The subcellular distribution of rat liver serine–pyruvate aminotransferase

Kathleen V. ROWSELL, Lamia M. R. AL-NAAMA* and Peter BENETT

Department of Medical Biochemistry, The Medical School, Manchester University, Manchester M13 9PT, U.K.

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1. The subcellular distribution of L-serine–pyruvate aminotransferase activity in rat liver was investigated. About 80% was recovered from cell-free homogenates in a 'total-particles' fraction and the remainder in the cytosol. 2. Subfractionation of the particles by differential sedimentation and on sucrose density gradients showed a distribution for serine–pyruvate aminotransferase activity closely matching that observed for mitochondrial marker enzymes. 3. A study of the solubilization of enzymes from combined subcellular particles by digitonin at various concentrations also indicated a common subcellular location for serine–pyruvate aminotransferase and established mitochondrial enzymes. 4. The increase in liver serine–pyruvate aminotransferase activity induced by glucagon injection was accounted for as an increased mitochondrial activity.

Accounts of the subcellular distribution of rat liver L-serine–pyruvate aminotransferase (EC 2.6.1.51) vary. Without presenting supporting data Rowsell *et al.* (1972a) report the enzyme as wholly particulate. Snell (1975, 1976) assigned 90% of the activity to homogenate particles with 10% cytosolic and presented evidence that, in suckling rats at least, the particle association is with mitochondria. Noguchi *et al.* (1976, 1978) report recoveries of 16% and 80% for the cytosol and combined particles respectively, 50–55% of the particle-bound enzyme being in the peroxisomes with the remainder in the mitochondria. Hoshino *et al.* (1977) claimed that about 75% of the activity is in the cytosol and about 25% in the mitochondria, though the latter was a crude mitochondrial fraction (as described in the present paper) containing presumably also lysosomes and peroxisomes.

For a proper consideration of the role of serine–pyruvate aminotransferase in liver metabolism, reliable knowledge as to its subcellular location is required. For example, it has been noted that enzymes catalysing the reactions for the sequences [glyoxylate → glycine → serine] and [hydroxypyruvate → D-glycerate → 2-phosphoglycerate] are largely located in rat liver mitochondria (Rowsell *et al.*, 1972a). [Kitagawa & Sugimoto (1979) reported a cytosolic location for D-glycerate dehydrogenase, the enzyme responsible for the reduction of hydroxypyruvate, but deficiencies in the assay method employed make this a dubious claim]. If the enzyme

for the linking reaction serine → hydroxypyruvate were also principally sited in the mitochondria, it would be possible and reasonable to visualize a sequence of reactions leading towards glucose formation from glyoxylate, glycine or serine, the overall operation being facilitated by the proximity of all the enzymes concerned. The subcellular distribution of rat liver serine–pyruvate aminotransferase was re-investigated.

Methods

Preparation of homogenates

Male Sprague–Dawley rats (200–250 g) starved for 24 h were stunned and bled to death from the blood vessels of the neck. To avoid contamination of the liver homogenates with blood cells, immediately the liver was perfused *in situ* with Ca²⁺-free Locke's solution for 3–4 min. Perfusion with Ca²⁺-free saline also softens the intercellular cement and facilitates the disruption of the tissue (Anderson, 1953). The liver was then quickly removed into a tared beaker in ice, weighed and finely chopped with scissors. The preparation of homogenates (10% w/v) in unbuffered 0.4 M-sucrose was as described by Rowsell *et al.* (1972a). Under these conditions, in our experience, mitochondria at least, judging by their retention of glutamate dehydrogenase and appearance under the electron microscope, are more reliably maintained intact than when homogenates are prepared in 0.25 M-sucrose, with or without additives.

* Present address: University of Basrah, Basrah, Iraq.

Subcellular fractionation

All centrifugation procedures were performed at 2–4°C and all fractions were stored on ice until required for enzyme activity assays. Differential sedimentation was carried out in the 8 × 25 ml angle rotor of the MSE65 centrifuge or, for the isolation of nuclei, in the 8 × 50 ml swing-out rotor of the Mistral 4L centrifuge. For density-gradient fractionation the 3 × 23 ml and 3 × 70 ml swing-out rotors of the MSE65 were employed, or the B-14 zonal rotor.

Differential sedimentation fractionation

In separate experiments the following fractionations were made. (1) The homogenate was centrifuged at 150 000 *g* (r_{av} . 6.9 cm) for 100 min to give a clear supernatant cytosol fraction and a sedimented total-particles fraction. (2) A nuclear fraction was sedimented at 550 *g* (r_{av} . 7.7 cm) for 2 min leaving a supernatant containing other subcellular particulate components with the cytosol. The nuclear fraction was washed three times by resuspension in 0.4 M-sucrose containing 0.2 mM-CaCl₂ and recentrifugation as described above before a final resuspension in the same medium. (3) Nuclei were sedimented as described in (2) and discarded. The supernatant was centrifuged at 30 000 *g* (r_{av} . 6.9 cm) for 60 min to obtain a clear cytosol fraction and sedimented particles. The latter were resuspended in 0.4 M-sucrose and centrifuged at 15 400 *g* (r_{av} . 6.9 cm) for 10 min to sediment a crude mitochondrial fraction leaving a portion of the microsomes in the supernatant. (4) After removing the nuclei the remaining supernatant was centrifuged at 61 600 *g* (r_{av} . 6.9 cm) for 15 min to obtain a cytosol fraction containing light microsomes and a sedimented particles fraction. The latter was resuspended in 0.4 M-sucrose and centrifuged at 11 500 *g* (r_{av} . 6.9 cm) for 10 min to sediment a crude mitochondrial fraction leaving microsomes in the supernatant.

A Teflon homogenizer operated by hand was used to resuspend the sedimented fractions. The original homogenizing medium was used except with the sedimented nuclei.

Density-gradient fractionation

Sucrose gradients were used in attempts to separate mitochondria, lysosomes and peroxisomes. In some experiments, to facilitate the separation of lysosomes, rats were pre-injected with Triton WR-1339 (supplied by Hilton Davis Chemicals Ltd., Newcastle-upon-Tyne, U.K.) as described by Leighton *et al.* (1968). A 10% (w/v) homogenate was prepared and centrifuged at 500 *g* for 3 min to remove nuclei. The supernatant remaining was recentrifuged at 26 700 *g* (r_{av} . 9.4 cm) for 10 min to sediment a particulate fraction (described as 'crude mitochondria') for application to a sucrose gradient.

Continuous sucrose gradients were constructed

with an LKB Ultragrad 11300 gradient-making device and discontinuous gradients by pipetting discrete layers of sucrose solution. Details are given in the Results section. After centrifugation, sucrose solution, at the highest concentration for the gradient used, was introduced through the bottom of the tubes at 1.5 ml/min and fractions were collected from the top by using an ISCO density gradient fractionator model 185 (Instrumental Specialties Co., Lincoln, NE, U.S.A.). Alternatively fractions were isolated with a modified Beckman tube slicer as described by Turner (1964). The sucrose concentration (% w/w) of each fraction recovered was measured at 20°C on an Abbé refractometer, designed to read directly both refractive index and the associated sucrose concentration, then by referring to appropriate calibration curves the sucrose concentration (% w/v) and density were derived.

Zonal centrifugation

For each experiment a crude mitochondrial fraction was prepared from three rats, not pre-treated with Triton WR-1339, and resuspended to obtain the equivalent of 0.5 g of original tissue/ml in 55% (w/v) sucrose. A portion (30 ml) was applied over a sucrose gradient [57–70% (w/v), 520 ml] cushioned by 80 ml of 70% (w/v) sucrose, in the zonal rotor. An overlay of 50 ml of 50% (w/v) sucrose completed the loading procedure. After centrifugation at 58 000 *g* (r_{av} . 4.5 cm) for 3 h, 70% (w/v) sucrose was pumped into the periphery inlets at 20 ml/min and 20 ml fractions were collected from the centre.

Digitonin treatment of particles

A 10% (w/v) digitonin solution was prepared by heating digitonin in 0.4 M-sucrose. This was mixed with 0.4 M-sucrose in centrifuge tubes to obtain a series of 2.0 ml portions containing 0–50 mg of digitonin/ml which were then cooled to 0°C. To each tube 8.0 ml of a rat liver crude mitochondrial preparation was added, and, after mixing by gentle swirling, tubes were left at 0°C for 15 min. The tubes were then centrifuged at 30 000 *g* (r_{av} . 9.4 cm) for 30 min. The supernatant and the resuspended pellets were then taken for enzyme assays.

Enzyme assays

To measure the recovery of different subcellular components the following marker enzymes were assayed: lactate dehydrogenase (EC 1.1.1.27; cytosol), glucose 6-phosphatase (EC 3.1.3.9; microsomal), acid phosphatase (EC 3.1.3.2; lysosomal), glutamate dehydrogenase (EC 1.4.1.2) and cytochrome oxidase (EC 1.9.3.1) (mitochondrial), urate oxidase (EC 1.7.3.3), D-amino acid oxidase (EC 1.4.3.3) and catalase (EC 1.11.1.6) (peroxisomal); DNA was used as a marker for

nuclei. Except as detailed below, assay procedures were as described by Rowsell *et al.* (1972a).

Before making assays for glutamate dehydrogenase activity, all subcellular fractions were left at 0°C for 3 h with 0.1% (v/v) Triton X-100. The activity thus measured was the same as that assayed after sonication at 0–2°C for 10 min [with maximum power on the MSE Ultrasonic (disintegrator) Model 150W], but activation by Triton was more convenient where several fractions required assay. Cytochrome oxidase was assayed by observing the re-oxidation of reduced cytochrome *c*, as described by Smith (1960), with fraction samples clarified by sonication or by treatment with Triton X-100 as described above. D-Amino acid oxidase was assayed by measuring the pyruvate formed aerobically from D-alanine. Assay mixtures (8.0 ml) contained 20 mM-D-alanine, 50 mM-potassium phosphate buffer, pH 7.4, and tissue preparation and were incubated at 37°C under oxygen. Samples (2.0 ml) were removed at 0, 15 and 30 min into 2.0 ml of 10% (w/v) trichloroacetic acid. After centrifugation supernatants were analysed for pyruvate with lactate dehydrogenase. Catalase was assayed by measuring the decrease in H₂O₂ absorbance at 240 nm, as described by Lück (1963). The catalase was solubilized by pretreating fraction samples at 0°C with 1% (v/v) Triton X-100 and in the presence of 0.33% bovine serum albumin to protect the enzyme from inactivation by the detergent (see Leighton *et al.*, 1968). Samples were diluted to a suitable concentration with 0.05 M-potassium phosphate buffer, pH 7.0, immediately before assay.

L-Serine-pyruvate aminotransferase was assayed as described by Rowsell *et al.* (1979) and L-alanine-glyoxylate aminotransferase by measuring the pyruvate formed with lactate dehydrogenase in the presence of Tris at pH 8.4, as described by Rowsell *et al.* (1972b).

Checks were made on the reliability of the assay procedures, for use in subcellular fractionation studies, and some sucrose interference with the serine-pyruvate aminotransferase assay, (i.e. with the determination of hydroxypyruvate), was found. When blank assays (no tissue extract) were conducted with samples of sucrose at high concentration (>60%), a slight re-oxidation of NADH was observed (a decrease in A_{340} of about 0.003) when glycerate dehydrogenase was added to HClO₄-treated, neutralized assay samples. This was attributable apparently to hydrolysis products of sucrose obtained on exposure to HClO₄. The effect was not seen with sucrose made up and maintained at neutrality. To correct for this in subcellular-fractionation studies, a blank serine-pyruvate aminotransferase assay was made for each fraction investigated using the same volume and sucrose concentration as in the test assay.

Table 1. *Whole homogenate enzyme activities in rat liver*
Activities are expressed as μmol of substrate utilized or product formed/min per g of liver $\pm$ S.E.M. (no. of observations), except for catalase activity which is given as mmol/min per g. DNA concentrations were 2220 ± 200 ($n = 3$) $\mu\text{g/g}$ of liver.

Enzyme	Activity
Lactate dehydrogenase	302 ± 14 (10)
Glutamate dehydrogenase	33.5 ± 2.2 (20)
Cytochrome oxidase	28.5 ± 2.2 (12)
Glucose 6-phosphatase	7.43 ± 0.7 (12)
Acid phosphatase	6.42 ± 0.3 (16)
Urate oxidase	0.75 ± 0.08 (12)
D-Amino acid oxidase	0.32 ± 0.10 (6)
Catalase	552 ± 1.5 (4)
Alanine-glyoxylate aminotransferase	3.76 ± 0.38 (12)
Serine-pyruvate aminotransferase	0.083 ± 0.004 (20)

Except with cytochrome oxidase and catalase all enzyme activity values were based on linear progress curves. Whole homogenate activities for the enzymes assayed in the present work are given in Table 1.

Results

There was no significant difference in serine-pyruvate aminotransferase activity when homogenates were made from the same rat liver in 0.4 M-sucrose or in 0.1 M-potassium phosphate buffer, pH 7.4. When the activity was assayed on an homogenate freshly prepared in 0.4 M-sucrose and after 3 h storage at 0°C there was no significant decrease (<2%). It appeared there would be little loss of activity during fractionation.

Subcellular fractions obtained by differential sedimentation

Reproducibly somewhat more than 80% of the serine-pyruvate aminotransferase activity was recovered in a total-particles fraction and the remainder in the cytosol [Table 2, (1)]. Samples of a total-particles preparation were sonicated at sub-maximal power for periods up to 15 min. After centrifugation at 160 000 *g* for 100 min the supernatant and the resuspended pellet were assayed for serine-pyruvate aminotransferase. A solubilization of the enzyme was observed but at a somewhat slower rate than for glutamate dehydrogenase; yet the recovery of serine-pyruvate aminotransferase in the cytosol clearly exceeded that of glutamate dehydrogenase and of other particle marker enzymes. We conclude that the serine-pyruvate aminotransferase activity found in the cytosol is located there in the intact liver cell; it does not originate in the disruption of a subcellular organelle in the course of homogenization or fractionation.

Table 2. *Enzyme activities of subcellular fractions isolated by differential sedimentation*
 Fractions were prepared from rat liver homogenates by differential sedimentation as described in the Methods section [procedures (1)–(4)]. Percentages of the original whole homogenate values are given.

Experiment	Serine-pyruvate amino-transferase	Lactate dehydro-genase	Glutamate dehydro-genase	Cytochrome oxidase	Glucose 6-phosphatase	Acid phosphatase	Urate oxidase	DNA
(1)								
Total-particles fraction	82	12	98	99	99	93	92	
Cytosol fraction	17	90	0.4	0	2	6	0	
Recovery	99	102	98.4	99	101	99	92	
(2)								
Nuclear fraction	6	5	4	2	0	4		76
Supernatant fraction	92	92	94	86	82	98		25
Recovery	98	97	98	88	82	102		101
(3)								
Crude mitochondrial fraction	69	15	81		21	66		
Microsomal fraction	12	15	12		65	20		
Cytosol fraction	25	78	6		9	19		
Recovery	106	108	99		95	105		
(4)								
Crude mitochondrial fraction	69	7	84		16	70		
Microsomal fraction	10	15	5		68	24		
Cytosol + light microsomal fraction	19	74	7		28	15		
Recovery	98	96	96		112	109		

A small proportion of the serine-pyruvate aminotransferase activity was recovered in nuclear fractions [Table 2, (2)] and in microsomal fractions [Table 2, (3) and (4)] but contamination with marker enzymes for other subcellular components casts doubt on a genuine location there. The results in Table 2 suggest a distribution for serine-pyruvate aminotransferase between the cytosol and particles mainly other than nuclei or microsomes. A major association with crude mitochondrial fractions was regularly observed. These fractions contained a high proportion of the mitochondria and the lysosomes and would also be expected to include peroxisomes.

Subcellular fractions obtained by centrifugation on density gradients

Crude liver mitochondrial fractions prepared from rats pretreated with Triton WR-1339 were subjected to fractionation on a sucrose gradient designed to maximize the separation of lysosomes from mitochondria. The bulk of the acid phos-

phatase was then recoverable in the upper fractions accompanied by relatively little serine-pyruvate aminotransferase activity (Table 3). Other experiments were made with gradients designed to increase the separation of mitochondria from peroxisomes (Table 4). In neither type of experiment was it possible to obtain mitochondria uncontaminated with peroxisomes, but repeatedly it was observed that the distribution of serine-pyruvate aminotransferase activity along the gradients fairly closely paralleled those of the mitochondrial marker enzymes and this was not observed with the peroxisomal or lysosomal markers.

Zonal centrifugation experiments were made in further attempts to improve the separation of mitochondria and peroxisomes. As indicated by the distribution of glutamate dehydrogenase and cytochrome oxidase, mitochondria were recovered in a relatively restricted band centred on a density of 1.20 g/ml. However, the peroxisomal marker enzymes (catalase, D-amino acid oxidase and urate

Table 3. Enzyme activities of subcellular fractions isolated by centrifugation on a sucrose gradient designed to separate lysosomes and mitochondria

A crude mitochondrial fraction was prepared from a rat pre-injected with Triton WR-1339, as described in the Methods section, and resuspended to obtain the equivalent of 0.2 g of original tissue/ml in 0.4 M-sucrose. Portions (10 ml) were applied over continuous sucrose gradients [15.4–80% (w/v), 52 ml] and after centrifugation at 93 000 g (*r*_{av}, 9.4 cm) for 90 min fractions were collected sequentially from the top of the gradient. Enzyme activities recovered are presented as percentages of crude mitochondrial activities and as relative concentrations (*R*), i.e. % recovery/% gradient volume.

Fraction			Glutamate dehydrogenase		Cytochrome oxidase		Acid phosphatase		Urate oxidase		Serine-pyruvate aminotransferase	
Density (g/ml)	Volume (ml)	% of total volume	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>
(a)	1.05	13	21	0	0	0	0	7.5	0.36	0	0	0
(b)	1.10	18	29	1.6	0.06	1	0.03	76	2.6	0	0	4
(c)	1.15	13	21	1.0	0.05	2	0.10	7.0	0.33	0	0	12
(d)	1.23	18	29	93	3.2	93	3.2	9.7	0.33	84	2.9	81

Table 4. Enzyme activities of subcellular fractions isolated by centrifugation on a sucrose gradient designed to separate mitochondria and peroxisomes

A crude mitochondrial fraction was prepared as described in Table 2. It was resuspended to obtain the equivalent of 0.2 g of original tissue/ml in 50% (w/v) sucrose, density 1.19 g/ml. Portions (15 ml) were applied over continuous sucrose gradients [58–70% (w/v), 35 ml] and then overlaid with 12 ml of 0.4 M-sucrose. After centrifugation at 78 000 g (*r*_{av}, 11.3 cm) for 180 min, fractions were collected from the top of the gradient. Enzyme activities are expressed as in Table 3.

Fraction			Glutamate dehydrogenase		Cytochrome oxidase		Acid phosphatase		Urate oxidase		D-Amino acid oxidase		Serine-pyruvate aminotransferase	
Density (g/ml)	Volume (ml)	% of total volume	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>
(a)	1.19	31.5	51	60	1.18	55	1.08	88	1.73	36	0.71	53	1.04	55
(b)	1.23	4.7	8	27	3.38	31	3.84	2	0.25	14.5	1.8	10	1.25	27
(c)	1.25	5.5	9	12	1.30	16	1.78	2	0.22	34	3.73	34	3.78	15

oxidase) were recovered to some degree throughout the gradient. Total enzyme recoveries were >90%, and we can only conclude that with this technique, in our hands, the peroxisomes had suffered damage. Nevertheless the findings were again consistent with a mitochondrial location for serine-pyruvate aminotransferase. Its activity distribution, together with that of alanine-glyoxylate aminotransferase, closely matched those of the mitochondrial marker enzymes.

Differential extraction of particle-bound enzymes by digitonin

Crude mitochondrial preparations were treated with different concentrations of digitonin in 0.4 M-sucrose. After centrifugation, supernatants and resuspended pellets were assayed for serine-pyruvate aminotransferase activity and for mitochondrial and peroxisomal marker enzymes (Fig. 1). Assays for urate oxidase were not included since, as

a peroxisomal 'core' enzyme (see de Duve & Baudhuin, 1966), it was expected it would not be solubilized by digitonin.

There was some release of catalase from the particles even in the absence of digitonin. (This suggests that there will be a loss of this enzyme from the peroxisomes in the course of subcellular fractionation and that catalase is not therefore an ideal peroxisomal marker.) But at the lowest digitonin concentration investigated the release of catalase was greatly increased and for 5 mg of digitonin/ml there was almost a total loss from the particles with an equivalent recovery in the supernatant. The loss of D-amino acid oxidase from the particles showed the same dependence on digitonin but unaccountably no activity was measurable in the supernatants.

Higher concentrations of digitonin were needed to bring about the solubilization of glutamate dehydrogenase, and for increasing digitonin concen-

Fig. 1. Release of enzyme activities from rat liver particles by digitonin

As described in the Methods section, samples of a crude mitochondrial fraction in 0.4 M-sucrose were treated with digitonin at the concentration indicated. After centrifugation, assays were made on the resuspended pellet and the supernatant. Enzyme activities recovered are presented as percentages of crude mitochondrial activities. ●, Serine-pyruvate aminotransferase; ○, alanine-glyoxylate aminotransferase; ■, glutamate dehydrogenase; ▲, catalase; △, D-amino acid oxidase.

tration the release of serine-pyruvate aminotransferase and alanine-glyoxylate aminotransferase closely paralleled that of glutamate dehydrogenase. The results were consistent with the suggestion that particulate serine-pyruvate aminotransferase is located in the same subcellular organelle as glutamate dehydrogenase. Along with the zonal centrifugation findings they also confirm the report of a mitochondrial location for alanine-glyoxylate aminotransferase (Rowsell *et al.*, 1972a).

Subcellular distribution in glucagon-injected rats

Some rats were given a single intraperitoneal injection of glucagon (Eli Lilly & Co. Ltd., Basingstoke, U.K.), 0.25 mg/100 g body wt., and killed 24 h later. As expected (see Rowsell *et al.*, 1969; Rowsell *et al.*, 1973; Snell & Walker, 1974), serine-pyruvate aminotransferase activity measured in crude liver homogenates had increased, to about 11 times the normal value. When homogenates were prepared in 0.4 M-sucrose and centrifuged to separate a total-particles fraction from the cytosol, it was observed that virtually all the increase in serine-pyruvate aminotransferase activity could be accounted for by an increase in the particle-bound enzyme. There was no evidence of any effect of glucagon, with respect to the whole homogenate activity or subcellular distribution, on any of the marker enzymes.

Crude mitochondrial fractions were prepared from glucagon-injected rats and were further fractionated on sucrose gradients. Experiments were made to maximize the separation of lysosomes and mitochondria (Table 5) and, subsequently, with a modified procedure, in attempts to increase the separation of mitochondria and peroxisomes (Table 6). As in experiments with normal rats (cf. Tables 3 and 4) enzyme assays on the fractions obtained showed a fairly close matching between the distribution of serine-pyruvate aminotransferase activity and those of the activities for the mitochondrial marker enzymes, but not with the distribution of either the lysosomal or the peroxisomal marker.

Discussion

For all the subcellular-fractionation experiments, in percentage distribution, the particle-bound serine-pyruvate aminotransferase activity was fairly closely paralleled by the activities of the mitochondrial marker enzymes. This matching in distribution was not observed for any other subcellular organelle. Nothing like a proper separation of mitochondria and peroxisomes was ever achieved but, in a number of experiments, fractions were obtained in which the recovery of serine-pyruvate aminotransferase activity far exceeded the recovery of peroxisomal marker enzymes and vice versa. If we can disregard the notion that a significant portion of the particle-bound serine-pyruvate aminotransferase is associated with a specialized subfraction of the peroxisomes, the subcellular fractionation experiments, we must conclude, point to the mitochondria as the major site of activity in rat liver cells.

Digitonin may be used to isolate submitochondrial particles (Schnaitman & Greenwalt, 1968; Greenwalt, 1974). If sufficiently dilute the digitonin acts only to disrupt the outer membrane (Schnaitman *et al.*, 1967) leaving the inner membrane intact

Table 5. *Enzyme activities of subcellular fractions isolated from glucagon-injected rats on a sucrose gradient designed to separate lysosomes and mitochondria*

A crude mitochondrial fraction was prepared from two rats pre-injected with Triton WR-1339 (see the Methods section) and 24 h after glucagon injection (see the Results section). It was resuspended to obtain the equivalent of 0.2 g of original tissue/ml in 0.4 M-sucrose. Portions (5 ml) were applied over discontinuous sucrose gradients [40–80% (w/v), 15 ml] and, after centrifugation at 93 000 *g* (r_{av} , 9.4 cm) for 180 min, fractions were isolated with the use of a tube slicer. Enzyme activities are as expressed in Table 3.

	Fraction			Glutamate dehydrogenase		Cytochrome oxidase		Acid phosphatase		Urate oxidase		Serine-pyruvate aminotransferase	
	Density (g/ml)	Volume (ml)	% of total volume	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>
(a)	1.05	5.3	26.5	0	0	0	0	38	1.4	0	0	0	0
(b)	1.10	1.5	7.5	1.1	0.15	2.8	0.37	34	4.5	0	0	1.5	0.20
(c)	1.19	2.3	11.5	29	2.52	41	3.57	13	1.13	8.2	0.71	32	2.78
(d)	1.20	5.5	27.5	64	2.33	52	1.89	8	0.29	33	1.20	63	2.29
(e)	1.23	2.4	12.0	2.3	0.19	2.7	0.22	0	0	28	2.33	3.2	0.27
(f)	1.27	3.0	15.0	0	0	0	0	0	0	10	0.67	0	0

Table 6. *Enzyme activities of subcellular fractions isolated from glucagon-injected rats on a sucrose gradient designed to separate mitochondria and peroxisomes*

A crude mitochondrial fraction was prepared from two rats 24 h after glucagon injection (see the Results section) but without Triton pre-treatment. It was resuspended to obtain the equivalent of 0.2 g of original tissue/ml in 50% (w/v) sucrose. The fractionation procedure was as described in Table 5 except that the discontinuous sucrose gradient used was 60–80% (w/v). Enzyme activities are expressed as in Table 3.

	Fraction			Glutamate dehydrogenase		Cytochrome oxidase		Acid phosphatase		Urate oxidase		Serine-pyruvate aminotransferase	
	Density (g/ml)	Volume (ml)	% of total volume	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>	(%)	<i>R</i>
(a)	1.19	7	35	91	2.60	92	2.63	81	2.31	32	0.90	86	2.46
(b)	1.23	4	20	4.5	0.23	1.5	0.08	12	0.60	9.1	0.46	8.7	0.44
(c)	1.24	3	15	1.3	0.09	0.9	0.06	5	0.33	19	1.27	1.5	0.10
(d)	1.25	1.7	8.5	0	0	0	0	0	0	12	1.41	0	0
(e)	1.27	4.3	21.5	0	0	0	0	0	0	10	0.47	0	0

and enclosing the matrix enzymes. As the concentration of digitonin is increased the inner membrane too becomes permeable or is disrupted and the matrix contents leak out (Schnaitman *et al.*, 1967; Cassidy *et al.*, 1972). Digitonin is known to act also on the peroxisomal membrane to release soluble enzymes such as D-amino acid oxidase and catalase, though not urate oxidase, which is associated with the insoluble core of the peroxisomes (de Duve & Baudhuin, 1966). It appears from the present experiments that the critical concentration of digitonin required to solubilize the mitochondrial matrix enzymes (e.g. glutamate dehydrogenase) is higher than that needed for the liberation of the soluble peroxisomal enzymes. It is also clear that the release of serine-pyruvate aminotransferase, with the application of increasing concentrations of digi-

tonin, much more closely parallels the release of the mitochondrial matrix marker than it does that of the peroxisomal enzymes. The results of the digitonin experiments agree with the present findings on separated subcellular fractions in showing mitochondria as the likely site of the particle-bound serine-pyruvate aminotransferase in rat liver.

The present finding that the bulk (>80%) of rat liver serine-pyruvate aminotransferase activity is particle-bound accords with the observations of Snell (1975, 1976) and Noguchi *et al.* (1976). In contrast Hoshino *et al.* (1977) found the larger proportion (about 75%) to be cytosolic. This disagreement may be attributable to the differences in the homogenization medium employed. Hoshino *et al.* (1977) used 0.1 M-sucrose containing 0.1 M-potassium phosphate buffer, pH 7.6, and 1 mM-

EDTA. We have used 0.4M-sucrose with no addition, basing this procedure on earlier findings that salt additions to the sucrose are conducive to the leakage of mitochondrial enzymes (Turner, 1964).

We have no satisfactory explanation for the discrepancy between present findings and those of Noguchi *et al.* (1978) who report a somewhat higher recovery of serine-pyruvate aminotransferase in peroxisomes than in mitochondria after zonal centrifugation on a sucrose gradient.

Our results, indicating a distribution of liver serine-pyruvate aminotransferase activity between mitochondria (about 80%) and cytosol (about 20%), are essentially in agreement with Snell's (1975, 1976) findings for suckling rats. It appears that this distribution is not a special feature of the neonatal stage of development. And the distribution, we find, is still between mitochondria and cytosol when liver serine-pyruvate aminotransferase activity is greatly increased in rats by glucagon injection; the additional activity is apparently in the mitochondria. Fukushima *et al.* (1978) also conclude that this activity is largely located in mitochondria from both normal and glucagon-treated rats, but insufficient detail is given on the distribution of serine-pyruvate aminotransferase and marker enzymes to allow a really critical assessment of their findings.

With the rat some observations have suggested serine-pyruvate aminotransferase as the liver enzyme principally involved in initiating glucose formation from serine, but other findings favour serine dehydratase (see e.g. Rowsell *et al.*, 1973; Sandoval & Sols, 1974). This uncertainty appears to be resolved by the results of experiments made on perfused rat livers and with the rat *in vivo* which indicate that gluconeogenesis from serine is predominantly via hydroxypyruvate in the normally-fed rat but that a much higher rate (approx. $\times 8$) in 24 h starved rats depends on almost equal contributions via pyruvate and hydroxypyruvate (Bhatia *et al.*, 1975). Present work suggests that serine conversion into hydroxypyruvate may occur largely in the mitochondria, and the increase in mitochondrial serine-pyruvate aminotransferase activity in response to glucagon is consistent with a gluconeogenic role for the enzyme sited there, though this is not apparently the mechanism responsible for an enhanced flux via hydroxypyruvate in starved rats. The improved capacity for glucose formation from serine after 24 h starvation is not accompanied by any increase in liver homogenate serine-pyruvate aminotransferase activity. One explanation would be that the portion of the extra gluconeogenesis

attributable to hydroxypyruvate formation was due to activation of the aminotransferase and this possibility deserves investigation with both the mitochondrial and cytosolic enzymes.

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