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Two Forms of Aspartate Aminotransferase in Rat Liver and Kidney Mitochondria

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1. Butan-1-ol solubilizes that portion of rat liver mitochondrial aspartate aminotransferase (EC 2.6.1.1) that cannot be solubilized by ultrasonics and other treatments. 2. A difference in electrophoretic mobilities, chromatographic behaviour and solubility characteristics between the enzymes solubilized by ultrasonic treatment and by butan-1-ol was observed, suggesting the occurrence of two forms of this enzyme in rat liver mitochondria. 3. Half the aspartate aminotransferase activity of rat kidney homogenate was present in a high-speed supernatant fraction, the remainder being in the mitochondria. 4. A considerable increase in aspartate aminotransferase activity was observed when kidney mitochondrial suspensions were treated with ultrasonics or detergents. 5. All the activity after maximum activation was recoverable in the supernatant after centrifugation at 105000*g* for 1hr. 6. The electrophoretic mobility of the kidney mitochondrial enzyme was cathodic and that of the supernatant enzyme anodic. 7. Cortisone administration increased the activities of both mitochondrial and supernatant aspartate aminotransferases of liver, but only that of the supernatant enzyme of kidney.

Recent studies have established the presence of two different forms of GOT* in rat liver (Boyd, 1961; Eichel & Bukovsky, 1961; Katunama, Matsuzawa & Huzino, 1962; Morino, Kagamiyama & Wada, 1964). They differ in electrophoretic mobility (Boyd, 1961; Eichel & Bukovsky, 1961), K_m (Katunama *et al.* 1962) and immunological characteristics (Morino *et al.* 1964). One form is localized in mitochondria and the other is present in the supernatant fraction (Boyd, 1961; Eichel & Bukovsky, 1961). Attempts to solubilize the mitochondrial enzyme by treatment with detergents, ultrasonic treatment, disintegration in a Waring Blendor or freezing-and-thawing resulted in a severalfold increase in activity (Bhargava & Sreenivasan, 1965, 1966*a*,*b*). The increased activity was associated with enzyme that had been solubilized, but the original activity remained pellet-bound (Bhargava & Sreenivasan, 1966*b*) and further treatments of the pellet did not result in activation or solubilization of GOT. Recently Boyd (1966) reported quantitative solubilization of the mitochondrial enzyme by treatment with butan-1-ol. It was therefore decided to test whether butan-1-ol could solubilize the pellet-bound enzyme and, if so, whether pellet-bound enzyme solubilized by butan-1-ol is the same as or different from the enzyme solubilized by other treatments.

A study of GOT in kidney was undertaken to examine the possibility of tissue-specific differences with respect to the occurrence of GOT. The effect of cortisone administration on mitochondrial and supernatant GOT enzymes of rat liver and kidney was also investigated, since there are conflicting reports (Sergio & Cordoba, 1963; Kenney, 1962; Goldstein, Stella & Knox, 1962; Sheid & Roth, 1965; Barnaberi & Serni, 1962) with regard to the induction of liver GOT enzymes by cortisone administration and none for the kidney. It was also decided to test whether there are any tissue-specific differences in induction of the two forms of GOT brought by cortisone.

* Abbreviation: GOT, aspartate aminotransferase (L-aspartate–2-oxoglutarate aminotransferase, EC 2.6.1.1).

EXPERIMENTAL

Preparation of subcellular fractions, treatment of mitochondria, determination of GOT activity, paper electrophoresis and butan-1-ol treatment. Wistar-strain rats maintained on laboratory stock diet were used throughout. Kidneys were perfused via the dorsal aorta with chilled 0·9% NaCl. They were then cut into small pieces with scissors and homogenized in chilled 0·25 M-sucrose in a Teflon–glass homogenizer. The preparation of subcellular fractions (at 2–4°) was carried out as described by Schneider (1948). Isolation of liver mitochondria, determination of GOT activity, treatment with

detergents, ultrasonic treatment and paper electrophoresis were done as described in previous papers (Bhargava & Sreenivasan, 1965, 1966*a*,*b*). GOT activity units are expressed as μmoles of oxaloacetate formed during incubation at 37° for 10 min. and are given as means ± S.E.M. of four independent observations. Butan-1-ol treatment of the pellet obtained after centrifugation of treated mitochondrial suspension and of intact mitochondria was carried out as described by Boyd (1966).

Column chromatography. Column chromatography on a 30 cm. × 1 cm. column of CM-cellulose was carried out after activation and equilibration with 0·1 M-sodium phosphate buffer, pH 6·8, with a gradient of 0·1–1 M-NaCl for elution; 3 ml. fractions were collected.

Cortisone administration. This was done intraperitoneally by injecting cortisone acetate (4 mg./100 g. body wt.) in 0·9% NaCl. Rats were killed after 4–6 hr.

RESULTS AND DISCUSSION

Solubilization and electrophoretic mobility of pellet-bound GOT. The mitochondrial GOT that cannot be solubilized by ultrasonics and other treatments (Bhargava & Sreenivasan, 1966*b*) and remains in the pellet on centrifugation at 105000 *g* for 1 hr. was solubilized by treatment with butan-1-ol (Table 1). The pellet enzyme was almost completely solubilized when the final concentration of butan-1-ol was 20% (v/v) (Table 1).

The paper-electrophoretic mobility of mitochondrial GOT solubilized by ultrasonic treatment (GOT_1) was slower than that of pellet-bound GOT solubilized by butan-1-ol (GOT_2) (Fig. 1).

When the intact mitochondria were treated with butan-1-ol no increase in GOT activity was observed, though the solubilization achieved was similar to that of pellet-bound GOT with butan-1-ol. This suggests that butan-1-ol may be inactivating GOT_1. The effect of increasing the butan-1-ol concentration on the GOT activity of the supernatants obtained from ultrasonically treated mitochondria was therefore studied. The results in Table 2 suggest that at a final concentration of 20% (v/v) butan-1-ol inhibited almost all the activity of the supernatant.

If butan-1-ol inhibits the GOT activity, then a decrease in total GOT activity would be expected when ultrasonically treated mitochondrial suspensions are treated with butan-1-ol. The results in Table 3 show that this is indeed the case. With increasing butan-1-ol concentration there is a decrease in the GOT activity of ultrasonically treated mitochondrial suspension. The decrease in the GOT activity of the sediment obtained after centrifugation of butan-1-ol-treated mitochondrial suspension is apparently due to solubilization of the pellet enzyme. The supernatant GOT activity represents the sum of GOT_1 that is becoming inactivated and GOT_2 that is becoming solubilized with increasing concentrations of butan-1-ol.

Column chromatography of liver mitochondrial GOT. Further confirmation of the existence of two forms of GOT in mitochondria having different electrophoretic mobilities, with butan-1-ol specifi-

Table 1. *Effect of butan-1-ol concentration on the solubilization of pellet-bound GOT of liver mitochondria*

A mitochondrial pellet, obtained by centrifugation of ultrasonically treated mitochondrial suspension, was treated with increasing concentrations of butan-1-ol after suspension in 0·25 M-sucrose, and the GOT activities were determined in whole suspensions and the sediments and supernatants obtained by centrifugation at 105000 *g* for 1 hr. For further details see the text.

Concn. of butan-1-ol (v/v)	GOT activity (units)		
	In pellet suspension	In sediment	In supernatant
0	181·8	181·8	0
5	181·8 ± 15·9	129·5 ± 17·4	46·2 ± 6·8
10	181·8 ± 14·4	92·4 ± 14·4	78·0 ± 9·8
15	181·8 ± 21·2	47·7 ± 6·8	122·0 ± 16·6
20	174·3 ± 23·4	14·4 ± 4·5	162·9 ± 21·9

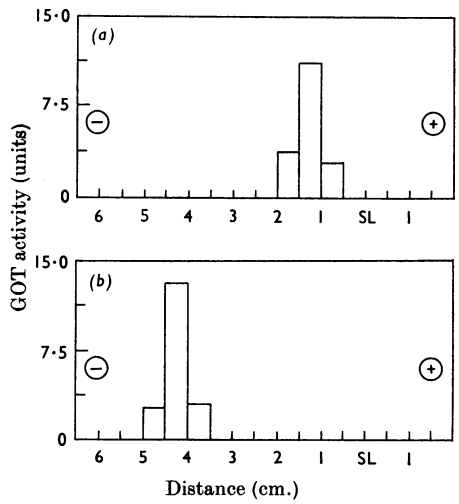

Fig. 1. Electrophoretic mobilities of liver mitochondrial GOT solubilized by ultrasonic treatment and of pellet-bound GOT solubilized by butan-1-ol. Mitochondria from 1 g. of liver were suspended in 2 ml. of 0·25 M-sucrose and treated ultrasonically at 15 kcyc./sec. for 1 min., then centrifuged at 105000 *g* for 1 hr.; the supernatant was spotted for electrophoresis (*a*) and the pellet was treated with 20% (v/v) butan-1-ol and the supernatant spotted for electrophoresis (*b*). The GOT activity in 0·5 cm. segments of the electrophoretograms was measured as described in the text. SL, Starting line.

cally inhibiting one of these, was obtained from chromatographic studies on CM-cellulose. These studies showed a difference in elution pattern: GOT_2 was less firmly bound to the column and was eluted earlier, whereas GOT_1 was more firmly held and eluted later, when the sodium chloride concentration was raised (Fig. 2). When the ultrasonically treated mitochondrial GOT was treated with butan-1-ol at a final concentration of 10% (v/v) and chromatographed, both forms of GOT were present, whereas with 20% (v/v) butan-1-ol only GOT_2 was present.

Two forms of GOT in rat liver mitochondria. These results therefore provide further experimental evidence that there are two forms of GOT in rat liver mitochondria, and that these, besides differing in their binding to membrane, have different electrophoretic, chromatographic and solubility characteristics.

Eichel & Bukovsky (1961) failed to find any differences in pH optima between readily measurable mitochondrial enzyme and that which was unmasked and solubilized by blending. We also were unable to find any such differences between pellet-bound enzyme and the enzyme solubilized by ultrasonic treatment.

It is relevant to point out that more than one form of non-mitochondrial GOT has been reported in cell sap (Decker & Rau, 1963; Martinez-Carrion, Riva, Turano & Fasella, 1965). A close resemblance exists between the two GOT enzymes reported in the present paper and the two glutamate dehydrogenases in mitochondria, differing in degree of attachment and termed tightly and loosely bound, reported by Hirschberg, Snider & Osnos, (1964). Two forms of malate dehydrogenase in liver mitochondria have also been reported (Kitto, Wassarman, Michjeda & Kaplan, 1966). Also, Wilson (1967) has suggested that the assayable and latent forms of hexokinase may have different binding sites in rat brain mitochondria.

Table 2. *Effect of butan-1-ol concentration on the GOT activity of the supernatant obtained by centrifugation of ultrasonically treated liver mitochondria*

Mitochondria obtained from 1g. of liver were suspended in 3ml. of 0·25M-sucrose and treated ultrasonically at 15kcyc./sec. for 1min., then centrifuged at 105000*g* for 1hr., and the supernatant was treated with increasing concentrations of butan-1-ol. For further details see the text and Table 1.

Concn. of butan-1-ol (v/v)	GOT activity in supernatant (units)
0	181·8
5	163·6±15·1
10	113·6± 9·8
15	60·6± 8·3
20	15·9± 6·2
25	—

Table 4. *Intracellular distribution of GOT activity in rat kidney*

Perfused rat kidneys were homogenized in 0·25M-sucrose and subcellular fractions were isolated by differential centrifugation. For further details see the text.

Fraction	GOT activity (units/g. fresh wt. of kidney)	Distribution (%)
Homogenate	4·90±0·40	100
Nuclei	0·35±0·06	7·1
Mitochondria	1·50±0·23	30·7
Microsomes	0·31±0·08	6·2
Supernatant	2·65±0·32	53·0

Table 3. *Effect of butan-1-ol concentration on the GOT activity of ultrasonically treated liver mitochondrial suspension*

Mitochondria obtained from 1g. of liver were suspended in 3ml. of 0·25M-sucrose and treated ultrasonically at 15kcyc./sec. for 15min., and the resulting suspension was treated with increasing concentrations of butan-1-ol. The GOT activity was determined in treated whole suspensions and the sediments and supernatants obtained after centrifugation at 105000*g* for 1hr. For further details see the text and Table 2.

Concn. of butan-1-ol (v/v)	GOT activity (units)		
	In whole ultrasonically treated mitochondrial suspension	In sediment	In supernatant
0	371·2±29·5	181·8±15·9	194·6±20·5
5	319·1±23·5	159·1±17·4	137·1±15·9
10	252·3±31·1	115·9±12·9	125·0±22·0
15	197·9±23·5	67·4±12·1	144·0±15·9
20	179·5±19·7	17·4±8·3	153·8±23·5
25	173·5±20·5	—	167·4±22·7

The previous observation (Bhargava & Sreenivasan, 1965, 1966*a*,*b*) that GOT_1 but not GOT_2 is altered *in vivo* as a result of mitochondrial damage caused by administration of carbon tetrachloride suggests that there are separate control mechanisms for the two forms; further work will be needed to establish this.

Intracellular distribution of kidney GOT activity. The results in Table 4 show that 53% of the GOT activity of kidney homogenate is localized in the supernatant fraction and that 30% is present in the mitochondrial fraction. The activity observed in nuclei and microsomes might be due to contamination with mitochondria.

Solubilization and electrophoretic mobility of kidney GOT. A severalfold increase in enzyme activity was observed when kidney mitochondria were treated with deoxycholate, Triton X-100 or digitonin or when ultrasonically treated (Fig. 3). However, maximum activation per mg. of mitochondrial protein could be obtained by much less drastic treatment than that used with liver mitochondria (Bhargava & Sreenivasan, 1966*a*), suggesting that the enzyme in kidney is more loosely

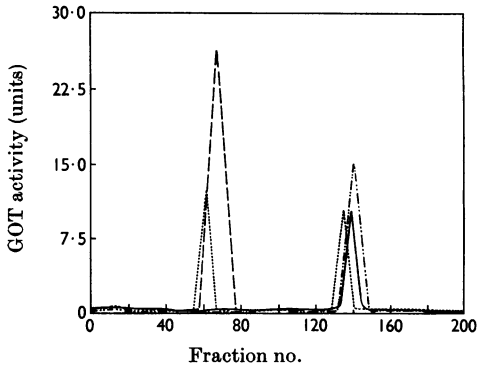

Fig. 2. Elution patterns of liver mitochondrial GOT enzymes after various treatments. Mitochondria obtained from 3g. of liver were suspended in 3ml. of 0·25 M-sucrose and treated ultrasonically at 15kcyc./min. for 1·5min., then centrifuged at 105000*g* for 1hr. A 1ml. sample of the supernatant obtained was chromatographed on the CM-cellulose column (——). The pellet was treated with butan-1-ol and the supernatant obtained after centrifugation and dialysis was chromatographed (••—••). The ultrasonically treated mitochondrial suspension was also treated with butan-1-ol at 10% or 20% (v/v) concentration and the supernatants (------, 10%-butan-1-ol-treated; ——, 20%-butan-1-ol-treated) obtained after centrifugation and dialysis were chromatographed. Fractions of volume 3ml. were collected and GOT activity was determined as described in the text.

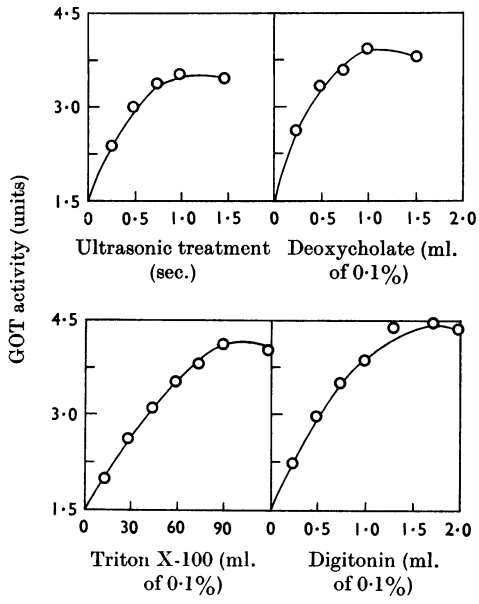

Fig. 3. Effect of various treatments on mitochondrial GOT of rat kidney. Mitochondria obtained from 1g. of kidney were suspended in 1ml. of 0·25 M-sucrose and various treatments were carried out as described in the text.

Table 5. *Activation and solubilization of kidney mitochondrial GOT by various treatments*

Samples (5ml.) of 1:20 mitochondrial suspensions were treated as described in Fig. 1 and then centrifuged at 105000*g* for 1hr., and GOT activity was determined in whole mitochondrial suspension, sediment and supernatant. For further details see the text and Table 1.

Treatment	GOT activity (units)		
	In mitochondrial suspension	In sediment	In supernatant
Nil	1·51±0·15	1·51±0·15	—
Ultrasonic treatment for 90sec.	4·16±0·53	—	3·94±0·75
Deoxycholate (1·5ml. of 0·1%)	4·47±0·83	0·37±0·22	4·31±0·98
Triton X-100 (1ml. of 0·1%)	3·56±0·53	—	3·49±0·45
Digitonin (1ml. of 0·1%)	3·94±0·68	—	3·71±0·68

Table 6. *Effect of cortisone administration on mitochondrial and supernatant GOT enzymes of rat liver and kidney*

A single dose of cortisone was administered intraperitoneally. Rats were killed after 4–6 hr., and the isolation of mitochondrial and supernatant fractions and determination of GOT activity were done as described in the text.

	GOT activity (units/g. of kidney)		GOT activity (units/g. of liver)	
	Mitochondria	Supernatant	Mitochondria	Supernatant
Control	1·74±0·15	2·42±0·37	152·3±31·1	144·0±24·2
Cortisone administered	1·86±0·30	4·47±0·37	240·2±47·7	267·4±38·6

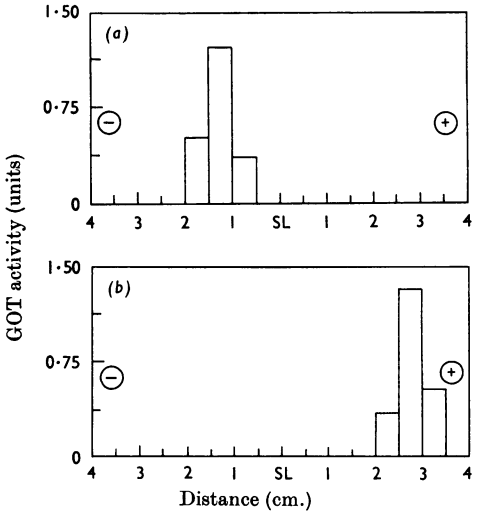

Fig. 4. Electrophoretic mobilities of mitochondrial and supernatant GOT enzymes of rat kidney. Mitochondria obtained from 5g. of kidney were suspended in 2ml. of 0·25M-sucrose and ultrasonically treated for about 2min., then centrifuged at 105000*g* for 1hr.; 0·1ml. was spotted in the centre of the paper (*a*). A 0·2ml. sample of supernatant equivalent to 2g. of kidney was also spotted (*b*). The measurement of GOT activity in 0·5cm. segments of the electrophoretograms was carried out as described in the text. SL, Starting line.

bound. It is, however, also possible that kidney mitochondria are more easily damaged than those from liver. When the treated mitochondrial suspensions, where maximum activation is achieved, were centrifuged at 105000*g* for 1hr., all the activity was recovered in the supernatant (Table 5). This suggests that the latency observed with kidney mitochondria is solely due to permeability barriers and that once the mitochondrial membrane is broken there is maximum substrate–enzyme contact and therefore activity. With liver, however, when the treated mitochondrial suspensions were centrifuged a certain amount of activity always remained pellet-bound and could not be solubilized further by these treatments (Bhargava & Sreenivasan, 1966*b*). Thus kidney, unlike liver, does not have two forms of mitochondrial GOT.

The solubilized kidney mitochondrial enzyme has cathodic mobility on the paper electrophoretogram, compared with the anodic mobility of the kidney supernatant GOT (Fig. 4), as with the liver GOT enzymes.

Effect of cortisone administration on rat liver and kidney GOT enzymes. Cortisone administration leads to an increase in both the mitochondrial and the supernatant liver enzymes, but in the kidney it specifically induces only the supernatant enzyme under the experimental conditions used (Table 6). When cortisone is added *in vitro* to mitochondrial and supernatant fractions there is no increase in activity, suggesting that the increased activity obtained is due not to activation but perhaps to induction, as has been suggested by Segal, Rosso, Hopper & Weber, (1962) and Sheid & Roth (1965). The induction of this enzyme in the supernatant might explain the increased gluconeogenesis brought about by cortisone administration (Gavosto, Pileri & Brusca, 1957; Rosen, Roberts, Budnik & Nichol, 1958), since (1) all the gluconeogenic enzymes are localized in this fraction, and (2) the increased transamination will mean increased channeling of products of amino acids like α-oxoglutarate and oxaloacetate into the carbon pool from which glucose is made (Gavosto *et al.* 1957; Rosen *et al.* 1958).

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