Richardson, E. M., Bulaschenko, H. & Dohan, F. C. (1958). *J. clin. Endocrin.* **18**, 666.
Richardson, E. M., Touchstone, J. C., Dohan, F. C., Bulaschenko, H., Landolt, I. & Applin, W. (1955). *J. clin. Invest.* **34**, 285.
Rimini, E. (1901). *Gazz. chim. ital.* **31**, II, 84.
Romani, J. D. (1956*a*). *C.R. Soc. Biol., Paris*, **150**, 644.
Romani, J. D. (1956*b*). *C.R. Soc. Biol., Paris*, **150**, 887.
Romani, J. D. & Ableaux-Fernet, M. (1956). *Ann. Endocr., Paris*, **17**, 789.
Romani, J. D., Bugard, P. & Fischer, G. (1956). *C.R. Soc. Biol., Paris*, **150**, 1755.
Struck, H. (1956). *Mikrochim. Acta*, p. 1277.
Talalay, P., Fishman, W. H. & Huggins, C. (1946). *J. biol. Chem.* **166**, 757.
Tompsett, S. L. (1953). *J. clin. Path.* **6**, 74.
Touchstone, J. D., Bulaschenko, H. & Dohan, F. C. (1955). *J. clin. Endocrin.* **15**, 760.
Touchstone, J. C., Bulaschenko, H., Richardson, E. M. & Dohan, F. C. (1954). *Arch. Biochem. Biophys.* **52**, 284.

Biochem. J. (1961) **81**, 434

The Intracellular Distribution, Latency and Electrophoretic Mobility of L-Glutamate–Oxaloacetate Transaminase from Rat Liver

BY J. W. BOYD
Biochemistry Department, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge

(*Received* 20 *April* 1961)

Previous studies of the distribution of L-glutamate–oxaloacetate transaminase in subcellular fractions, obtained by differential centrifuging of liver homogenates, have all shown that this enzyme is present in both supernatant and mitochondria, with a somewhat higher percentage of total activity in the supernatant fraction (Müller & Leuthardt, 1950; Asada, 1958; Gaull & Villee, 1960). There are no previous reports of investigations of the latency and activation of L-glutamate–oxaloacetate transaminase in mitochondria, although an increase of activity in liver homogenates during storage has been reported (Schmidt, Schmidt & Wildhirt, 1958) and aging is known to activate latent mitochondrial enzymes. Green, Leloir & Nocito (1945) reported that purified L-glutamate–oxaloacetate transaminase could be separated by electrophoresis into three components and that activity was associated with two of these. It has recently been shown (Fleisher, Potter, Wakim, Pankow & Osborne, 1960) that two L-glutamate–oxaloacetate transaminases with different electrophoretic mobilities have different substrate affinities and pH-dependence. The following experiments show a relationship between the electrophoretic mobility of the enzyme and its localization within the cell.

EXPERIMENTAL

Enzyme-assay procedures

Several procedures were used to assay L-glutamate–oxaloacetate transaminase. Units of transaminase activity were defined as μmoles of oxaloacetate or L-glutamate formed/hr. at 25°.

L-Glutamate–oxaloacetate-transaminase assay by the method of Reitman & Frankel (1957). The amount of oxaloacetate formed during incubation for 1 hr. at 37° was measured colorimetrically with 2:4-dinitrophenylhydrazine. The results were converted into μmoles of oxaloacetate formed/hr. at 25° (King, 1960).

L-Glutamate–oxaloacetate-transaminase assay by the method of Karmen (1955). In this method the rate of formation of oxaloacetate is determined, reduced diphosphopyridine nucleotide (DPNH) and malic dehydrogenase being added to the assay system so that DPNH is oxidized as oxaloacetate is produced. The resulting decrease in extinction at 340 mμ was followed in a Unicam SP. 500 spectrophotometer; the progress curve was linear. A fall in E of 0·01 is equivalent to the formation of 4·83 μm-moles of oxaloacetate in the reaction mixture. The temperature was maintained at 25° by use of a cell housing through which water from a thermostat was circulated. A higher concentration of L-aspartate, 66·6 mM, was used than that in the original method (33·3 mM). The concentration of α-oxoglutarate was 6·7 mM.

It was found that in the presence of NH_4^+ ions some glutamic-dehydrogenase activity is measured in the Karmen method. The commercial malic dehydrogenase added in this test contains $(NH_4)_2SO_4$, 9 mM final concentration. To correct for this the oxidation of DPNH was followed in the presence of all reactants except L-aspartate and this control rate was subtracted from the final rate after adding aspartate, allowance being made for dilution. A correction of up to 10% was sometimes necessary. A slight inhibition of transamination, 2–5%, due to these NH_4^+ ions, was ignored.

L-Glutamate–oxaloacetate-transaminase assay by the method of Cammarata & Cohen (1951). The rate of oxaloacetate production is measured spectrophotometrically at 280 mμ. Preliminary experiments showed that the substrate concentrations in the original descriptions by Cammarata & Cohen (1951) were suboptimum for mitochondrial preparations; hence they were both increased from the

Table 1. *Activation of glutamate–oxaloacetate transaminase in rat-liver homogenates*

The values are means of three or four different experiments. Units are μmoles of DPNH oxidized or oxaloacetate produced/hr./mg. of liver (wet wt.) at 25°. Tramsaminase was assayed by the method of Karmen (1955). For experimental details see text.

	Water homogenate	0·25 M-Sucrose homogenate		
		Untreated	Waring Blendor, 4 min.	Ultrasonic disintegrator, 1 min.
Glutamic dehydrogenase	2·32	0·71	2·88	2·35
L-Glutamate–oxaloacetate transaminase	5·30	1·55	5·75	5·43

20 mM of the original method, L-aspartate to 133 mM and α-oxoglutarate to 50 mM. Preincubation with pyridoxal phosphate was omitted because only crude enzyme preparations were being assayed. 0·1 M-Potassium phosphate buffer, pH 7·5, was used, as in the other methods, instead of 0·05 M-phosphate buffer, pH 7·4. It was found necessary in this assay to include blanks when testing mitochondria to allow for decrease in E, due presumably to swelling of these particles during the assay. The routine blank adopted contained mitochondrial preparation suspended in the phosphate buffer.

L-Glutamate–oxaloacetate-transaminase assay by the method of Müller & Leuthardt (1950). The glutamic acid formed in a given time is measured by the glutamic-decarboxylase method of Krebs (1948). This method was modified by the use of a higher concentration of Cetavlon, as advised by Meister, Sober & Tice (1951).

A further modification was the use of higher substrate concentrations, namely 100 mM-L-aspartate and 50 mM-α-oxoglutarate. Incubation was for 30 min. at 25°. The reaction was stopped by immersion of the reaction mixture for 5 min. in a boiling-water bath. This method gave results linear with respect to enzyme concentration only up to approx. 1·5 μmoles of glutamic acid/hr. An unincubated enzyme-substrate mixture was used as the routine blank.

Glutamic-dehydrogenase assay. Glutamic dehydrogenase was estimated spectrophotometrically with the following reaction mixture: 87 μM-DPNH; 73 mM-potassium phosphate buffer, pH 7·5; 0·167 M-$(NH_4)_2SO_4$; 0·2 ml. of enzyme preparation. After equilibration the reaction was started by the addition of 6·7 mM-sodium α-oxoglutarate, pH 7·5, bringing the total volume to 3 ml. E at 340 mμ was plotted against time in minutes and the result calculated as μmoles of DPNH oxidized/hr. at 25°.

Separation of enzyme fractions from rat liver

Subcellular fractionation. Differential centrifuging of homogenates of rat liver in 0·25 M-sucrose was performed as described by Hogeboom (1955). Mitochondria were washed twice. Ultrasonic disintegration of subcellular fractions was carried out with a MSE–Mullard Ultrasonic Disintegrator, 60 w, with a frequency of approx. 20 kcyc./sec., for 1 min. This instrument was tuned to give maximum agitation in the tissue preparation, which was kept immersed in ice-cold water.

Electrophoretic fractionation. The liver extract for electrophoresis was prepared by homogenizing rat liver in 10 vol. of ice-cold water in a Waring Blendor for 4 min. The insoluble material was removed by centrifuging for 30 min. at 20000g in a MSE refrigerator centrifuge. The supernatant was freeze-dried and redissolved in the electrophoresis buffer when required. In this extract approximately 60% of the original transaminase activity was recovered.

Paper electrophoresis was carried out on Whatman no. 541 paper on a Durram-type apparatus with 30 mM-Na_2HPO_4–KH_2PO_4 buffer, pH 7·5, I 0·1, 7 v/cm., 2 mA for 15 hr. at 4°.

Zone electrophoresis in a sucrose-density gradient was performed with a column electrophoresis apparatus, type LKB 3340, manufactured by LKB-Produkter, Fabriksaktiebolag, Stockholm, Sweden. 5 mM-Na_2HPO_4–KH_2PO_4 buffer, pH 7·5, I 0·03, was used with 650 v, 9 mA for 14 hr. at 15°.

Materials

Purified malic dehydrogenase, α-oxoglutaric acid and DPNH were obtained from C. F. Boehringer und Soehne, Mannheim, W. Germany. L-Aspartic acid was obtained from L. Light and Co. Ltd., L-glutamic acid and D-aspartic acid from Roche Products Ltd. and oxaloacetic acid from Sigma Chemical Co., St Louis, Mo., U.S.A. All these substances were used without further purification.

RESULTS

Latency of L-glutamate–oxaloacetate transaminase in rat-liver homogenates. Table 1 shows the effect of various treatments on the activity of transaminase in rat-liver homogenates. Parallel determinations were made of glutamic dehydrogenase. This was selected as a reference enzyme since it is established as a latent mitochondrial enzyme (Bendall & de Duve, 1960).

Hypo-osmoticity (homogenizing in water), mechanical disintegration of subcellular particles (5 min. in Waring Blendor) and ultrasonic disintegration (1 min.) produced a great increase in activity as measured by the Karmen method. Incubation at 37° for several hours, storage at 4° for several days or treatment with the detergent Triton X-100, 0·06% (w/v), also caused this activation observed by the Karmen method.

The rates of activation of glutamic dehydrogenase and transaminase by mechanical disintegration, are shown in Fig. 1. The explanation of the

activation of glutamic dehydrogenase suggested by Bendall & de Duve (1960) is that the enzyme is largely inaccessible to its substrates until the mitochondrial membrane is damaged. They have shown that this enzyme is liberated into solution by blending but that the increased activity after exposure to media of low concentration is not in solution. Table 2 shows that transaminase is similarly rendered soluble by mechanical and ultrasonic disintegration and not by hypo-osmotic media.

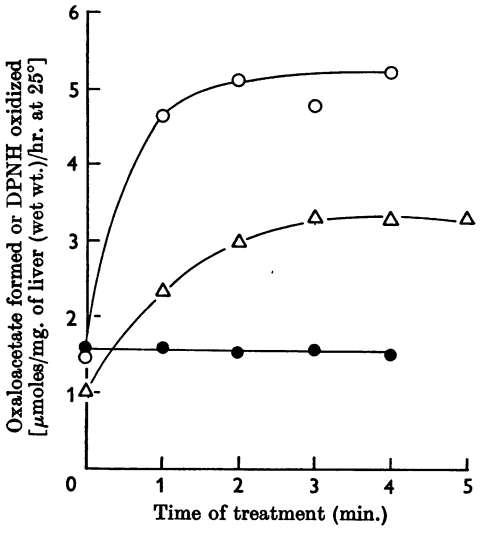

Fig. 1. Effect of mechanical disintegration in a Waring Blendor of a homogenate of rat liver in 0·25 M-sucrose on the glutamic-dehydrogenase and L-glutamic–oxaloacetate-transaminase activities. △, Glutamic dehydrogenase; ●, transaminase (method of Reitman & Frankel, 1957); ○, transaminase (method of Karmen, 1955).

The next step was to see if this latent transaminase activity was attached to certain subcellular particles as with glutamic dehydrogenase.

Intracellular localization of L-*glutamate–oxaloacetate transaminase.* Rat-liver homogenates were fractionated by differential centrifuging and the various cell fractions assayed for transaminase activity before and after ultrasonic disintegration. Once again glutamic dehydrogenase was used as a reference enzyme. Table 3 shows that the latent transaminase resides mainly in the mitochondrial fraction. With the Karmen method there is an increase of 120 % in activity in mitochondria after disintegration.

Electrophoretic mobility of L-*glutamate–oxaloacetate transaminase on paper.* For these experiments samples (5 μl.) of liver extract equivalent to 5 mg. of liver (wet wt.) were used. After electrophoresis some strips of paper were stained for protein with 1 % Light Green and others were cut into sections 5 mm. wide and the protein was eluted with 0·15 M-sodium chloride. These eluates were then assayed for transaminase. Over 90 % of the activity could be recovered from the paper. The

Table 2. *Solubility of* L-*glutamate–oxaloacetate transaminase in liver homogenates after activation procedures*

Transaminase was assayed by the method of Karmen (1955). Units are μmoles of oxaloacetate produced/hr./mg. of liver at 25°. For experimental details see text.

Treatment	Activity before centrifuging	Centrifuged for 30 min., 20000*g*		Recovery (%)
		Deposit	Supernatant	
Water homogenate	4·20	3·10	0·81	93
Sucrose homogenate				
Untreated	2·04	2·19	0·73	142
Waring Blendor, 4 min.	5·63	2·00	2·84	86
Ultrasonic disintegrator, 1 min.	6·15	1·58	3·92	90

Table 3. *Distribution of glutamic dehydrogenase and* L-*glutamate–oxaloacetate transaminase in subcellular fractions of rat-liver homogenates, showing the effect of subsequent ultrasonic disintegration*

Units are μmoles of DPNH oxidized or oxaloacetate formed/hr./mg. of liver (wet wt.) at 25°. Transaminase was assayed by the method of Karmen (1955) and glutamic dehydrogenase as described in the text.

Enzyme	Glutamic dehydrogenase		L-Glutamate–oxaloacetate transaminase	
Ultrasonic disintegration (min.) ...	—	1	—	1
Whole homogenate	0·51	1·66	3·10	6·24
Nuclear fraction	0·06	0·17	0·27	0·71
Mitochondrial fraction	0·56	1·56	1·45	3·25
Microsomal fraction	0·06	0·02	0·19	0·30
Supernatant fraction	0·13	0·04	1·31	1·23
Recovery in fraction (%)	159	108	104	88

results (Fig. 2) confirm the findings of Fleisher *et al.* (1960) that L-glutamate–oxaloacetate-transaminase activity separates into two peaks. One peak (fraction A) was not far from the point of application; the other peak (fraction B) was further from this point; both had moved towards the anode. Fraction A was in a similar position to γ-globulin, and fraction B to α-globulin, of bovine serum on a parallel strip. This suggests that if allowance were made for endosmosis then fraction A would be shown to have moved towards the cathode. It is demonstrated in Fig. 3 that the transaminase of mitochondrial extract after electrophoresis is almost all in one peak, that corresponding to fraction A. The transaminase of supernatant fraction forms a major peak corresponding to fraction B. Its minor fraction A peak could be due to contamination with transaminase from mitochondria during homogenization.

Substrate affinities and pH-*dependence of* L-*glutamate–oxaloacetate transaminase.* Fleisher *et al.* (1960) showed that the two transaminases which they had separated from canine heart had different substrate affinities and pH-dependence curves. Table 4 shows that by the Karmen (1955) method the substrate affinities of two electrophoretic transaminases from rat liver also differ markedly. Moreover, the Michaelis constants of mitochondrial transaminase correspond fairly closely with those of fraction A. The supernatant transaminase and fraction B also correspond in this respect. Fig. 4 shows that with the Karmen method mitochondrial transaminase and fraction A are similar to one another and differed from supernatant transaminase and fraction B in their pH-dependence curves. Both the supernatant transaminase and fraction B have only 40% of maximum activity at pH 6·0. These results demonstrate the presence in the cell of two L-glutamate–oxaloacetate transaminases with different properties, one located principally in the mitochondria and the other in the supernatant fraction.

Comparison of methods of L-*glutamate–oxaloacetate-transaminase assay.* There is a large discrepancy between the results obtained by the method of Reitman & Frankel (1957) and those by the method of Karmen (1955) (Figs. 1, 2). The method of Reitman & Frankel gave very low results for fraction A. Table 5 shows that this method gave a similarly low estimate of the transaminase activity of mitochondria.

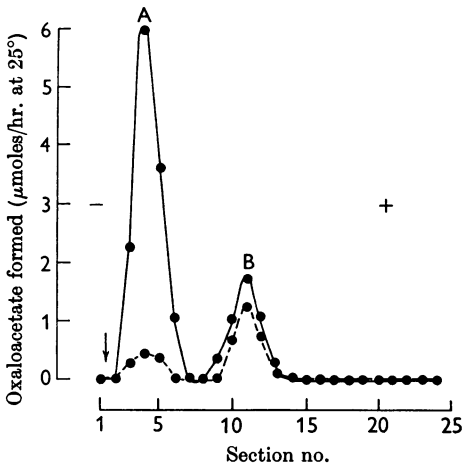

Fig. 2. Distribution of L-glutamate–oxaloacetate transaminase after paper electrophoresis of crude liver extract. A preparation containing 18·9 units (Karmen, 1955, method) and 8·9 units (Reitman & Frankel, 1957, method) of transaminase was applied near the cathode as indicated by the arrow, and recoveries after electrophoresis were 92 and 99% respectively. ●—●, Transaminase by method of Karmen (1955); ●- - -●, transaminase by method of Reitman & Frankel (1957).

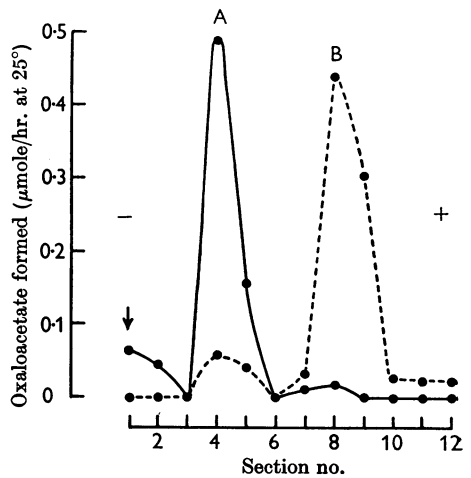

Fig. 3. Distribution of L-glutamate–oxaloacetate transaminase after paper electrophoresis of supernatant fraction (●- - -●) and mitochondrial extract (●—●) from rat liver. The two curves have been superimposed on one another so that their points of application (shown by an arrow) coincide. The supernatant fraction was obtained by centrifuging a 10% sucrose homogenate at 20000*g* for 30 min. The mitochondrial extract was prepared by subjecting the resuspended pellet, after washing it twice, to ultrasonic disintegration (1 min.) and removing the insoluble material by centrifuging at 20000*g* for 30 min. These preparations were applied in 5 μl. samples. Purified bovine serum albumin (1%) was added to the buffer used in order to stabilize the enzyme. The sections of paper were 10 mm. wide and were added directly to the assay mixture because of the low concentration of enzyme. Otherwise the experimental details were as described in the text.

Table 4. *Michaelis constants for* L-*glutamate–oxaloacetate transaminase in fractions from rat liver*

The Karmen method was used and K_m values obtained from Lineweaver–Burk plots. All points fell satisfactorily near a straight line. Fractions A and B were obtained by paper electrophoresis of rat-liver extract.

Transaminase fraction	No. of units of enzyme used (μmoles of oxaloacetate/hr. at 25°)	K_m for α-oxoglutarate (mM with 33·3 mM-L-aspartate)	K_m for L-aspartate (mM with 6·66 mM-α-oxoglutarate)
Mitochondrial*	1·4	1·00	0·47
Fraction A	1·3	0·95	0·36
Supernatant	0·6	0·20	2·15
Fraction B	0·5	0·16	3·70

* Activated by ultrasonic disintegration for 1 min.

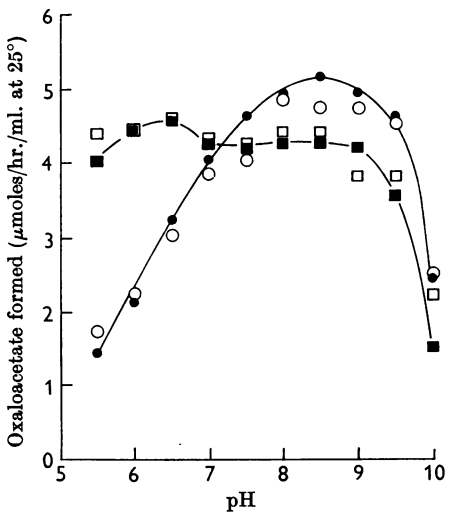

Fig. 4. Effect of change of pH on activity of L-glutamate–oxaloacetate transaminase as assayed by the method of Karmen (1955). The pH was altered from 7·5 by the addition of small amounts of N-HCl or N-NaOH to the substrates. □, Mitochondrial fraction (activated by ultrasonic disintegration); ○, supernatant fraction; ■, fraction A; ●, fraction B. Fractions A and B were obtained by density-gradient electrophoresis.

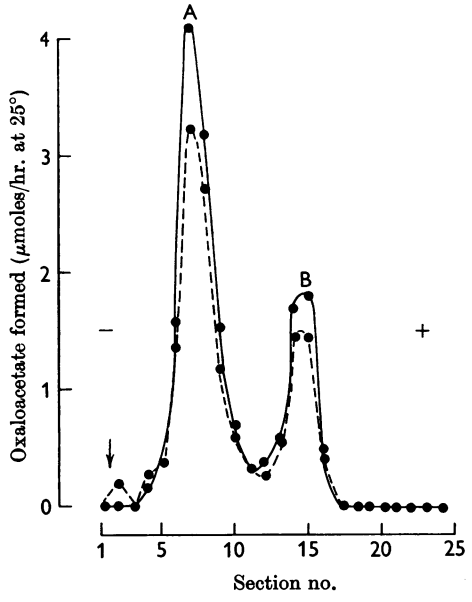

Fig. 5. Distribution of L-glutamate–oxaloacetate transaminase after paper electrophoresis of crude liver extract. A preparation containing 16 units by the method of Karmen (1955) was applied near the cathode as indicated by the arrow; 104% of this activity was recovered. ●—●, Transaminase (method of Karmen, 1955); ●- - -●, transaminase (method of Cammarata & Cohen, 1951, modified as described in the text).

In an attempt to resolve the difference between the results of the method of Reitman & Frankel and the method of Karmen, the subcellular and electrophoretic fractions were assayed by the method of Cammarata & Cohen (1951). The results in Fig. 5 and Table 5 show a close resemblance to the results of the method of Karmen. In the method of Cammarata & Cohen substrate concentrations were increased because with 20 mM-α-oxoglutarate only 70% of the total activity of fraction A was measured and 20 mM-L-aspartate was suboptimum for fraction B, giving only 80% of total activity. The α-oxoglutarate concentration needed was much higher than in the Karmen method, indicating that fraction A had a much lower affinity for α-oxoglutarate in the assay procedure of Cammarata & Cohen than in the Karmen method. The same was true, but to a much lesser extent, with fraction B. On the other hand, affinity for L-aspartate was not significantly affected by the assay procedure.

The progress curves with the Cammarata & Cohen method were linear for fraction B and supernatant transaminases but non-linear for fraction A and mitochondrial transaminase (Fig. 6). The progress curves for all transaminase fractions were linear in the Karmen method in which oxaloacetate

Table 5. *Distribution of* L-*glutamate–oxaloacetate transaminase in subcellular fractions of rat-liver homogenates assayed by different methods and the effect of ultrasonic disintegration*

Units are μmoles of oxaloacetate or glutamate formed/hr./mg. of liver (wet wt.) at 25°.

Assay method	Cammarata & Cohen (1951)		Reitman & Frankel (1957)		Müller & Leuthardt (1950)	
Ultrasonic disintegration (min.) ...	—	1	—	1	—	1
Whole homogenate	2·32	4·74	1·28	1·58	1·05	1·12
Nuclear fraction	0·34	0·94	0·05	0·05	—	—
Mitochondrial fraction	0·56	3·44	0·29	0·38	0·25	0·57
Microsomal fraction	0·40	0·38	0·01	0·02	—	—
Supernatant fraction	0·80	0·76	1·11	1·10	0·67	—
Recovery in fractions (%)	91	116	95	98	—	—

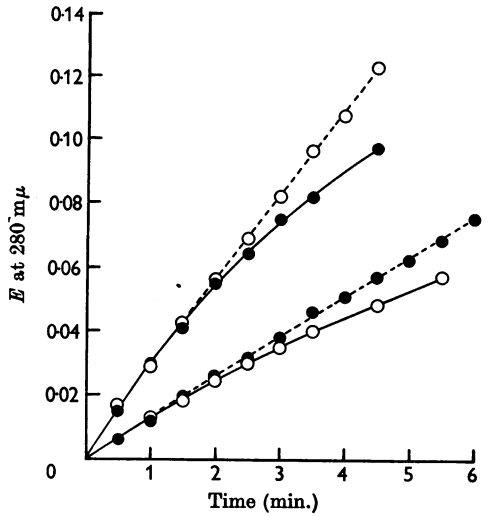

Fig. 6. Progress curves for L-glutamate–oxaloacetate-transaminase fractions assayed by a modification of the method of Cammarata & Cohen (1951). ●—●, Mitochondrial transaminase (activated by ultrasonic disintegration); ○—○, fraction A; ○- - -○, supernatant transaminase; ●- - -●, fraction B.

formed is removed. These results suggest that when oxaloacetate accumulates it inhibits transamination by fraction A and mitochondrial transaminase. The finding that affinities for α-oxoglutarate are lower in the Cammarata & Cohen method, particularly with mitochondria and fraction A, indicates that the oxaloacetate inhibition may be partially competitive.

It is concluded that the failure of the method of Reitman & Frankel can be attributed to the low α-oxoglutarate concentration (2 mM) and to inhibition by oxaloacetate.

L-Glutamate formation by L-*glutamate–oxaloacetate-transaminase fractions.* In order to prove that oxaloacetate measured in the above-described methods was formed by a transamination reaction, it was necessary to show that L-glutamate was being

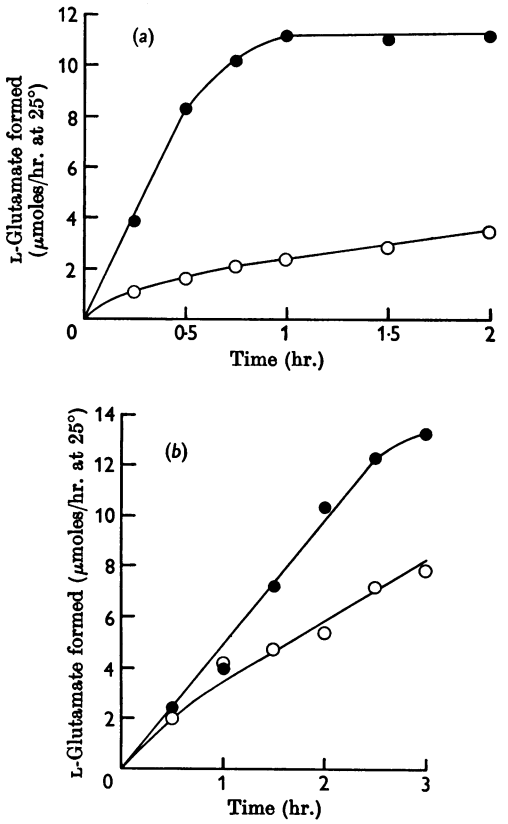

Fig. 7. (*a*) Effect of oxaloacetate reduction on activity of fraction A. A sample of fraction A capable of producing 14 μmoles of oxaloacetate/hr., as measured by the method of Karmen (1955), was assayed for L-glutamate production by means of glutamic decarboxylase (○). Acceleration was achieved by the addition of 5 μg. of purified malic dehydrogenase and 10 μmoles of DPNH (●). (*b*) A similar experiment with fraction B capable of producing 4·6 μmoles of oxaloacetate/hr. as measured by the method of Karmen (1955). The fractions used in this experiment were obtained by density-gradient electrophoresis, details of which are given in the text.

formed at the same rate. For this purpose the method of Müller & Leuthardt (1950) was examined. The concentrations of substrates were found to be suboptimum and were increased. Nevertheless, the glutamate formed in this method was much less than the oxaloacetate formed in the methods of Karmen and Cammarata & Cohen with both mitochondria and supernatant fractions (Table 5) and with electrophoretic fractions (Fig. 7). However, owing to the relative insensitivity of this assay procedure, much higher concentrations of oxaloacetate were accumulating than in the method of Cammarata & Cohen. When purified malic dehydrogenase and DPNH were added to the reaction mixture to reduce the oxaloacetate during the reaction, as in the Karmen method, glutamate production by fraction A and fraction B reached the concentrations expected and had a linear progress curve as long as DPNH was available (Fig. 7). The rate of L-glutamate production over the linear part of the curve for fraction A was 16·5 μmoles/hr. compared with 14 μmoles of oxaloacetate/hr., whereas the respective rates for fraction B were 4·9 and 4·6 μmoles/hr.

DISCUSSION

Hird & Rowsell (1950) pointed out that preoccupation with soluble transaminase preparations had tended to divert attention from the more general transamination reactions of the cell. They found that the glutamate–phenylpyruvate transaminase was located solely in the insoluble-particle fraction of rat-liver homogenates, and that transamination to form tyrosine, alanine and aspartate was also catalysed by these particle suspensions. The experiments reported here show that previous work on the distribution of L-glutamate–oxaloacetate transaminase in the cell has led to the under-estimation of the importance of transamination between L-aspartate and α-oxoglutarate in mitochondria. The main reason for this underestimation appears to be the latency of this transaminase in undamaged mitochondria. A contributory cause has been the failure of certain assay methods to measure the true activity of mitochondrial L-glutamate–oxaloacetate transaminase. This failure is shown to be due in part to the fact that mitochondrial transaminase differs in substrate affinities and other properties from supernatant transaminase. In three of four commonly used assay procedures substrate concentrations were found to be suboptimum, particularly for the mitochondrial enzyme. Furthermore, transamination from aspartate is inhibited as oxaloacetate produced accumulates. This inhibition, which is apparently partly due to oxaloacetate competing with α-oxoglutarate for the enzyme, affects the mitochondrial enzyme more than the supernatant one. Only the Karmen (1955) method, in which oxaloacetate formed is reduced to malate by added malic dehydrogenase and DPNH, gives linear progress curves at the optimum rate with mitochondrial transaminase. The results from the Karmen (1955) and modified Cammarata & Cohen (1951) methods show that there is three or four times as much L-glutamate–oxaloacetate transaminase in the mitochondria after activation as in the supernatant fraction of liver cells.

A large number of investigators have studied changes in L-glutamate–oxaloacetate-transaminase activity in the tissues and body fluids of patients with various diseases and of experimental animals subjected to various interferences, e.g. treatment with hormones. Unfortunately, as well as using the unsatisfactory assay procedures discussed above, many workers in this field have used fresh isoosmotic tissue preparations kept refrigerated and stored for the minimum time. Hence changes in activity due to changes confined to the mitochondrial enzyme may have been underestimated, whereas elevations in activity recorded may have been merely due to unmasking of latent enzyme.

The discovery that mitochondrial transaminase differs from supernatant transaminase in substrate affinities, pH-dependence and electrophoretic mobility allows an interesting parallel to be drawn between L-glutamate–oxaloacetate transaminase and malic dehydrogenase, which has recently been shown to exist in two forms, one localized in the mitochondria and the other in the supernatant fraction of rat-liver homogenates (Thorne, 1960). Further work is necessary to ascertain whether the two types of enzyme are in fact distinctly different proteins. Sophianopaulus & Vestling (1960) have claimed that the supernatant form of malic dehydrogenase may be converted into the mitochondrial form simply by treatment with butan-1-ol.

It is possible that further purification might reveal the existence of other types of L-glutamate–oxaloacetate transaminase. In preliminary experiments it has been found that whereas fraction B and supernatant fractions of transaminase can react only with L-aspartate, both fraction A and mitochondrial fractions react also with D-aspartate although at a slower rate and with lower affinity. It has not yet been found whether this results from lack of stereospecificity in the mitochondrial enzyme or whether there is a further enzyme which reacts with the D-amino acid.

SUMMARY

1. Methods of L-glutamate–oxaloacetate-transaminase assay (with α-oxoglutarate and L-aspartate as substrates) have been compared and modified. Maximum activity could be readily achieved only

with the assay system of the method of Karmen (1955).

2. L-Glutamate–oxaloacetate transaminase has been shown to be present in rat-liver mitochondria in latent form. After activation the activity of transaminase in mitochondria is three to four times that in the supernatant fraction.

3. Activation has been achieved by using hypo-osmotic media, incubation at 37°, storage at 4°, treatment with Triton X-100, mechanical disintegration and ultrasonic disintegration. With ultrasonic and mechanical disintegration, but not with hypo-osmotic treatment, this activation is accompanied by release of the enzyme in soluble form.

4. It has been shown that mitochondrial transaminase differs from supernatant transaminase in its substrate affinities, pH-dependence and electrophoretic mobility.

5. Two L-glutamate–oxaloacetate transaminases separated electrophoretically from rat-liver extract appear to correspond to the mitochondrial and supernatant enzymes respectively.

6. Transamination from L-aspartate is inhibited by the oxaloacetate produced.

I wish to acknowledge with thanks the helpful advice and criticism received from Dr G. D. Greville, and the technical assistance given by Miss A. Saltmarsh and Mr R. Braham. My thanks are also due to Dr A. D. Bangham and Mr R. J. Hall for advice and assistance with the electrophoretic procedures.

REFERENCES

Asada, M. (1958). *Med. J. Osaka Univ.* **9**, 45.
Bendall, D. S. & de Duve, C. (1960). *Biochem. J.* **74**, 444.
Cammarata, P. S. & Cohen, P. P. (1951). *J. biol. Chem.* **193**, 53.
Fleisher, G. A., Potter, C. S., Wakim, K. G., Pankow, M. & Osborne, D. (1960). *Proc. Soc. exp. Biol., N.Y.*, **103**, 229.
Gaull, G. & Villee, C. A. (1960). *Biochim. biophys. Acta*, **39**, 560.
Green, D. E., Leloir, L. F. & Nocito, V. (1945). *J. biol. Chem.* **161**, 559.
Hird, F. J. R. & Rowsell, E. V. (1950). *Nature, Lond.*, **166**, 517.
Hogeboom, G. S. (1955). In *Methods in Enzymology*, vol. 1, p. 16. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Karmen, A. (1955). *J. clin. Invest.* **34**, 131.
King, J. (1960). *J. med. Lab. Technol.* **17**, 1.
Krebs, H. A. (1948). *Biochem. J.* **43**, 51.
Meister, A., Sober, H. A. & Tice, S. V. (1951). *J. biol. Chem.* **189**, 591.
Müller, A. F. & Leuthardt, F. (1950). *Helv. chim. acta*, **33**, 268.
Reitman, S. & Frankel, S. (1957). *Amer. J. clin. Path.* **28**, 56.
Schmidt, E., Schmidt, F. W. & Wildhirt, E. (1958). *Klin. Wschr.* **36**, 172.
Sophianopaulus, A. J. & Vestling, C. S. (1960). *Biochim biophys. Acta*, **45**, 400.
Thorne, C. J. R. (1960). *Biochim. biophys. Acta*, **42**, 175.

Biochem. J. (1961) **81**, 441

Characterization of Tissue Alkaline Phosphatases and their Partial Purification by Starch-Gel Electrophoresis

By D. W. MOSS, DIANA M. CAMPBELL, EFFIE ANAGNOSTOU-KAKARAS* and E. J. KING
Postgraduate Medical School, London, W. 12

(*Received* 1 *May* 1961)

Attempts have been made to assign the alkaline phosphatase present in abnormal amounts in human blood plasma in certain diseases to an origin in a given tissue by a study of the properties of the enzyme increment. These attempts have yielded only inconclusive results. [References may be found in the recent review by Gutman (1959).] Studies of this nature are impeded by the presence in serum of inhibitors such as albumin (Henneman, Rourke & Jackson, 1955) and bile acids (Bodansky, 1937), of activators, such as metal cations, and of a factor of an unknown nature which appears to be present in icteric serum (Cantarow, 1940). Amino acids may inhibit or activate depending on their concentration (Bodansky, 1946). The properties of alkaline phosphatase in serum may reflect therefore not so much the properties of the enzyme as those of its environment.

Electrophoresis on starch gel (Smithies, 1955) permits a refined separation of the serum proteins

* Present address: Evangelismos Hospital, Athens, Greece.