Transaminations with Pyruvate and other α-Keto Acids

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It was considered that limitations in analytical technique had previously directed attention largely to transamination reactions involving L-glutamate and α-oxoglutarate. By means of paper-partition chromatography (e.g. Consden, Gordon & Martin, 1944), other amino acid–α-keto-acid pairs could be studied for transamination.

Results now presented show that in liver preparations of some animal species, L-α-amino acids other than L-glutamate will transaminate with α-keto acids other than α-oxoglutarate. Pyruvate especially has been investigated as an amino acceptor. Evidence is given that transaminations with pyruvate do not involve α-oxoglutarate–L-glutamate as an intermediate amino carrier. For the L-α-amino acids concerned, transamination with pyruvate takes place more readily than aerobic oxidation.

A preliminary report of this work has been published (Rowsell, 1951).

MATERIALS AND METHODS

L-α-Amino acids, α-keto acids and tissue preparations used were obtained or made, in general, as described in the earlier paper (Rowsell, 1956).

Pure sodium α-oxobutyrate and α-oxo*iso*caproate were generously provided by Dr A. Meister, Bethesda, U.S.A. When 5 μl. samples of 0·05M L-alanine were run on paper chromatograms a trace ninhydrin-reacting impurity was observed, running just behind alanine in water-saturated phenol. This may have been threonine (see Dent, 1948). According to chromatograms other amino acids used were pure.

Particle preparations were thoroughly washed, at least five times by redispersion and resedimentation at 10000 g in about 50 ml. of 0·1M phosphate, pH 7·7. Usually particles from each rat liver were finally made up to 20 ml. in the same buffer. The clarified supernatant measured 20–25 ml.

Incubations. These were carried out in test tubes at 37° under N_2, for 1 hr. Incubations (vol. 2 ml.) included 1·0 ml. of tissue preparation, 0·02M α-keto acid and 0·03M L-α-amino acid. (Any differences from these conditions are indicated.) Reactions were stopped by placing the tubes in boiling water for 3 min. The coagulated protein was centrifuged down and the clear supernatants were taken for chromatographic analysis. These were stored at −15° until used.

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Chromatographic technique. This was, in general, as described in the earlier paper (Rowsell, 1956). It has been reported that there is considerable destruction of α-amino acids by reaction with phenol, when this solvent is removed from papers by heating (Brush, Boutwell, Barton & Heidelberger, 1951). Papers were therefore dried at room temperature in a draught of air. Estimations of amino acids, formed by transamination, were made from chromatograms by visual comparison with standards, except for one experiment (Table 2) where a photodensitometer was employed, (see Rowsell, 1956).

D-α-*Amino acid oxidase.* This enzyme was extracted from 2 g. of sheep-kidney acetone-dried powder (Keilin & Hartree, 1936) by shaking with 20 ml. of 0·017M sodium pyrophosphate buffer, pH 8·3, for 45 min. at 37°. After centrifuging, the supernatant was dialysed against the same buffer.

RESULTS

Transaminations to pyruvate with rat-liver particles

Transamination reactions between α-oxoglutarate and many L-α-amino acids have been demonstrated with washed-particle preparations of rat liver (Rowsell, 1956). With such preparations, as a first approach, pyruvate was investigated as a possible amino acceptor with a number of L-α-amino acids. It was observed chromatographically that 0·3–0·5 mg. of alanine was formed in 1 hr. with pyruvate and L-leucine, L-phenylalanine, L-norleucine, L-histidine or L-methionine, but none with D-phenylalanine, D- or L-valine or DL-isoleucine. Controls with pyruvate or with each L-α-amino acid tested, as sole substrate, gave no observable formation of alanine. Controls with buffer replacing the liver preparation, or with boiled-liver preparation, gave negative results. (After 24 hr. at 0° a preparation had lost none of its activity, at least with L-leucine.)

Possibility of transamination to pyruvate via glutamate. The most obvious explanation of these results was that enzymes exist in rat liver which are able to catalyse the transfer of the α-amino group of some L-α-amino acids directly to pyruvate. However, evidence for the wide scope of transamination to α-oxoglutarate in animal tissues (Hird & Rowsell, 1950; Cammarata & Cohen, 1950) made it necessary to give consideration to an alternative explanation. The L-α-amino acid might first transfer its α-amino group to α-oxoglutarate (reaction i), and the glutamate thus formed then react with pyruvate to

Table 1. *Transamination to pyruvate, α-oxoglutarate and oxaloacetate from L-α-amino acids with rat-liver particles*

Incubation conditions and estimations are as described under Methods; incubation period was 1 hr., except (a), which was 45 min.

Alanine, glutamate or aspartate formed from pyruvate, α-oxoglutarate or oxaloacetate, respectively, with L-α-amino acids (mg./2·0 ml.)

α-Oxo acid	Control, no amino acid	Leucine	Phenyl-alanine	Methionine	Valine	Glutamate	Aspartate
(a) Pyruvate	0	0·4	0·5	0·4	0	—	0
α-Oxoglutarate	0	0·2	1·0–2·0	0·2	0·1	—	2·0–3·0
(b) Pyruvate	0	0·5	—	—	—	About 2	0
α-Oxoglutarate	0	<0·3	—	—	—	—	About 2
(c) Pyruvate	0	0·5–1·0	About 1	—	—	—	—
α-Oxoglutarate	<0·1	0·3	About 2	—	—	—	—
Oxaloacetate	0	0	0·5	—	—	About 2	—

form alanine (reaction ii), each amino transfer being catalysed by the appropriate transaminase, and the coupling made possible by a trace of glutamate or α-oxoglutarate remaining in the particle preparation, despite the thorough washing procedure.

(i) L-α-Amino acid + α-oxoglutarate→
                α-keto acid + L-glutamate.

(ii) L-Glutamate + pyruvate→
                α-oxoglutarate + L-alanine.

O'Kane & Gunsalus (1947) have shown that in pig-heart extracts transamination from aspartate to pyruvate proceeds in this fashion, by a coupling of the aspartate–α-oxoglutarate and glutamate–pyruvate transaminations.

Incubations were carried out to compare pyruvate and α-oxoglutarate as amino acceptors with L-α-amino acids, in the presence of rat-liver particles (Table 1 (a)). Under the same conditions 3·0 ml. of the rat-liver preparation was incubated with 3·0 ml. of 0·05 M phosphate buffer, pH 7·7. In this case the protein was precipitated by the addition of 10 ml. of warm ethanol and removed by centrifuging. The supernatant was evaporated to dryness at 50–60° under reduced pressure and the solid residue dissolved in 1·5 ml. of 0·2 M acetate buffer, pH 4·5. A sample (1·0 ml.) of this solution and 1·0 ml. of the L-methionine–pyruvate incubation extracts were tested manometrically with an active *Clostridium welchii* L-glutamic decarboxylase (Gale, 1948). No L-glutamate was measurable in either extract. L-Glutamate–pyruvate transamination is more powerfully catalysed in rat liver than transamination with α-oxoglutarate and L-leucine or L-methionine (Rowsell, 1956). It would be expected, therefore, if the two-step mechanism indicated above were operative, that the pace-making step in overall transamination to pyruvate with these amino acids would be reaction (i), namely trans-

amination to α-oxoglutarate. In fact it would be expected that transamination by this two-stage process to pyruvate would be much less than that observed to α-oxoglutarate, since the trace concentration of L-glutamate (none was detectable on the chromatograms or by decarboxylase) or α-oxoglutarate would give a pace-making reaction much slower than the transfer to α-oxoglutarate at 0·02 M. The reverse was found with L-leucine and L-methionine [Table 1 (a); see also (b) (c)]. In molar concentrations the ratio of alanine to glutamate formation is even greater.

Transamination with α-oxoglutarate is more rapid with L-aspartate than with other L-α-amino acids, for rat-liver particles (Table 1 (a); Rowsell, 1956). If the observed transaminations with pyruvate proceeded via transamination to α-oxoglutarate then it would be expected that L-aspartate would be a particularly effective amino donor. However, no transamination was observed with L-aspartate and pyruvate (Table 1 (a)).

A preparation which catalysed L-aspartate–α-oxoglutarate and L-glutamate–pyruvate transaminations gave no alanine formation with L-aspartate and pyruvate. Evidently not enough L-glutamate or α-oxoglutarate was present to couple the two transaminations. The same preparation was active in promoting transaminations from L-leucine to pyruvate and α-oxoglutarate (Table 1 (b)).

Again, if the reaction sequences (i) and (ii) were operative it would be expected that transaminations with oxaloacetate to form aspartate would be similarly catalysed. However, a preparation which promoted transamination with L-leucine and pyruvate was ineffective with L-leucine and oxaloacetate. Insufficient L-glutamate or α-oxoglutarate remained on the particles to couple the observed transamination reactions:

(iii) L-Leucine + α-oxoglutarate → L-glutamate,

(iv) L-Glutamate + oxaloacetate → L-aspartate,

as shown in Table 1 (c). It was unlikely, therefore, that alanine formation from L-leucine and pyruvate with the same preparation had occurred by a coupling of reactions (i) and (ii), which suggested that direct transamination was involved. Moreover, it was necessary to consider the possibility that aspartate had been formed from oxaloacetate and phenylalanine [Table 1 (c)] by direct transamination. In all incubations with oxaloacetate some alanine was formed. This probably occurred by β-decarboxylation of oxaloacetate to form pyruvate, followed by transamination from the L-α-amino acids.

Transaminations to pyruvate with soluble proteins of rat liver

Clarified supernatants of rat-liver homogenates catalysed the formation of alanine from pyruvate with L-leucine, L-phenylalanine, L-methionine, L-tyrosine or L-histidine, but not with D-phenylalanine, L-valine, L-tryptophan, glycine, DL-isoleucine or L-serine. Controls with boiled supernatant gave negative results. With the same preparations transamination to α-oxoglutarate was not observed with L-leucine (Fig. 1), L-phenylalanine or L-methionine.

In one experiment alanine and glutamate, formed by transamination, were estimated with a recording photodensitometer (Table 2). Although glutamate was measurable it was much less than alanine formation. There was no diminution in pyruvate-transaminase activity of the preparation after pre-incubating for 1 hr. at 37°, and the decrease in the rate of alanine formation after the first 30 min. may indicate that equilibrium positions were being approached. Values of Q_T are given to indicate the order of transamination rates with pyruvate. No O_2 consumption above control values was observed when incubation extracts from this experiment were tested with D-amino acid oxidase (Table 3). The oxidase was active with quantities of

DL-alanine close to those estimated for alanine formation in the transamination experiments. The use of a method measuring L-alanine specifically and directly is preferable, but the absence of D-alanine implies that only the L-isomer is formed by transamination.

It would be convenient to have a dry, stable preparation of rat liver, from which the enzyme or enzymes catalysing transaminations with pyruvate could be extracted as desired. However, an extract of rat-liver acetone-dried powder (Keilin & Hartree, 1936) in 0·1 M phosphate, pH 7·7, did not catalyse the formation of alanine from pyruvate with L-phenylalanine or L-leucine. With the same extract transaminase activity between L-phenylalanine and α-oxoglutarate was observed.
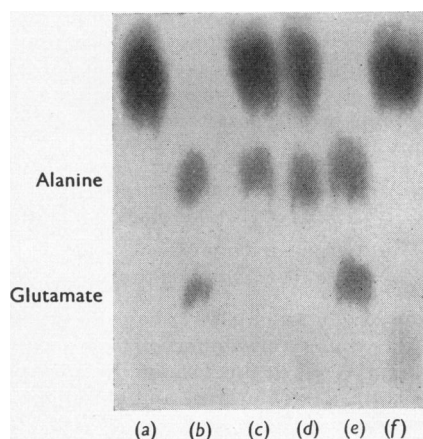

Fig. 1. Transamination with dialysed rat-liver supernatant to form alanine from pyruvate and (c) L-leucine or (d) L-phenylalanine. None was formed with (a) L-leucine alone. No transamination occurred with α-oxoglutarate and (f) L-leucine. (b) 0·5 mg./2·0 ml. and (e) 1·0 mg./2·0 ml. each of alanine and glutamate. Ascending chromatogram run in water-saturated phenol.

Table 2. *Transamination to pyruvate and α-oxoglutarate with a soluble preparation of rat liver*

Incubations were under the described conditions, with a dialysed clarified supernatant of rat-liver homogenate (fresh liver wet wt., 10·3 g.; 31 ml. of supernatant obtained, with dry wt. 15 mg./ml.). After separation on paper chromatograms with propanol–water, amino acid formation was estimated with a recording photodensitometer. Values of Q_T are given in parentheses. In (b) the supernatant had been pre-incubated for 1 hr. at 37°.

| | | | Alanine or glutamate formed from pyruvate or α-oxoglutarate respectively with L-α-amino acids (mg./2·0 ml.) | | |
α-Keto acid	Incubation period (min.)	Control, no amino acid	Leucine	Phenyl-alanine	Methionine
(a) Pyruvate	0	—	0	—	—
	30	—	0·3 (10·1)	0·3 (10·1)	0·4 (13·4)
	60	—	0·4	—	—
	90	0	0·5	0·7	0·5
α-Oxoglutarate	90	<0·05	0·1	0·2	0·05
(b) Pyruvate	90	0	0·5	0·7	0·5

Comparison of transamination to pyruvate with aerobic oxidation of L-α-amino acids

Transamination to pyruvate has been compared with O_2 uptake for a number of L-α-amino acids with the same liver preparations. In the manometric

Table 3. Absence of D-isomer in alanine formed by transamination

Warburg manometers were assembled to measure O_2 uptake at 37°. (i) Flasks contained 1·5 ml. of 0·067 M sodium pyrophosphate buffer, pH 8·3, and 1·0 ml. of substrate or 0·05 M phosphate, pH 7·7; D-amino acid oxidase (0·5 ml.) was added from the side bulb. (ii) Flasks contained 1·0 ml. of 0·067 M sodium pyrophosphate, pH 8·3, and 2·0 ml. of substrate or 0·05 M phosphate, pH 7·7; D-amino acid oxidase (0·3 ml.) was added from the side bulb. DL-Alanine, (i) and (ii), was in 0·05 M phosphate, pH 7·7. Centre wells contained 0·2 ml. of 40% NaOH and filter paper, and all vessels were gassed with O_2.

	O_2 uptake (μl./hr.)
(i) Control	3·5, 7·5
DL-Alanine (0·3 mg.)	22·5, 21·0
L-Phenylalanine–pyruvate incubation extract (1·0 ml.) (Table 2 (b))	7·0
L-Methionine–pyruvate incubation extract (1·0 ml.) (Table 2 (b))	4·0
Calc. for 100% oxidation of D-isomer in 0·3 mg. of DL-alanine	18·8
(ii) Control	4·0, 5·5
DL-Alanine (0·6 mg.)	37·5, 33·0
L-Phenylalanine–pyruvate incubation extract(1·0ml.)(Table 2(a)) + L-Methionine–pyruvate incubation extract (1·0 ml.) (Table 2 (a))	2·0
Calc. for 100% oxidation of D-isomer in 0·6 mg. of DL-alanine	37·6

experiments, except for the absence of pyruvate and the substitution of O_2 as gas phase, the same conditions were employed as for transamination. In no case, neither with washed particles nor with a supernatant preparation, was there any significant O_2 uptake above that of the control, but transaminations to pyruvate were observed in parallel experiments (Table 4). The formation of 0·4 mg. of alanine by transamination and the consumption of 50 μl. of O_2 are equivalent for the deamination of L-α-amino acids.

Transamination from L-alanine to α-keto acids

In the presence of washed rat-liver particles 0·03 M L-alanine was incubated with (a) phenylpyruvate, (b) α-oxoisocaproate and (c) α-oxobutyrate, each 0·025 M. In (a) 1 mg. of phenylalanine and in (b) 0·4 mg. of leucine were formed. Chromatograms of (c) showed the formation of a ninhydrin-reacting compound running just ahead of alanine and just behind valine in water-saturated phenol, and in propanol–water. Published R_F values (Dent, 1948) suggest that this compound was α-amino-n-butyrate. No transamination was observed with α-oxoisocaproate or α-oxobutyrate when D-alanine, L- or DL-aspartate was substituted for L-alanine. L-Aspartate–α-oxoglutarate transamination was catalysed by the same preparation. The absence of leucine or α-amino-n-butyrate formation with L-asparate was regarded as evidence for transamination with L-alanine without α-oxoglutarate and L-glutamate as intermediates.

Table 4. Comparison of O_2 consumption and transamination to pyruvate with rat-liver preparations

Oxygen consumption was measured in Warburg manometers with 0·2 ml. of 50% KOH and filter paper in centre wells, and O_2 as gas phase. For both O_2 consumption and transamination, 2·0 ml. incubations, including 1·0 ml. of tissue preparation and 0·03 M L-α-amino acid, were at 37° for 1 hr. (except O_2 uptake (a): 80 min.). Transamination incubations also included 0·02 M pyruvate; they were in test tubes under N_2.

Rat-liver preparation		O_2 consumption (μl.)	Alanine formed with pyruvate (mg./2·0 ml.)
(a) Washed particles	Control, no amino acid	29·5	0
	L-Leucine	25·5	0·4
	L-Phenylalanine	31·0	0·7
	L-Methionine	28·0	0·5
	L-Valine	28·0	0
	L-Histidine	—	0·3
(b) Washed particles	Control, no amino acid	14·5	0
	L-Leucine	11·5	0·4
	L-Phenylalanine	11·0	0·5
	L-Histidine	11·5	0·3
	DL-Isoleucine	—	0
(c) Dialysed supernatant	Control, no amino acid	3·5	0
	L-Leucine	3·5	0·4
	L-Phenylalanine	2·0	0·4
	L-Histidine	3·5	0·2
	DL-Serine	—	0
	DL-Isoleucine	—	0

Table 5. *Transamination to pyruvate with liver preparations of other animal species*

For details of tissue preparations, see Rowsell (1956).

Animal	Liver preparation	Alanine formed from pyruvate and amino acid (mg./2·0 ml.)				
		Control, no amino acid	L-Leucine	L-Phenyl-alanine	DL-Methionine	L-Valine
Mouse	Washed particles	0	0·5–1·0	0·5–1·0	—	—
	Dialysed supernatant	0	0	0	—	—
Pig	Washed particles	0	0	0	0	0
	Dialysed supernatant	0	0	0	0	0
Pigeon	Dialysed whole homogenate	0	0·1	0·5	—	—
Chicken	Washed particles	0	0	0	0	0

Distribution of pyruvate transaminase

Rat tissues. Neither washed particles nor supernatant of rat kidney catalysed the conversion of pyruvate into alanine, when incubated with L-leucine, L-valine, L-phenylalanine or DL-methionine, although the particles caused glutamate formation from α-oxoglutarate with the same amino acids in a parallel experiment (Rowsell, 1956). Dialysed whole homogenates of rat brain (total vol., 8 ml.), heart (6 ml.), and 7 g. of thigh muscle (8 ml.) gave negative results with L-leucine or L-phenylalanine and pyruvate.

Livers of different animals. Liver preparations from a number of other animal species have been tested for their ability to catalyse transaminations with pyruvate. The results of this limited survey are given in Table 5.

Transaminations not involving glutamate ⇌ α-oxoglutarate or alanine ⇌ pyruvate

With clarified supernatants of rat-liver homogenates, incubations were carried out to test α-oxobutyrate, phenylpyruvate, and α-oxo*iso*caproate, each 0·025M, as amino acceptors with 0·03M L-α-amino acids, other than glutamate and alanine, as amino donors. The formation of α-amino-*n*-butyrate from α-oxobutyrate with L-phenylalanine, L-leucine and L-methionine was observed. A trace of leucine was formed with L-phenylalanine and α-oxo*iso*caproate as substrates, and 1–2 mg. of phenylalanine with L-leucine and phenylpyruvate. Leucine and phenylalanine were separated on a paper-strip ascending chromatogram (20 in. long) in propanol–water. No phenylalanine was formed when DL-serine or DL-isoleucine was substituted for L-leucine. No transaminations were observed with boiled-liver preparations or with α-keto acids as sole substrate.

DISCUSSION

Present results show the formation of alanine from pyruvate and some L-α-amino acids, with rat-liver particles. Evidence is given that this occurred not via transamination to α-oxoglutarate (by a coupling of reactions (i) and (ii), see Results) but probably by direct transamination. Unambiguous demonstration of transaminations directly to pyruvate is given by the findings that clarified soluble preparations of rat liver and washed particles of mouse liver catalysed transaminations with certain L-α-amino acids and pyruvate, but did so poorly or not at all with α-oxoglutarate. Similar consideration has not been given to the possibility that for some L-α-amino acids in rat liver transamination with α-oxoglutarate may require pyruvate or alanine as an intermediate amino carrier. Only a few L-α-amino acids have been tested for transamination with pyruvate in tissues other than liver. However, the indication so far is that liver is the only tissue able to catalyse transaminations with pyruvate.

Quastel & Witty (1951) observed transamination from L-orthinine to pyruvate with liver and kidney homogenates of a number of animal species. Awapara & Seale (1952) reported transaminations with pyruvate and some L-α-amino acids in rat heart, liver, kidney and ventral prostate. However, in both investigations unwashed whole homogenates were used, and it is possible that some, at least, of the transfers they observed were mediated by α-oxoglutarate–L-glutamate. This could account for a number of discrepancies between the observations of Awapara & Seale (1952) and present findings. For example, they found that L-aspartate, DL-serine and DL-valine were active in transamination with pyruvate in rat liver, whereas in the present study with washed particles or dialysed supernatant no transamination was observed with these amino acids. On the other hand, with the thorough washing procedures applied in the present study it is possible that a necessary activator was removed (e.g. pyridoxal phosphate), with the result that some transaminations with pyruvate have been underestimated or not observed at all.

With rat-liver and rat-kidney preparations, it has been shown that transamination with α-oxoglutarate is at a higher level than aerobic oxidation, for all the L-α-amino acids tested (Rowsell, 1956).
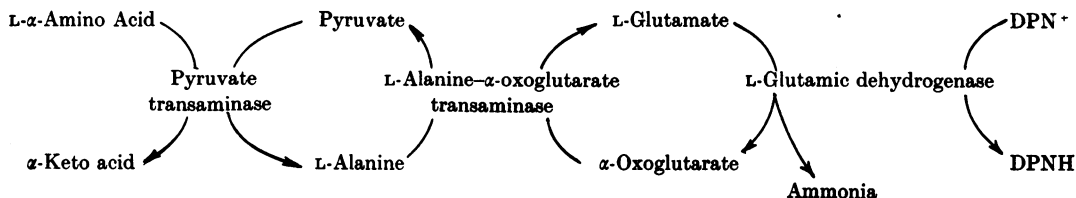
Fig. 2. Hypothetical metabolic scheme for oxidative deamination of some L-α-amino acids in liver.
DPN+, Oxidized diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide.

Present work with rat liver shows that, for those L-α-amino acids active in this respect, transamination to pyruvate also is at a higher level than aerobic oxidation (Table 4).

One of the special metabolic functions of liver is the oxidative deamination of L-α-amino acids (Van Slyke & Meyer, 1913; Krebs, 1933; Maddock & Svedberg, 1938; Svedberg, Maddock & Drury, 1938). In view of present findings, a variant of the hypothesis that L-α-amino acids are oxidatively deaminated via transamination to α-oxoglutarate (Braunstein, 1939, 1947) may be considered. For some L-α-amino acids, and with some animal species, oxidative deamination in the liver may include, as a first step, transamination to pyruvate according to the hypothetical metabolic scheme in Fig. 2.

Glutamate–pyruvate transamination has been found much more active in liver (Q_T 46) than in other tissues of the rat (Q_T 2–13) (Cohen & Hekhuis, 1941). Values of Q_{O_2} for L-glutamate oxidation are greater with liver and kidney (49·3 and 24·7, respectively) than with other tissues of the rat (4·9–10·3) (Copenhaver, McShan & Meyer, 1950). Thus, the enzymes which could catalyse deamination via alanine and then glutamate are present in rat liver, and particularly active in that tissue. Pyruvate and α-oxoglutarate, or alanine and glutamate, would be necessary as intermediate amino carriers on this hypothesis. Metabolic processes leading to their formation are well known, and moreover they are found in fresh rat liver (Frohman, Orten & Smith, 1951; Awapara, 1949). However, investigations of a different kind are necessary to determine the mechanism of oxidative deamination *in vivo*. Although the enzymes and carriers are present for deamination of L-α-amino acids via transamination to α-oxoglutarate or to pyruvate, they may not be integrated functionally in the necessary way in intact tissues. For example, it has been demonstrated that L-tyrosine oxidation with fresh rat-liver preparations (Knox & LeMay-Knox, 1951) and acetone-dried-powder extracts (La Du & Greenberg, 1951; Schepartz, 1951) is dependent on initial transamination to form *p*-hydroxyphenylpyruvate. Neuberger (1948), how-ever, made observations indicating that some at least of the L-tyrosine metabolized in man and the rat is degraded via 2:5-dihydroxyphenylalanine and 2:5-dihydroxyphenylethylamine. Again, though brain contains a powerful L-glutamate-oxidation system (Weil-Malherbe, 1936) and a very active L-aspartate–α-oxoglutarate transaminase (Cohen & Hekhuis, 1941), brain slices will not oxidise L-aspartate (Weil-Malherbe, 1936).

Experiments with ^{15}N-labelled L-α-amino acids with perfused organs or slices, with observation of the rate of incorporation of the [15]N α-amino group into glutamate, alanine and other amino acids, and its rate of liberation as ammonia, could make a decisive contribution to our understanding of the physiological mechanism of oxidative deamination.

Only very few of the possible amino acid–α-keto acid pairs have been tested for transamination in the present exploratory study, and for the most part with only roughly quantitative technique. Nevertheless, it is believed that a much wider scope for transamination reactions in animal tissues is indicated than has usually been considered. Rudman & Meister (1953) have reported transamination with many α-keto acid–L-α-amino acid pairs not involving α-oxoglutarate or L-glutamate with dialysed extracts of strains of *Escherichia coli*.

SUMMARY

1. Alanine formation has been observed chromatographically from pyruvate and some L-α-amino acids, with sedimented particles and with soluble proteins of fresh rat-liver homogenates.

2. Evidence has been obtained that transaminations with pyruvate do not proceed via transamination to α-oxoglutarate.

3. For L-α-amino acids which take part in the reaction, transamination with pyruvate is more rapid than aerobic oxidation in rat-liver preparations.

4. The possibility of oxidative deamination via transamination to pyruvate, for some L-α-amino acids in rat liver, is discussed.

5. Transaminations with L-alanine and some α-keto acids have been observed.

6. Fresh rat-liver preparations catalyse transaminations with L-α-amino acid–α-keto acid pairs, not involving glutamate–α-oxoglutarate or alanine–pyruvate.

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(+)-(S-Methyl-L-cysteine S-Oxide) in Cabbage

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For some years work in our laboratory has been aimed at identifying in plant juices compounds of low molecular weight containing residues of chemically bound amino acids which are set free by hydrolysis with acid (Synge, 1951; Synge & Wood, 1954; Ellfolk & Synge, 1955). Most of this work has been with grass extracts. However, we have cursorily examined extracts from a number of other plants. The results were generally not much different from those with grass, but with fractions from cabbage the treatment with hot acid led to a striking increase in the intensity of the spot in the position of valine on our two-dimensional paper chromatograms.

In order to isolate the parent substance from the unhydrolysed juice we first tried fractionation by electrical transport in a diaphragm cell (Synge, 1951). At pH 6–7 the substance migrated, though less readily than aspartic and glutamic acids, into the acetic acid compartment of the four-compartment cell, while in dilute acetic acid in the three-compartment cell it migrated into the cathode compartment, though less readily than the ordinary neutral amino acids. Experiments were then made with paper ionophoresis. A sample strip from the paper was coloured with ninhydrin; the zones so located were cut out, eluted and the eluates hydrolysed. Our substance was associated with a ninhydrin-staining zone due, it seemed, to an ampholyte intermediate in behaviour between the dicarboxylic and neutral amino acids. On two-dimensional paper chromatograms, this material occupied position *A* (Fig. 2). Accordingly, to isolate the material in larger quantities we tried displacement chromatography on a cation-exchange resin (cf. Westall, 1950). The diffusate of the juice could be applied to the column without preliminary concentration and much anionic and neutral material removed by washing with water; on displacement with ammonia the substance emerged early from the column, contaminated chiefly with aspartic and glutamic acids, serine, threonine and glutamine. It was readily separated from these amino acids by partition chromatography with phenol–water. The product crystallized readily, and represented (best yield) 4·4 % of the N of the cabbage diffusate (non-