and 6 times more catalase activity than nonparenchymal cells while the nonparenchymal cell preparation contained almost exclusively M-type pyruvate kinase and 6 times more peroxidase activity than parenchymal cells indicating the purity of these cell preparations. Serine:pyruvate aminotransferase was clearly detectable only in the parenchymal cell preparation when cells were obtained from normal rat liver and the activity in parenchymal cells was increased more than 10-fold when rats were treated with glucagon. These results indicated that serine:pyruvate aminotransferase in the liver is localized mainly in the parenchymal cells. More than 70% of serine:pyruvate aminotransferase activity in parenchymal cells was recovered in a particulate fraction which was sedimented by centrifugation at 25,000 × g.

**Intercellular distribution of serine:pyruvate aminotransferase**

Parenchymal and nonparenchymal cells were isolated from normal and glucagon-treated rat livers as described under "Experimental Procedures." The activity of pyruvate kinase was determined at a phosphoenolpyruvate concentration of 0.25 mM in the presence or absence of 0.5 mM fructose 1,6-diphosphate (FDP). Assay conditions of other enzymes are described under "Experimental Procedures."

**Table I**

<table>
<thead>
<tr>
<th>Source of homogenate</th>
<th>Peroxidase</th>
<th>Catalase</th>
<th>Pyruvate kinase</th>
<th>Serine:pyruvate aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/min/mg protein</td>
<td>µmol/min/mg protein</td>
<td>FDP</td>
<td>FDP</td>
</tr>
<tr>
<td>Whole liver</td>
<td>7.2</td>
<td>488.3</td>
<td>15.9</td>
<td>175.4</td>
</tr>
<tr>
<td>Parenchymal cells</td>
<td>4.8</td>
<td>444.3</td>
<td>8.4</td>
<td>117.4</td>
</tr>
<tr>
<td>Nonparenchymal cells</td>
<td>29.4</td>
<td>70.9</td>
<td>27.5</td>
<td>30.4</td>
</tr>
</tbody>
</table>

*ND, negligible.*

approximately 100 nmol/min/g of liver wet tissue in agreement with previous workers (8, 10). When glucagon was administered, serine:pyruvate aminotransferase in the liver increased with a lag phase of approximately 3 h and reaching levels 7 to 10 times those of control animals after about 12 h. Kidney and other tissues examined including brain, heart, lung, thymus, spleen, stomach, intestine, testis, adrenal gland, and muscle showed little or no serine:pyruvate aminotransferase activity and in the kidney no increase of the enzyme activity was observed after glucagon administration (Fig. 1).

![Fig. 1](left). Time course of increase in serine:pyruvate aminotransferase activity produced by glucagon administration. Rats were injected intraperitoneally with 0.25 mg of glucagon per 100 g body weight as described under "Experimental Procedures." Control rats received 5 mM NaOH instead of glucagon solution. At the time indicated rats were killed and livers and kidneys were removed and immediately homogenized with 9 volumes of mannitol/sucrose solution. Serine:pyruvate aminotransferase activity was expressed as nanomoles per minute per gram of liver wet tissue in agreement with earlier reports (8, 10). When glucagon was administered, serine:pyruvate aminotransferase in the liver increased with a lag phase of approximately 3 h and reaching levels 7 to 10 times those of control animals after about 12 h. Kidney and other tissues examined including brain, heart, lung, thymus, spleen, stomach, intestine, testis, adrenal gland, and muscle showed little or no serine:pyruvate aminotransferase activity and in the kidney no increase of the enzyme activity was observed after glucagon administration (Fig. 1).

![Fig. 2](center and right). First hydroxylapatite (a) and Sephadex G-150 (b) chromatographies of serine:pyruvate aminotransferase. Detailed procedures are described in the text. Aliquots (0.1 ml) of each fraction were assayed for serine:pyruvate aminotransferase (●) and protein (○).
**Induction of Serine:Pyruvate Aminotransferase**

*Intramitochondrial distribution of serine:pyruvate aminotransferase*

Catalase, succinate dehydrogenase, and monoamine oxidase were used as marker enzymes of peroxisome and inner and outer membranes of mitochondria, respectively. Submitochondrial fractionation and determination of enzymatic activities were carried out as described under "Experimental Procedures." Numbers in parentheses represent recoveries from whole mitochondria in each fraction.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Serine:pyruvate aminotransferase</th>
<th>Succinate dehydrogenase</th>
<th>Monoamine oxidase</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td>71.3</td>
<td>13.3</td>
<td>13.3</td>
<td>7.8</td>
</tr>
<tr>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td></td>
</tr>
<tr>
<td>Outer mem.</td>
<td>6.3</td>
<td>13.3</td>
<td>13.3</td>
<td>7.8</td>
</tr>
<tr>
<td>(8.8)</td>
<td>(15.1)</td>
<td>(18.1)</td>
<td>(20.0)</td>
<td></td>
</tr>
<tr>
<td>Mitoplast</td>
<td>37.5</td>
<td>12.0</td>
<td>12.0</td>
<td>8.5</td>
</tr>
<tr>
<td>(52.5)</td>
<td>(80.7)</td>
<td>(82.5)</td>
<td>(35.0)</td>
<td></td>
</tr>
<tr>
<td>Inter mem.</td>
<td>17.5</td>
<td>1.8</td>
<td>1.8</td>
<td>3.3</td>
</tr>
<tr>
<td>(24.5)</td>
<td>(57.3)</td>
<td>(0)</td>
<td>(10.0)</td>
<td></td>
</tr>
</tbody>
</table>

**Purification of Serine-Pyruvate Aminotransferase from Glucagon-treated Rat Liver Mitochondria**

Glucagon was injected intraperitoneally to seven rats (200 g body weight) at 24-h intervals in an amount of 0.25 mg/100 g body weight each time. After 2 h rats were killed and livers (40 g in total) were removed. All subsequent procedures were carried out at 0 to 4°C.

**Step 1: Sonic Extraction of Serine-Pyruvate Aminotransferase from Mitochondria**—Mitochondrial fraction prepared as described under "Experimental Procedures" was suspended in 50 ml of mannitol/sucrose solution and stored at −20°C. The thawed mitochondrial suspension was mixed with 150 ml of 10 mM Tris/Cl (pH 7.5), stirred at 0°C for 30 min and subjected to sonication for five periods of 15 s with 30-s intervals using a Branson ultrasonic disintegrator at maximum power. The suspension was centrifuged for 20 min at 25,000 × g. The pellet was resuspended in 50 ml of 10 mM Tris/Cl (pH 7.5) and the sonication and centrifugation steps were repeated. The supernatants were combined. The recovery of the serine:pyruvate aminotransferase activity of this step was over 95%.

**Step 2: Ammonium Sulfate Fractionation**—To a gently stirred mitochondrial extract solid ammonium sulfate was slowly added to 25% saturation. After standing for 1 h the supernatant was collected by centrifugation at 12,000 × g for 15 min. The supernatant was made 0.1 mM with respect to pyridoxal 5-phosphate and then solid ammonium sulfate was added to 65% saturation. The solution was kept constant at pH 7.5 with 1 M NaOH during the addition. After standing for 1 h, the precipitate was collected by centrifugation at 12,000 × g for 15 min and was successively extracted with 100 ml each of 0.5, 0.5, 50, 45, 40, and 35% saturated ammonium sulfate solution containing 0.1 mM pyridoxal 5-phosphate (pH 7.5). Serine:pyruvate aminotransferase was recovered mainly in the 55 and 50% ammonium sulfate extracts. The extracts were combined and made 80% saturation with respect to ammonium sulfate. After standing for 1 h, the precipitate was collected by centrifugation and dissolved in 30 ml of 10 mM Tris/Cl (pH 8.0), and the resulting clear solution was dialyzed overnight against 3 litters of the same buffer.

**Step 3: DEAE-cellulose Chromatography**—The dialyzed enzyme solution (36 ml) was applied on a DEAE-cellulose column (2.3 × 13 cm) which had been equilibrated with 10 mM Tris/Cl (pH 8.0) and then elution was performed with a linear gradient between 200 ml of 10 mM Tris/Cl (pH 8.0) containing 0.1 mM pyridoxal 5-phosphate and 0.5 mM dithiothreitol in a mixing chamber and 200 ml of the starting buffer containing 0.3 M KCl in a reservoir. Serine:pyruvate aminotransferase activity was detected in the eluate when the KCl concentration reached approximately 0.12 M. The fractions containing enzyme activity over 49 nmol/min/ml were pooled.

**Step 4: Hydroxylapatite Chromatography**—The pooled eluate from the DEAE-cellulose step was directly applied on a hydroxylapatite column (2.3 × 10 cm) which had been equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 0.5 mM dithiothreitol. The column was washed with 150 ml of 0.1 mM potassium phosphate buffer (pH 7.4) containing 0.1 mM pyridoxal 5-phosphate and 0.5 mM dithiothreitol. Elution was then carried out with a linear gradient between 200 ml each of 0.1 M and 0.4 M potassium phosphate buffer (pH 7.4) both containing 0.1 mM pyridoxal 5-phosphate and 0.5 mM dithiothreitol. Elution profile is shown in Fig. 2a. The active fractions (44 to 50) were pooled and concentrated to 4.5 ml by the use of a collodion bag (SM-13290, Bartorius membrane filter).

**Step 5: Sephadex G-150 Gel Filtration**—The concentrated enzyme solution was applied on a Sephadex G-150 column (1.2 × 103 cm) which had been equilibrated with 10 mM potassium phosphate buffer (pH 7.4) containing 0.1 M KCl, 0.1 mM pyridoxal 5-phosphate, and 0.5 mM dithiothreitol. Elution was carried out with the same buffer. Serine:pyruvate aminotransferase was eluted as a single symmetrical peak as shown in Fig. 2b. The active fractions (27 to 33) were pooled.

**Step 6: Second Hydroxylapatite Chromatography**—The pooled eluate from Sephadex G-150 was rechromatographed on a hydroxylapatite column (1 × 10 cm) which had been equilibrated with 10 mM potassium phosphate buffer (pH 7.4) both containing 0.1 M KCl, 0.1 mM pyridoxal 5-phosphate, and 0.5 mM dithiothreitol. Elution profile is shown in Fig. 2c. The active fractions (44 to 50) were pooled.

**Ouchterlony Double Diffusion Analysis and Immunoelectrophoresis**

An antibody against the purified serine:pyruvate aminotransferase (Step 6) was prepared in rabbits as described under "Experimental Procedures." Upon Ouchterlony double diffusion analysis, the anti-serine:pyruvate aminotransferase γ-globulin produced a single connecting band of precipitin.
TABLE III
Purification of serine:pyruvate aminotransferase from glucagon-treated rat liver mitochondria

Details of the purification and assays are described in the text and under "Experimental Procedures."

<table>
<thead>
<tr>
<th>Step</th>
<th>Volume</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>mg</td>
<td>umol/min</td>
<td>pmol/min/mg protein</td>
<td>%</td>
</tr>
<tr>
<td>Mitochondrial extract</td>
<td>250</td>
<td>1375.0</td>
<td>14.91</td>
<td>10.8</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>86</td>
<td>207.2</td>
<td>10.64</td>
<td>55.1</td>
<td>77.0</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>80</td>
<td>57.6</td>
<td>6.57</td>
<td>114.0</td>
<td>44.0</td>
</tr>
<tr>
<td>1st hydroxylapatite</td>
<td>50</td>
<td>7.0</td>
<td>3.19</td>
<td>456.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Sephadex G-150</td>
<td>15</td>
<td>1.0</td>
<td>1.47</td>
<td>1466.8</td>
<td>9.8</td>
</tr>
<tr>
<td>2nd hydroxylapatite</td>
<td>10</td>
<td>0.5</td>
<td>1.15</td>
<td>2300.8</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Fig. 3. Analysis of purified serine:pyruvate aminotransferase by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis was carried out with 25 μg of the purified enzyme according to the method of Weber and Osborn (42). Denstometric analysis of the gel was carried out at 660 nm. Bromphenol blue (BPB) represents the position of the tracking dye.

between the purified enzyme and mitochondrial extracts of glucagon-treated rat liver as shown in Fig. 4a. When applied in large quantities, the supernatant fraction from glucagon-treated rat liver and mitochondrial extracts from normal liver were also shown to make a faint fused precipitin line with the purified enzyme (Fig. 4b). The completeness of connections of the precipitin band indicated that serine:pyruvate aminotransferase molecules in mitochondrial extracts of control rat liver and the cytosol fraction of glucagon-treated rat liver are immunologically identical or similar to the enzyme purified from glucagon-treated rat liver mitochondria. It has not yet been decided, however, whether the enzyme detected in the cytosol fraction of glucagon-treated rat liver are immunologically identical or similar to the enzyme purified from glucagon-treated rat liver mitochondrial extracts of control rat liver and the cytosol fraction of glucagon-treated rat liver. When the purified enzyme and the mitochondrial extracts from glucagon-treated rat liver were subjected to immunoelectrophoresis, smooth and single precipitin lines were made as shown in Fig. 4c. These results also indicated that the enzyme preparation employed as antigen was practically homogeneous and the antibody produced is specific for serine:pyruvate aminotransferase.

Quantitation of Antigen by Immunotitration

A quantitative immunotitration was set up as shown in Fig. 5. It can be seen that the equivalence points for the titrations namely 2.3 nmol/min of enzyme activity/25 μl of the antibody are the same for mitochondrial extracts and homogenates from both normal and glucagon-treated rat livers. When control γ-globulin was added instead of anti-serine:pyruvate aminotransferase γ-globulin, various amounts of enzyme activity added were quantitatively recovered in the supernatant fract-
TABLE IV

Relative rate of serine:pyruvate aminotransferase synthesis in normal and glucagon-treated rat livers

At the onset of experiment rats were injected intraperitoneally with 0.25 mg of glucagon/100 g body weight and then subjected to fasting. After 12 h the same amount of glucagon was injected for the second time. Control rats received the same amounts of 5 mM NaOH instead of glucagon solution. Six hours after the second injection, 0.25 mcCi of \(^{3}H\)leucine was injected intraperitoneally. The rate of serine:pyruvate aminotransferase induction was not necessarily maximum under this condition. Rats were killed 3 h later and the radioactive incorporated into trichloroacetic acid-precipitable and immunoprecipitable proteins in homogenate and mitochondria of the liver were determined as described under "Experimental Procedures." The data represent the mean of the two rats. (The value of individual rat differed less than 10% from each other.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enzyme activity</th>
<th>(^{3}H)leucine incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Total activity</td>
</tr>
<tr>
<td>Homogenate</td>
<td>nmol/min/mg protein</td>
<td>(\mu)mol/min/ liver</td>
</tr>
<tr>
<td>Control</td>
<td>0.84</td>
<td>0.575</td>
</tr>
<tr>
<td>Glucagon</td>
<td>4.98</td>
<td>3.430</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Control</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>Glucagon</td>
<td>10.32</td>
</tr>
</tbody>
</table>

As a measure of relative rate of enzyme synthesis the extent of \(^{3}H\)leucine incorporation into protein which is precipitated by anti-serine:pyruvate aminotransferase \(\gamma\)-globulin was determined in a pulse-labeling experiment according to the principle developed by Schimke (45, 46). \(^{3}H\)leucine was injected intraperitoneally to glucagon-treated and control rats, and 3 h later the acid-precipitable and immunoprecipitable radioactivities in liver homogenates and mitochondrial fraction were determined as described in the legend to Table IV and under "Experimental Procedures." The amount of isotope incorporated into trichloroacetic acid-precipitable liver protein was approximately 1% of the total radioactivity injected and differed less than 10% between control and glucagon-treated rats. The amount of \(^{3}H\)leucine incorporated into immunoprecipitable protein in the glucagon-treated rat liver was 10 times the amount in the control rat liver (Table IV), indicating that glucagon markedly increases the rate of synthesis of serine:pyruvate aminotransferase protein under the conditions employed. The increase in the activity of serine:pyruvate aminotransferase by glucagon injection was approximately 6-fold. The recoveries of enzyme activity and the immunoprecipitable labeled protein in the mitochondrial fraction were 50 and 30%, respectively, in this experiment.

In order to assure that the radioactivity precipitated by the antibody is mainly serine:pyruvate aminotransferase, the immunoprecipitates were solubilized and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When the immunoprecipitate obtained from the glucagon-treated rat livers in the isotope incorporation experiments (Table IV) were solubilized by heating at 90° for 3 to 5 min in 10 mM sodium phosphate buffer (pH 7.2) containing 1% sodium dodecyl sulfate and 1% 2-mercaptoethanol. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the solubilized immunoprecipitates and the determination of radioactivity in gel slices were carried out as described under "Experimental Procedures." Immunoprecipitates obtained from 0.5 ml of glucagon-treated liver homogange (○) and that obtained from 1.5 ml of control liver homogenate (□) were subjected to electrophoresis. BPB, bromphenol blue.

Fig. 7 (right). Degradation rate of serine:pyruvate aminotransferase in normal rat liver. Rats were treated as specified for the control rats in the experiment shown in Table IV except that animals were killed at the time indicated. The radioactivity incorporated into serine:pyruvate aminotransferase was determined as described under "Experimental Procedures." In the case of control rat, however, only less than 30% of the radioactivity of the immunoprecipitate migrated with serine:pyruvate aminotransferase; a major portion of radioactivity in the immunoprecipitate was thus mainly due to nonspecific trapping. Similar data were obtained using mitochondrial extracts from normal and glucagon-treated rat livers. These results indicated that the difference in the rate of serine:pyruvate aminotransferase synthesis in the control and glucagon-treated rat livers is much greater than that estimated in Table IV.

As a measure of the rate of enzyme degradation, the rate of loss of radioactivity from prelabeled enzyme was determined in normal rats where the rates of synthesis and degradation of serine:pyruvate aminotransferase are presumably in equilibrium. As shown in Fig. 7, the degradation of the enzyme protein was a slow process and a preliminary estimate suggested a half-life of about 30 h. It was thus difficult to explain the rate of accumulation of serine:pyruvate aminotransferase in glucagon-treated rat liver mitochondria by the inhibitory effect of this hormone on the degradation process of the enzyme.

DISCUSSION

The basal level of serine:pyruvate aminotransferase activity in livers of normal adult rats is relatively low and the majority of the enzyme is found in the mitochondrial matrix fraction of parenchymal cells. After administration of glucagon, slow but large increases in serine:pyruvate aminotransferase activity are observed and the majority of the increased aminotransferase activity is also found in the mitochondrial soluble matrix of parenchymal cells. Previous studies by
Hoshino et al. (11) with inhibitors of RNA and protein synthesis suggested that the increase in the serine:pyruvate aminotransferase activity is due to changes in enzyme synthesis.

In the present investigation serine:pyruvate aminotransferase has been purified from mitochondrial fraction of glucagon-treated rat liver to near homogeneity as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Ouchterlony double diffusion analysis and immunoelectrophoresis. The availability of the near homogeneous serine:pyruvate aminotransferase preparation has permitted us immunohistochemical studies on the changes in quantity of enzyme protein as well as on its synthesis and degradation caused by glucagon administration. The results of immunohistochemical analysis indicated that the increase in the level of serine:pyruvate aminotransferase activity in the liver seen after glucagon administration is determined by changing quantities of the enzyme protein. Possible involvement of increases in catalytic efficiency per enzyme molecule may be ruled out from the fact that a same amount of enzyme activity was removed with a fixed amount of the specific antibody from mitochondrial extracts and homogenates of both control and glucagon-treated rat livers. Pulse-labeling experiments with [3H]leucine together with the determination of the degradation rate of the enzyme protein in normal rat liver indicated that the change in the rate of synthesis of serine:pyruvate aminotransferase is the main factor responsible for the change in enzyme concentration.

It is generally suspected that a number of mitochondrial proteins is coded by nuclear genes, synthesized on cytoplasmic ribosomes, and subsequently imported into mitochondria (47). However, the information on the site of synthesis of individual mitochondrial proteins has been insufficient. The induction of serine:pyruvate aminotransferase by glucagon may provide a good system for the study of the turnover of a mitochondrial enzyme. Other mitochondrial enzymes, b-aminolevulinic acid synthase and ornithine aminotransferase, have been shown to be induced by porphyrogenic drugs such as 2-allylisopropylacetamide and 1,4-dihydro-3,5-dicarboxyoxindole (48, 49) and glucagon or high protein diets (50), respectively.

The physiological role of serine:pyruvate aminotransferase has not yet been fully understood. Experimental evidence so far reported has indicated that mammalian liver is equipped with alternative pathways initiated by serine dehydratase and serine:pyruvate aminotransferase, respectively, for the gluconeogenesis from serine (1-6, 8-13, 15, 18). In the normal adult rat liver, these two enzymes show relatively low activities (8-11, 51) and may participate in serine catabolism only to a small extent (18, 51). Serine dehydratase of rat liver has been noted to be induced under various gluconeogenic conditions such as fasting (10, 52), alloxan diabetes (53, 54), and injection of glucagon (55-57), adrenaline (57), or gluccorticoid (52, 53). In contrast, serine:pyruvate aminotransferase in the adult rat liver is not induced by fasting or cortisone (10) and high levels of this enzyme activity have been observed in suckling neonatal rat liver (8), adult rat liver injected with glucagon (9), or rabbit liver fed a high protein diet (58). Quantitative evaluation of the contribution of these two enzymes in gluconeogenesis from serine under different hormonal and dietary conditions remains to be elucidated. In the present study, parenchymal localization of serine:pyruvate aminotransferase, one of the presumed prerequisites for the gluconeogenic enzyme (24), has been fulfilled.

In a very recent report, Noguchi et al. (14) purified histidine:pyruvate aminotransferase from glucagon treated rat liver and showed that one of the isoenzymes of this aminotransferase is identical with serine:pyruvate aminotransferase. Characterization of serine:pyruvate aminotransferase purified in the present study is now in progress.

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