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Transaminations with L-Glutamate and α -Oxoglutarate in Fresh Extracts of Animal Tissues

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When the present work was begun current opinion was that biological transamination involved only glutamic, aspartic and cysteic acids and alanine (Cohen & McGilvery, 1949); subsequently Feldman & Gunsalus (1950), using bacterial extracts, demonstrated syntheses of phenylalanine and tyrosine by transaminations with L-glutamate, and Cammarata & Cohen (1950) observed transamination to α -oxoglutarate, with soluble extracts of freeze-dried pig tissues, from a wide range of L- α -amino acids.

We first investigated the possibility that L-glutamine, rather than L-glutamate, might function as an amino donor to some α -keto acids. With homogenates and washed sedimentable particles of rat liver, the formation of phenylalanine was observed chromatographically from phenylpyruvate and L-glutamine, but greater formation was indicated in L-glutamate controls. Transamination with *p*-hydroxyphenylpyruvate and L-glutamate, forming tyrosine, was also observed, and from some L- α -amino acids to α -oxoglutarate. Transaminases which catalyse the formation of phenylalanine and tyrosine were located in the sedimentable particles of fresh rat-liver homogenates, in particular in mitochondria. A preliminary report of this work has been published (Hird & Rowsell, 1950), and a detailed account is now presented.

The formation of leucine by transamination is also described. Transamination in other fresh tissue extracts is reported, and more quantitative data have been obtained with a recording photodensito-

meter on paper chromatograms, and with bacterial amino acid decarboxylases. Observations with the same tissue preparations indicate that transamination to α -oxoglutarate is a more powerful mechanism than aerobic oxidation, for the deamination of L- α -amino acids.

MATERIALS AND METHODS

Phenylpyruvic acid. This was synthesized from freshly distilled benzaldehyde via β -acetamidocinnamic acid (Herbst & Shemin, 1939). It was recrystallized from benzene.

p-Hydroxyphenylpyruvic acid was synthesized by the same procedure, with *p*-hydroxybenzaldehyde as starting material. The solid acids were kept in sealed tubes at -15° .

α -Oxoisocaproic acid was prepared by the enzymic deamination of D-leucine. An acetone-dried powder of sheep-kidney cortex was prepared and extracted with 0.1M sodium pyrophosphate buffer, pH 8.3, according to the method of Keilin & Hartree (1936). After dialysis against buffer, observations were made, with Warburg manometers, to determine conditions for a large-scale oxidative deamination. Then 1 g. of DL-leucine was dissolved in water and added to the bulk of the extract. Incubation was at 30° for 12 hr. with continuous aeration. (Samples were withdrawn for examination in manometers, and it was observed that a high level of oxidation was maintained for the whole period.) At the end of the incubation proteins were precipitated by heating, after adjusting to pH 3.0 with conc. HCl. Denatured protein was centrifuged down and the supernatant evaporated to dryness at 60° under reduced pressure. From the solid residue α -oxoisocaproic acid was brought into ether solution in a Soxhlet extractor. Evaporation of the ether solution produced a syrup which solidified *in vacuo*. The yield was poor (about 50 mg.) and it was therefore decided to use this crude product without further purification.

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Oxaloacetic acid (Wohl & Oesterlin, 1901) and α -oxoglutaric acid (Friedman & Kosower, 1946) were synthesized by Mr B. R. Slater of the Department of Biochemistry, Cambridge. Pyruvic acid was redistilled and stored at 0° as an 0.33M aqueous solution at pH 5.4. α -Keto acids were dissolved and brought to the pH of the tissue preparation immediately before use.

L- α -Amino acids were obtained from Roche Products Ltd., Welwyn Garden City. L-Glutamic and L-aspartic acids were neutralized before use. When L-tryptophan was used, 12.2 mg. of the solid was added to an incubation volume of 2.0 ml. to give a 0.03M solution. With L-tyrosine an excess of solid was used to give a saturated solution in the incubations. With DL-amino acids amounts were used to give the required concentration of the L-isomer.

Buffer solutions. Phosphate buffers were made up from appropriate mixtures of Na_2HPO_4 and KH_2PO_4 solutions.

Tissue preparations

Norwegian piebald rats were killed by bleeding, after stunning. The liver (about 9 g.) was immediately removed to crushed ice. It was finely chopped with scissors, homogenized in a Potter-Elvehjem homogenizer (Potter, 1949) with 10 ml. of 0.1M phosphate buffer, pH 7.4, at 0°, and strained through cotton gauze.

Sedimentable particles. The homogenate was centrifuged in the high-speed head of a refrigerated centrifuge (International) at 0° and 10 000 g for 30 min. The supernatant was set aside for further treatment or discarded. The sediment was usually washed three times by dispersion in 50 ml. of 0.1M phosphate, pH 7.4, followed by re-sedimentation (10 000 g for 20 min.). The sedimented particles were finally taken up to 15–20 ml. in 0.02M phosphate, pH 7.4. Experiments with particles preparations were started about 4 hr. after the death of the animal.

Supernatant fractions of rat-liver homogenates. These were clarified by centrifuging twice at 18 000 g in the refrigerated centrifuge for 1 hr., followed by gravity filtration through Whatman no. 42 paper. After dialysis against 0.1M phosphate buffer, pH 7.4, at 0–5°, and again centrifuging at 18 000 g, the volume was made up to 10–15 ml. with buffer. Experiments with supernatant fractions were normally started on the day following the death of the animal.

Mitochondria were prepared by differential centrifuging from rat liver homogenized in 0.88M sucrose, according to the procedure of Hogeboom, Schneider & Pallade (1948). After washing with 0.88M sucrose, the sedimented mitochondria were dispersed in 15 ml. of 0.02M phosphate, pH 7.8.

Rat-liver acetone-dried powder was prepared according to a method for pig liver (Keilin & Hartree, 1936). A soluble extract was obtained by grinding 3 g. of powder with 30 ml. of 0.1M phosphate buffer, pH 7.7, in a mortar. After extraction for 30 min. the mixture was centrifuged. The supernatant was poured off, and the sediment was dispersed in 30 ml. of buffer. Both fractions were dialysed against buffer.

Other tissues were treated as for rat liver to obtain suspensions of sedimentable particles, and supernatant fractions. Two rat kidneys were used to obtain 10 ml. of particles and 7 ml. of supernatant. Pig liver (10 g.; obtained from the abattoir) gave 15 ml. of both particles and supernatant. Two mouse livers, combined weight 5 g., gave 10 ml. of both particles and supernatant. A chicken liver

gave 20 ml. of particles, and a pigeon liver (5 g.) yielded 11 ml. of unfractionated dialysed homogenate. Except for pig liver all tissues were obtained from freshly killed animals.

Dry weights of tissue preparations were obtained after 4–5 hr. at 100°. Dry weights for buffer alone were subtracted.

Incubations were at 37° under N_2 . Except where otherwise indicated, test tubes were used and the total volume was 2.0 ml., including substrates and 1.0 ml. of tissue preparation. The reactions were stopped by the addition of warm ethanol, usually 8.0 ml. The denatured protein was removed by centrifuging and the supernatants were evaporated to dryness at 60–70° under reduced pressure. For chromatography, the solid residues remaining in the evaporation flasks were dissolved in a known volume (usually 2.0 ml.) of water.

For photodensitometer measurements, reactions were stopped by heating the test tubes in boiling water for 3 min. The denatured protein was centrifuged down and the supernatants were taken for chromatographic analysis.

All solutions awaiting analysis were stored at –15°.

Paper chromatography

The ascending-boundary technique was normally employed, with Whatman no. 1 paper. Collidine-lutidine (1:1, v/v) saturated with water (Dent, 1948), propan-1-ol-water (4:1, v/v) and phenol saturated with water (Dent, 1948) have been used to separate the monoaminomono-carboxylic α -amino acids from glutamate. Water-saturated phenol gave spots varying in area and intensity according to the amount of amino acid present. It was used where amino acid syntheses were assessed by visual comparison with known amounts. Propan-1-ol-water gave more compact spots and has been preferred for photodensitometer estimations. Water-saturated phenol and phenol-acetic acid-water (4:5:5, by vol.) have been used to separate glutamic and aspartic acids. Samples (1 or 5 $\mu\text{l.}$, or 10 $\mu\text{l.}$ for low concentrations) of incubation extracts and amino acid standards were run. Since only two amino acids were involved, short runs (about 7 in.) were enough usually to achieve adequate separation in the appropriate solvent. Papers were dried in a stream of warm air, sprayed with 0.25% ninhydrin in water-saturated *n*-butanol and heated at 80° for 10 min.

In some experiments more objective assessments have been made with a recording reflexion photodensitometer (Model 2SR, Joyce, Loebel and Co. Newcastle upon Tyne). After separation and colour development, strips have been cut out across the paper, including all glutamic acid spots. Absorptions have been plotted, and glutamic acid, formed by transamination, was evaluated by comparing the weights of paper cut out under the absorption curves. Examples are given of such curves (Fig. 1) to indicate the sensitivity of the method and the variation in the background absorption. Duplicate 5.1 $\mu\text{l.}$ samples of L-glutamate solutions, in the range 0–2.0 mg./2.0 ml. have been compared on the same chromatograms with the photodensitometer. Good agreement was observed but the amounts of glutamate applied and the absorptions observed were not in linear relationship. Moreover, variations were observed for the same amounts of glutamate on different paper chromatograms. It was necessary to run incubation extracts with standards close in concentration on the same chromatogram.

With the photodensitometer, incubation extracts have been matched with one of a series of standards in the range

Fig. 1. Typical absorption curves, from strips containing glutamate spots, plotted with a recording photodensitometer after separation on paper chromatograms. Weights of paper (mg.), cut out under absorption curves, are shown. Extracts of 2.0 ml. incubation samples (supernatants taken after heat coagulation and removal of protein): (a), (f) and (j), α -oxoglutarate sole substrate; α -oxoglutarate and (d) L-methionine, (h) L-tyrosine, and (l) L-leucine. [(a), (d), (f) and (h) with rat-liver particles; (j) and (l), rat-kidney particles.] Standards: glutamate/2.0 ml., (b) 0.1 mg., (c) 0.3 mg., (e) and (g) 0.5 mg., (i) and (k) 1.0 mg., (m) 2.0 mg.; 5.1 μ l. samples were run on ascending chromatograms, Whatman no. 1 paper, in propan-1-ol-water.

0–2.0 mg./1.0 ml. (differing by 0.1 mg./2.0 ml.). In order to work in its range of greatest sensitivity, incubation extracts were diluted, where necessary, to bring them below 2.0 mg. of glutamate/2.0 ml.

Manometric analyses of L-tyrosine and L-glutamate

The amounts formed or disappearing by transamination have been determined in some experiments, with specific bacterial decarboxylases (Gale, 1948).

An acetone-dried powder of *Streptococcus faecalis* (N.C.T.C., no. 6783), provided by Dr W. G. Crewther, was used in suspension in 0.2M sodium citrate-phosphate buffer, pH 5.5, as a source of specific L-tyrosine decarboxylase.

Washed suspensions of *Clostridium welchii* SR12 (N.C.T.C., no. 6784) were used as a specific L-glutamic acid decarboxylase. Cultures were provided by Dr E. F. Gale and the organism was grown for about 16 hr. at 30° (Gale, 1948).

For L-glutamic acid decarboxylase assay, reactions were stopped by adding 5 ml. of warm ethanol to each 2.0 ml. of reaction mixture. Denatured protein was centrifuged down and the supernatant retained. The sediment was re-extracted with 7 ml. of ethanol-water (5:2, v/v) and again centrifuged. The combined supernatants were evaporated to a small volume under reduced pressure at 50–60° and dried in

a desiccator. For manometric analysis, residues were dissolved in a known volume of 0.2M sodium acetate buffer, pH 4.6. Following this sequence of operations, 99% recovery was observed after adding 50 μ moles of L-glutamic acid to 1.0 ml. of washed rat-liver particles to give a total volume of 2.0 ml.

Q_T values (Cohen & Hekhuis, 1941) have been calculated in some cases; where possible (L-tyrosine and L-glutamate decarboxylase estimations), from manometric data, otherwise from the formula

$$\frac{\mu\text{moles of amino acid} \times 22.4}{\text{mg. dry wt. of tissue} \times \text{time in hr.}}$$

RESULTS

Formation of phenylalanine, tyrosine and leucine by transamination

Phenylpyruvate, *p*-hydroxyphenylpyruvate and α -oxoisocaproate, each 0.03M, were separately incubated for 1 hr. with 0.06M L-glutamate, with washed particles of rat liver. Chromatography indicated that phenylalanine, tyrosine and leucine respectively were formed (Fig. 2). Control incuba-

tions with each α -keto acid, or with each α -keto acid and ammonia (Fig. 2) or with L-glutamate as sole substrate, gave little or no amino acid synthesis. Controls with boiled-liver preparation gave no amination of the α -keto acids by L-glutamate.

The liver preparation had not been completely washed free of endogenous amino acids (Fig. 2). In subsequent experiments particles were more thoroughly washed (see Methods section). Chromatograms were then obtained on which the only amino acid spots visible were those of added substrates or reaction products.

With incubations under N_2 it is unlikely that there could have been any considerable oxidation of L-glutamate. Nevertheless, the possibility was investigated that the amination of the α -keto acids might require the metabolic products of L-glutamate oxidation, namely α -oxoglutarate, ammonia and reduced diphosphopyridine nucleotide (Dewan, 1938), rather than glutamate itself. The first experiment was repeated, with phenylpyruvate and *p*-hydroxyphenylpyruvate, with some different controls. These contained 0.03M phenylpyruvate or 0.03M *p*-hydroxyphenylpyruvate, 0.06M ammonium α -oxoglutarate and a metabolic supply of reduced diphosphopyridine nucleotide. This was

provided by 0.1M β -hydroxybutyrate and diphosphopyridine nucleotide (0.5 mg./ml.), after the observation had been made manometrically that washed particles of rat liver contain a powerful β -hydroxybutyrate dehydrogenase. Controls with phenylpyruvate or *p*-hydroxyphenylpyruvate as sole substrate were also included. Chromatography again showed amination of phenylpyruvate and *p*-hydroxyphenylpyruvate after incubation with 0.06M L-glutamate. Trace formations of phenylalanine and tyrosine were observed in the respective controls where ammonium α -oxoglutarate was present. Glutamate formation also was observed chromatographically in these controls, presumably from ammonia, α -oxoglutarate and β -hydroxybutyrate, by reactions of dehydrogenase coupled with diphosphopyridine nucleotide (Dewan, 1938). It was concluded that the observed amination of α -keto acids was by transamination from L-glutamate.

Comparison of sedimented particles with supernatant for transaminations with L-glutamate. With dialysed supernatants of rat-liver homogenates transaminations were observed chromatographically with pyruvate or oxaloacetate and L-glutamate, but not with phenylpyruvate or *p*-hydroxyphenyl-

Fig. 2. Transamination with L-glutamate and phenylpyruvate, *p*-hydroxyphenylpyruvate or α -oxoisocaproate. Rat-liver homogenate particles were twice sedimented in 30 ml. of 0.1M phosphate, pH 7.4, at 5000 *g* for 15 min. and then dispersed in 15 ml. of 0.01M phosphate, pH 7.4. Chromatogram shows the formation of (3) phenylalanine, (7) tyrosine, and (11) leucine, after incubating particles with 0.06M L-glutamate and (3) phenylpyruvate, (7) *p*-hydroxyphenylpyruvate and (11) α -oxoisocaproate, each 0.03M. (4) 0.4 mg. of L-phenylalanine, (8) 0.4 mg. of L-tyrosine and (12) 2.0 mg. of L-leucine were incubated under the same conditions. (1), (5) and (9) were controls with each α -keto acid as sole substrate, and (2), (6) and (10) with each α -keto acid and 0.06M-NH₄Cl. Amino acids from each incubation were extracted into 0.4 ml. of 0.01N-HCl. Samples (5 μ l.) were run on a descending chromatogram in water-saturated collidine-lutidine.

pyruvate. With washed particles transaminations were observed with each of these α -keto acids. For example, with a particulate preparation, on incubating phenylpyruvate, *p*-hydroxyphenylpyruvate and pyruvate, each 0.03 M, with 0.035 M L-glutamate, syntheses of about 1 mg. in 20 min. and 2 mg. in 40 min. of phenylalanine, tyrosine and alanine were indicated chromatographically. Tyrosine decarboxylase estimations showed a formation of 0.77 mg. of L-tyrosine in 20 min. and 1.46 mg. in 40 min. None was formed in a control incubation of 40 min. with *p*-hydroxyphenylpyruvate as sole substrate. (The standard L-tyrosine, 0.70 mg., gave 90 % of theoretical value.) Chromatograms indicated that the supernatant fraction did not catalyse the formation of phenylalanine or tyrosine, but in 40 min. gave about 2 mg. of alanine.

Tyrosine decarboxylase is specific for L-tyrosine (Gale, 1948). Apparently a large proportion, at least, of the tyrosine formed by transamination was the L-isomer.

Transaminations with rat-liver mitochondria. Incubations were carried out with a suspension of mitochondria in 0.01 M phosphate, pH 7.8, with 0.05 M L-glutamate and phenylpyruvate, *p*-hydroxyphenylpyruvate, pyruvate and oxaloacetate, each 0.025 M. In 40 min. 2 mg. of phenylalanine, 2 mg. of tyrosine, 1.0–2.0 mg. of alanine and 4 mg. of aspartate were formed. With another mitochondrial preparation [two extra washings and sedimentations, cf. Hogeboom *et al.* (1948)], the ability to catalyse the L-glutamate-pyruvate transamination had almost disappeared, although transamination reactions leading to the formation of about 1 mg. of phenylalanine, 0.5–1.0 mg. of tyrosine and 2.0–3.0 mg. of aspartate in 40 min. were still apparent.

Transaminations with α -oxoglutarate in liver and kidney preparations

Transaminations with L- α -amino acids and α -oxoglutarate, with preparations of rat liver and kidney (Table 1), and with liver preparations of other animal species (Table 2), have been studied chromatographically. Tissue dry weights were measured where glutamate formation was estimated with the photodensitometer, and Q_T values have been calculated.

Incubations with D-phenylalanine were included in one experiment. No formation of glutamate above the control was observed, with either particles or supernatant. (Results with L-amino acids in the same experiment are recorded in Table 1 (b).)

The results (Table 1) indicate that transaminations to α -oxoglutarate from L- α -amino acids other than alanine and aspartate are especially associated with the sedimentable particles of rat liver and kidney. An exceptional result was obtained in one experiment with rat liver, Table 1 (c). Transamina-

tion from L-tyrosine in particular, with the supernatant, was comparable in this case with that catalysed by the particles. It may be significant that here there was a delay of several hours before clarification of the homogenate supernatant could be started. The particles and supernatant of pig liver differed little in transaminase activity (Table 2). Cammarata & Cohen (1950) observed transamination to α -oxoglutarate from many L- α -amino acids, including L-phenylalanine and L-tyrosine, with soluble extracts of freeze-dried pig liver and kidney. It remains to be investigated how firmly the transaminases are attached to freshly prepared tissue particles.

Neither the particles nor the supernatant of mouse liver would catalyse transaminations from L-phenylalanine or L-leucine to α -oxoglutarate, though transaminations to pyruvate were observed (see Rowsell, 1956).

Oxygen consumption with L- α -amino acids in tissue preparations

To compare transamination to α -oxoglutarate, (i) with aerobic oxidation, (ii) as a deamination mechanism, O_2 consumption has been measured with L- α -amino acids, both washed particles and supernatants of rat-liver and -kidney homogenates being tested.

Except for the absence of α -oxoglutarate and the substitution of O_2 as gas phase, incubation conditions in the studies of O_2 consumption were the same as for transamination. In some experiments, preparations from the same animal were used for both transamination and aerobic oxidation [(c) and (d), Tables 1 and 3]. In many cases O_2 consumption in the presence of L- α -amino acids was less than in the control. Where additional O_2 consumption was observed it was small (Table 3; see also Table 4 of Rowsell, 1956). These results may be compared with transamination measurements [Table 1, especially (c) and (d)]. The formation of 1.47 mg. of glutamate by transamination (i) and the consumption of 112 μ l. of O_2 (ii) lead to the same deamination.

Transaminases for the L- α -amino acids tested in fresh rat liver and kidney appear to be much more effective than the aerobic L- α -amino acid oxidase extracted by Blanchard, Green, Nocito & Ratner (1944).

Equilibrium position for the L-phenylalanine- α -oxoglutarate transamination

The progressive formation of glutamate, during 4 hr. incubation, with rat-liver particles, 0.03 M L-phenylalanine and 0.03 M α -oxoglutarate, is

Table 1. *Transamination with α -oxoglutarate in rat-liver and rat-kidney preparations*

Incubations included 0.03 M L- α -amino acid and 0.03 M α -oxoglutarate [except (b): 0.02 M α -oxoglutarate]. Glutamate formed was separated on paper chromatograms and estimated with the photodensitometer, except in (a), where visual comparisons were made. Values of Q_T are given in parentheses. Albino rats were used, except in (a) (Norwegian piebald). Two livers were pooled to obtain 32 ml. of particles and 18 ml. of supernatant for (c), Table 3 (c) and Table 4. Four kidneys were pooled to obtain 22 ml. of particles for (d) and Table 3 (d). (b) Liver, weight 10.3 g., gave 25 ml. of particles and 31 ml. of supernatant. (c) Two kidneys, combined weight 1.8 g., gave 13 ml. of particles.

Tissue	Dry Incuba- tion wt. of (mg./ml.) (min.)	Control, α -oxo- glutarate alone	Phenyl- alanine	Tyrosine	Trypto- phan	Glutamate formed (mg./2.0 ml.)							Nor- leucine	Isoleucine	Alanine	Aspartate	
						Leucine	Valine	Methio- nine									
Rat liver																	
(a) Particles	—	0	1.0	0.5	0.7	0.2	0.1	0.2*	—	—	—	3	4				
Supernatant	—	0	0.2	0.1	0.1	0	0	0	—	—	—	4	3				
(b) Particles	23	<0.1	2.2 (9.3)	0.9 (3.6)	—	0.4 (1.3)	—	0.5 (1.8)	0.4* (1.3)	—	—	—	—				
Supernatant	15	<0.05	0.2	—	—	0.1	—	0.05	—	—	—	—	—				
(c) Particles	29	0.05	1.7 (8.7)	1.0 (5.0)	1.1 (5.5)	0.4 (1.8)	0.3 (1.3)	0.3 (1.3)	—	—	—	—	—				
Supernatant	23	0	0.3	0.9 (5.7)	0.3	0.1	0.1	0.05	—	—	—	—	—				
Rat kidney																	
(d) Particles	6	0.1	0.5 (10.2)	0.3 (5.1)	0.3 (5.1)	2.2 (53)	1.8 (43)	0.8 (18)	—	—	—	—	—				
Supernatant	11	0	0.1	—	—	0.3	0.2	0.05	—	—	—	—	—				
(e) Particles	4	0.05	0.5 (11.4)	—	—	1.9 (47)	2.0 (49)	0.5 (11.4)	0.6* (14)	1.6* (39)	—	—	—				

* DL-Amino acid.

Table 2. *Transamination with α -oxoglutarate in liver of different animal species*

Incubations included 0.02M α -oxoglutarate and 0.03M L- α -amino acid. Glutamate was estimated by visual comparison with known amounts, after separation on paper chromatograms.

Species	Liver preparation	Glutamate formed (mg./2.0 ml.)				
		Control, α -oxoglutarate alone	Phenyl- alanine	Leucine	Valine	Methionine
Pig	Particles	0.05	0.3	0.3	0.5	0.2
	Supernatant	0	0.3	0.3	0.3	0.1
Mouse	Particles	0	0	0	—	—
	Supernatant	0	0	0	—	—
Chicken	Particles	0.05	0.3	0.1	0.1	0.3
Pigeon	Whole dialysed homogenate	0.1	>1.0	>1.0	—	—

Table 3. *Effect of L- α -amino acids on O₂ consumption in rat-liver and rat-kidney preparations*

Oxygen consumption was measured in Warburg manometers at 37° with O₂ gas phase. There was 0.2 ml. of 40% NaOH and filter paper in centre wells. After equilibration, each L- α -amino acid was added from the side bulb to give a concentration 0.03M in 2.0 ml., including 1.0 ml. of tissue preparation. Albino rats were used.

Rat tissue	Preparation	Dry weight (mg./ml.)	O ₂ consumption (μ l./hr.)						
			Control, no amino acid	Phenyl- alanine	Tyrosine	Trypto- phan	Leucine	Valine	Methionine
(c) Liver	Particles	29	32.0	23.5	14.0	24.5	27.5	25.5	33.0
	Supernatant	23	13.0	17.0	11.0	18.5	14.5	14.5	16.5
(d) Kidney	Particles	6	18.0	16.5	7.5	21.5	20.0	12.0	20.0
(f) Kidney	Particles	5	10.0	12.0	10.0	10.0	13.5	14.0	16.0
	Supernatant	8	8.0	13.5	—	—	7.5	10.0	—

Table 4. *Equilibrium position for L-phenylalanine + α -oxoglutarate $\rightarrow$ glutamate*

A series of 2.0 ml. incubations with rat-liver particles contained 0.03M L-phenylalanine and 0.03M α -oxoglutarate, or α -oxoglutarate alone. After separation on paper chromatograms glutamate was measured with the photodensitometer.

Incubation period (min.)	Glutamate (mg./2.0 ml.)	
	α -Oxoglutarate alone	L-Phenylalanine + α -oxoglutarate
0	<0.05	0.3
30	—	1.3
60	0.1	1.9
120	0.1	2.8
240	0.3	3.4
Calc. for 100% transamination	—	8.8

shown in Table 4. The change in glutamate concentration in the second 2 hr. of the incubation was less than 20% of the total formed. It may be assumed from the results given in Table 6 that the enzyme activity was still at a high level after 3 hr. incubation. It seems that equilibrium conditions were being approached at the end of the incubation with about 40% transamination.

Transamination with L-phenylalanine and α -oxoglutarate assayed with L-glutamic decarboxylase

Effect of pH and salt concentration. The results (Table 5) show that over the range of pH and salt concentration tested there was no systematic variation in transaminase activity. Coagulation of the particles occurred (perhaps enzyme inactivation) during incubation with the highest salt concentration. For subsequent experiments a pH range of 7.4–7.7, with a buffer concentration of 0.05M phosphate was chosen.

Stability at 37°. Table 6 shows that there is little diminution of L-phenylalanine- α -oxoglutarate transaminase activity following 3 hr. incubation, and that after repeated washings substrates which are able to form L-glutamate on prolonged incubation remain attached to the particles.

Comparison of the rates of the forward and reverse reactions α -oxoglutarate + L-phenylalanine $\rightleftharpoons$ L-glutamate + phenylpyruvate. It is apparent from Fig. 3 that the forward and reverse reactions proceed at about equal rates. (Chromatograms showed formations of 0.5–1.0 and 1.0–2.0 mg. of phenylalanine at 30 and 60 min. respectively, for the 2.0 ml. samples of the L-glutamate-phenylpyruvate incubation.)

Table 5. *Effect of pH and salt concentration on L-phenylalanine- α -oxoglutarate transamination*

Sedimented washed rat-liver particles were suspended in 27 ml. of 0.1 M phosphate, pH 7.4. Five 5.0 ml. portions were centrifuged in the high-speed head. The re-sedimented particles were separately suspended in (a) 3.0 ml. of 0.1 M phosphate buffer of pH 6.6, 7.0, 7.4 and 7.7, and sodium pyrophosphate, pH 8.3; (b) 5.0 ml. of 0.02 M phosphate buffer, pH 7.6, at different KCl concentrations. Particle suspensions were incubated with L-phenylalanine and α -oxoglutarate, each 0.03 M, for 60 min. pH values, measured on duplicates of incubation mixtures, are recorded with incubation salt concentrations. Controls contained α -oxoglutarate as sole substrate. Residues from the incubations were dissolved in 2.0 ml. of 0.2 M acetate, pH 4.6, and 1.0 ml. samples were taken for decarboxylase assay. Tissue dry weights: (a) 25 mg./ml., (b) 27 mg./ml.

	L-Glutamate in 2.0 ml. incubation sample (as μ l. of CO ₂)	Q_T (controls subtracted)
(a) 0.05 M Phosphate, pH 7.56 (control)	8	—
0.05 M Phosphate, pH 7.00	92	—
0.05 M Phosphate, pH 7.30	120	—
0.05 M Phosphate, pH 7.56	126	4.9
0.05 M Phosphate, pH 7.65	124	—
0.05 M Sodium pyrophosphate, pH 8.3	118	—
(b) 0.01 M Phosphate, pH 7.6 (control)	6	—
0.01 M Phosphate, pH 7.6	126	—
0.01 M Phosphate, pH 7.6, + 0.02 M-KCl	148	—
0.01 M Phosphate, pH 7.6, + 0.03 M-KCl	145	—
0.01 M Phosphate, pH 7.6, + 0.05 M-KCl	142	—
0.01 M Phosphate, pH 7.6, + 0.1 M-KCl	162	5.8

Table 6. *Stability of L-phenylalanine- α -oxoglutarate transaminase at 37°*

Incubations (8.0 ml.) included (a) 4.0 ml. of freshly prepared washed rat-liver particles and (b) 4.0 ml. of the same preparation after 3 hr. at 37°, with L-phenylalanine and α -oxoglutarate, each 0.03 M. Controls contained α -oxoglutarate as sole substrate. Samples (3.0 ml.) were denatured after 30 and 60 min. For glutamate estimations at 0 min. 1.5 ml. of tissue preparation was denatured and the equivalent amounts of substrates were added. Residues from all samples were dissolved in 2.5 ml. of 0.2 M acetate, pH 4.6, and 1.8 ml. was taken for decarboxylase assay.

Substrates	Incubation period (min.)	L-Glutamate from 3.0 ml. incubation sample (as μ l. of CO ₂)	
		Obs.	Net
(a) L-Phenylalanine + α -oxoglutarate	0	8	—
	30	114	106
	60	204	190
α -Oxoglutarate, control	30	8	—
	60	14	—
(b) L-Phenylalanine + α -oxoglutarate	0	31	—
	30	132	96
	60	205	166
α -Oxoglutarate, control	30	36	—
	60	39	—

Transaminations with L- α -amino acids in rat-liver and rat-kidney preparations. A limited survey has been made of transamination rates with L- α -amino acids in rat-liver and rat-kidney preparations, with decarboxylase assays. The results are shown in Table 7.

Fig. 3. Comparison of rates of forward and reverse reactions: α -oxoglutarate + L-phenylalanine $\rightleftharpoons$ L-glutamate + phenylpyruvate. Incubations (vol. 12.0 ml.) included 6.0 ml. of washed rat-liver particles (tissue dry wt., 24 mg./ml.), with (a) L-phenylalanine and α -oxoglutarate, and (b) L-glutamate and phenylpyruvate (all 0.025 M). Residues from 2.0 ml. incubation samples were dissolved in 5.0 ml. of 0.2 M acetate, pH 4.6, and 1.0 ml. was taken for decarboxylase assay. Q_T values: (a) 10.5, (b) 8.1.

Table 7. *Decarboxylase assay of transamination with L- α -amino acids in rat-liver and rat-kidney preparations*

L- α -Amino acids and α -oxoglutarate, each 0.03 M, were incubated with rat-liver and rat-kidney preparations. Pyridoxal phosphate (10 μ g./ml.; a gift from Dr W. E. Knox) was added in incubations with acetone-dried-powder extracts (c) and (d). Control values with α -oxoglutarate as sole substrate have been subtracted. Q_T values in parentheses.

Tissue preparation	Tissue dry wt. (mg.)	Incubation period (min.)	L-Glutamate from each 2.0 ml. incubation sample (as μ l. of CO ₂)						
			Control, α -oxo-glutarate alone	Phenyl-alanine	Tyrosine	Tryptophan	Leucine	Valine	Glycine
Rat-liver particles	—	35	9	77	14	17	12	—	—
	16	60	9.5	71 (4.4)	28 (1.7)	0	4 (0.25)	—	—
Rat-liver acetone-dried powder	—	60	8.5	10.5 } 7*	29.5	4	0	0	—
	—	30	—	—	—	—	—	—	—
(d) Insoluble fraction	—	60	0	0	12.5	37.5	0	0	—
	—	30	—	—	—	—	—	—	—
Rat-kidney particles	4	60	0	17 (4.3)	12.5 (3.1)	10 (2.5)	94 (24)	83 (21)	10 (2.5)
	—	—	—	—	—	—	—	—	—

* Without pyridoxal phosphate.

DISCUSSION

Apart from those involving glutamate, aspartate and alanine, there have been doubts as to the metabolic importance of transamination reactions (Cohen, 1942, 1951; Cohen & McGilvery, 1949; Haurowitz, 1950). This can be assessed where quantitative comparisons are possible between metabolic rates of intact tissues and transamination rates, measured for extracts of the same tissues, and preferably where the enzyme preparations employed suffer minimum change in extraction. Studies with unwashed whole homogenates (e.g. Awapara & Seale, 1952) can give useful information on the overall amino transfers possible in tissues, but leave doubt as to the individual transamination steps involved. Fresh liver and kidney, for example, contain amino acids (Krebs, Eggleston & Hems, 1949; Awapara, 1949) and α -keto acids (Frohman, Orten & Smith, 1951) which may act catalytically as intermediate amino carriers (O'Kane & Gunsalus, 1947). Present results have been obtained mainly with washed particles extracted in the cold from fresh tissues.

The results in Table 5 suggest that pH and salt concentration are not critical factors for obtaining optimum transamination rates (at least for phenylalanine) with washed-particle preparations. Nor apparently is temperature. The L-phenylalanine- α -oxoglutarate transaminase is stable at 37° (Table 6) and decarboxylase estimations of L-tyrosine formation indicate that L-glutamate-*p*-hydroxyphenylpyruvate transamination is maintained, apparently undiminished, during a 40 min. incubation at 37°.

Comparison of data in Tables 1, 5 and 6 and in Fig. 3 with those of Table 4 (1.47 mg. of glutamate = 224 μ l. of CO₂) indicates that L-phenylalanine- α -oxoglutarate transamination is a long way from the equilibrium position after 1 hr. incubation, under present conditions. Assuming that the equilibrium position for other monoaminomonocarboxylic acids is comparable with that for L-phenylalanine and that accurately determined for L-alanine (Krebs, 1953), this has not been a limiting factor on rates measured in the present study.

The effect of varying substrate concentration has not been investigated, but it is of interest that L-tyrosine, present as a saturated solution (0.003–0.006 M, calculated from data of Dunn, Ross & Read, 1933), gave rates comparable with 0.03 M L-phenylalanine in some experiments (Table 1).

Pyridoxal phosphate may be an activator, and to a different extent with each L- α -amino acid tested for transamination (Cammarata & Cohen, 1950). It was not available except for one experiment (Table 7). It may be necessary to modify present conclusions on the distribution of transaminases

between different homogenate fractions, when these experiments are repeated with the addition of pyridoxal phosphate.

Many factors remain to be investigated to determine optimum conditions for the measurement of transamination rates in fresh tissue preparations. Under optimum conditions the relative rates for a series of L- α -amino acids with the same tissue preparations may be different. However, provisionally, present results suggest that (apart from those involving L-aspartate and L-alanine) transaminations with L-phenylalanine, L-tyrosine and L-tryptophan in rat liver, and with L-leucine and L-valine in rat kidney, are at a higher level in those tissues than with the other L- α -amino acids tested (Tables 1 and 7).

Great variability was observed in the relative rates of transamination for different L- α -amino acids, with liver preparations from different animals, with different methods of extracting transaminases (from fresh tissue or acetone-dried powder) or comparing liver with kidney (Tables 1 and 7). These findings suggest that there are many transaminases, perhaps one for each amino acid (see also Cammarata & Cohen, 1950). It is not to be expected, therefore, even under optimum conditions, that transaminations studied in fresh tissue preparations with individual amino acids will be so active as, say, succinate oxidation, which is on the main route for the metabolic oxidation of all carbohydrate and fat (see, for example, Krebs, 1954).

Accurate measurement of transamination rates has not been the purpose of the present exploratory investigation. Nevertheless, comparisons will be made with deamination rates observed in kidney and liver slices, to indicate that, for some L- α -amino acids, transamination rates in fresh liver and kidney extracts are of an order to warrant further study and consideration of a metabolic role. Present results were obtained mainly with the unfractionated particles of tissue homogenates. According to the sedimentation technique used they presumably contained mitochondria (cf. Hogeboom, Claude & Hotchkiss, 1946), but nuclei and cell debris were also present. [For rat liver the dry weight of material sedimented under these conditions is about 45% of the total dry weight of the fresh tissue (Dr I. T. Oliver, personal communication).]

For the oxidative deamination of 0.05 M L- α -amino acids with rat-kidney slices, Q_{NH_3} values of 2.53 for L-valine, 5.35 for L-leucine and 9.07 for L-phenylalanine have been observed (control values were subtracted for no added amino acid; data from Table 5, Krebs, 1933). Maximal Q_{NH_3} values of 3.01 for L-tyrosine and 3.13 for L-tryptophan were observed under the same conditions (data from Table 6, Krebs, 1933). Values of Q_T observed in the present work with rat-kidney particles for these

amino acids are comparable (considerably greater for L-leucine and L-valine; Tables 1 and 7).

It has been calculated from the data in Table 1 (e) that transamination rates are 93 and 97 μ moles of glutamate formed/g. wet weight of rat kidney/90 min. for L-leucine and L-valine respectively. For this calculation only the transaminase activity of the particles recovered after repeated sedimentations has been taken into account. This must involve an underestimation. [Complete data for calculating these rates is not available for other experiments with rat kidney, but it is evident that they are of the same order (Table 1 (d), Table 7).] These figures may be compared with values for ammonia formation by amino acid deamination with rat-kidney slices; Braunstein & Azarkh (1944) found 5 and 7 μ moles of ammonia/g. wet weight of rat kidney for L-leucine and L-valine respectively, for 1.5–2.5 hr. aerobic incubation at 37°.

Krebs (1933) measured urea formation as an index of deamination with rat-liver slices, and found Q_{urea} to be 0.38 for L-tyrosine, 0.05 for L-tryptophan. Values of Q_T observed with rat-liver particles may be compared (Table 1).

Present results show that the sedimentable material of fresh rat kidney and liver catalyses transamination with some L- α -amino acids much more rapidly than rat-kidney and rat-liver slices promote oxidative deamination. L-Glutamic dehydrogenase coupled with diphosphopyridine nucleotide is found in rat liver and kidney (Q_o , 49.3 and 24.7 respectively; Copenhaver, McShan & Meyer, 1950) and at higher levels than in other tissues (see also Euler, Adler, Gunther & Das, 1938). Thus, the enzymes required for oxidative deamination via transamination with α -oxoglutarate (suggested by Braunstein, 1939, 1947) are present in liver and kidney.

Consistent with this hypothesis, the present work shows that transamination to α -oxoglutarate is at a much higher level than direct aerobic oxidation for L- α -amino acids in fresh rat-liver and rat-kidney preparations (cf. Tables 1 and 3).

The highest transamination rates measured, with rat-kidney preparations, in the present study were with L-leucine and L-valine (Tables 1 and 7). Apart from L-glutamate, aspartate and alanine, highest rates of oxidative deamination have been observed with L-leucine and L-valine in rat-kidney slices (Braunstein & Azarkh, 1944). This correlation is again consistent with a transamination step in L- α -amino acid deamination.

Present work has demonstrated the formation of phenylalanine, tyrosine and leucine, from the corresponding α -keto acids, by transamination with L-glutamate in rat-liver preparations. These reactions may account for the amination of α -keto acids to form phenylalanine, tyrosine and leucine

observed in liver-perfusion experiments (Embden & Schmitz, 1910, 1911), and for the *in vivo* amination of α -keto acids apparent from feeding experiments (see Bubl & Butts, 1949). With a purified enzyme from rat liver, Meister & Tice (1950) have observed amination of α -keto acids with L-glutamine, without its prior deamination. Other experiments are necessary to assess the physiological significance of each system.

SUMMARY

1. Transamination reactions have been studied in freshly obtained extracts of liver and kidney mainly from the rat. To facilitate unambiguous interpretation of results, preparations have been washed free of endogenous substrates, particles by repeated sedimentation, soluble extracts by dialysis.

2. Formations of phenylalanine, tyrosine and leucine from the corresponding α -keto acids and L-glutamate, with particles of rat liver, have been observed chromatographically.

3. Transaminations with L- α -amino acids (other than alanine and aspartate) and α -oxoglutarate, and with the corresponding α -keto acids and L-glutamate, are associated with the sedimentable particles of fresh rat-liver and rat-kidney homogenates.

4. Quantitative studies with a photodensitometer on paper chromatograms, and with L-glutamic decarboxylase, show that glutamate formation with rat-liver and rat-kidney preparations is more rapid than aerobic oxidation, for all L- α -amino acids tested.

5. Values of Q_T for transamination with α -oxoglutarate with some L- α -amino acids, in rat kidney and liver, are comparable with Q_{NH_3} or Q_{urea} values for oxidative deamination in slices.

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