owing to iron toxicity, were found to contain four times the malic acid content of the healthy green leaves, whereas the citric acid in these leaves was too small to be accurately determined.

Whereas Pierce & Appleman (1943), who estimated oxalic, malic and citric acid by chemical means, frequently found that 70–80 % of the total acidity of the leaf could not be accounted for by these acids, no such discrepancies were found in the present studies, apart from skikimic acid in bracken and broad bean. Oxalic, malic or citric acid appears to be the major constituent. Fractions for fumaric, succinic, malonic or *trans*-aconitic acid were never present to any great extent. The *trans*-aconitic acid in these extracts probably arose from the conversion of *cis*-aconitic acid (Krebs & Eggleston, 1944).

From the results presented it would appear that a relation exists in leaves between the phosphorus:iron ratio, the potassium:calcium ratio and the citric acid:(malic+oxalic acid) ratio. The total organic acidity also appears to vary inversely with the total phosphorus in the leaf, as has been noted by others (Cooil, 1948; Vavrich, 1954; Ward & Petrie, 1940). Although the amount of carbonate has not been estimated in these leaves, its contribution to the cation-anion balance of the leaf is not very great (Scharrer & Jung, 1957).

In the previous part of this study it was shown that the free amino acids of leaf tissue were higher when the phosphorus content of the leaf was high (DeKock & Morrison, 1958). They therefore vary inversely as the total organic acids.

SUMMARY

1. Organic acids in healthy leaves and leaves chlorotic due to iron deficiency, heavy-metal

toxicity, ageing, virus-infection and genetical constitution were quantitatively studied.

2. All types of chlorotic leaves contained more citric acid relative to malic or oxalic acid, except in virus yellows in sugar beet where the reverse is true.

3. The citric acid:(malic+oxalic acid) ratio of leaves appears to vary as the phosphorus:iron and potassium:calcium ratios. The total amount of organic acid also varied inversely as the phosphorus content of the leaf.

4. Green leaves apparently contain large quantities of either malic or oxalic acid.

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The Uptake and Metabolism of Amino Acids by Slices of Carrot

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It has been shown that several amino acids disappear from a solution which is in contact with slices of carrot tissue (Birt & Hird, 1956). Further, Webster (1954), Rheinhold & Powell (1956) and Kursanov (1956) have studied the uptake of certain amino acids by various plant tissues. However,

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actual evidence for the transport of amino acids into plant tissues against a concentration gradient is limited to that provided for the accumulation of histidine by carrot slices (Birt & Hird, 1956).

In an investigation of the mechanism responsible for the uptake of amino acids by carrot slices, it is essential to assess the contribution made by the metabolic destruction of these compounds to the

apparent uptake as measured by disappearance from the external medium. Accordingly, the aim of the present investigation was to study the metabolic fate of a number of amino acids after they had been taken up by slices of carrot root. The term 'uptake' has been used to describe the disappearance from the external solution of the compound under investigation, that is adsorption on and entry into the tissue; the term 'accumulation' refers to the storage in the tissue of the chemically unaltered substance.

First, the proportions of the amino acids taken up that could be recovered from the tissue at the end of the experiment, and secondly, the effect of these compounds on the respiratory behaviour of the tissue, have been investigated. Thirdly, direct evidence for the metabolic destruction of certain amino acids was sought by using [^{14}C]amino acids, and collecting the $^{14}\text{CO}_2$ liberated during the period of uptake.

MATERIALS AND METHODS

Materials

The amino acids used in these and all subsequent experiments were commercial samples. DL-[2- ^{14}C]- and DL-[3- ^{14}C]-phenylalanine, and DL-[1- ^{14}C]leucine were obtained from Tracerlab Inc., Boston, Mass., U.S.A. L-[$^{14}\text{C}_6$]Phenylalanine was obtained from the Radiochemical Centre, Amersham, Bucks. The specific activities of the amino acids ranged from 0.3 to 1.1 mc/m-mole.

Methods of estimation

(i) Amino acids recovered from the tissue after uptake were estimated by the method of quantitative paper chromatography, described by Connell, Dixon & Hanes (1955). The following solvents and times of development were used with a descending system.

Propanol-water (4:1, v/v), 18 hr. for alanine; butanol-acetic acid-water (4:1:1, by vol.), (a) 7 hr. for leucine, isoleucine, phenylalanine, valine, methionine; (b) 36 hr. for glutamic and aspartic acid, histidine, ornithine, lysine (with a pad clipped to the bottom of the paper).

(ii) The disappearance of amino acids from the incubating media in uptake experiments was measured by direct development of the colour, without prior chromatographic separation, in samples (volume not exceeding 1 ml.) of the solution with 2 ml. of the ninhydrin reagent of Connell *et al.* (1955). The accuracy of this procedure was such that recoveries of $100 \pm 2\%$ were obtained.

The amount of ^{14}C taken up from solutions of labelled amino acids was calculated from the values for percentage disappearance of the amino acids, and the activity of the original solution determined directly by counting a sample of the [^{14}C]amino acid solution dried on a planchet. This procedure was adopted because of the greater accuracy and ease of the ninhydrin method of estimation, and had the advantage of avoiding errors due to the presence of ^{14}C -labelled compounds containing no amino nitrogen.

Tissue

The treatment of the tissue for use in recovery experiments did not differ from that described previously (Birt & Hird, 1956). However, for experiments with the Warburg apparatus it was found more convenient to prepare disks (0.5 cm. diam.) in place of the larger slices used in other experiments. The tissue was cut into slices (thickness 1 mm.) from which the disks were removed with a stainless-steel cork borer. The disks were washed in the usual way; when required for an experiment they were blotted lightly between filter paper before being placed in the Warburg flasks.

Experimental procedure

(a) *Recovery experiments.* Slices of tissue (2.5 g. fresh wt.) were incubated at 25° in a 50 ml. conical flask containing a solution (16 ml.) of the amino acid (5 mM). The flask was shaken at 150 oscillations/min. for a period of 6–7 hr.; at the end of this time, a sample (15 ml.) of the incubating solution was removed and made up to 25 ml. This is referred to as the 'final' solution. The variation of 1 hr. in the time of incubation for different amino acids was not easily avoidable; but, as no comparisons involving a standard time of uptake were made, this was not important. The tissue plus the residual incubating solution (1 ml.) still in the original conical flask was left standing overnight, at 5° , after addition of ethanol (9 ml.); after mixing, 8 ml. of the liquid was removed and placed in a 50 ml. standard flask. Next, the tissue was extracted five times with 70% (v/v) ethanol (9 ml.) with continual shaking at 40° for 0.5 hr.; 8 ml. of each successive extract was withdrawn. Preliminary experiments with L-histidine had shown that this procedure gave complete extraction; for example, successive extracts of a sample of tissue which had taken up $54 \mu\text{moles}$ of L-histidine yielded 28, 13, 4, 2, 3, 2 and $0 \mu\text{moles}$ of amino acid. The six extracts were combined and made up to 50 ml.

As a measure of the original concentration of the amino acid, a volume (15 ml.) of the amino acid equal to that used for uptake in the recovery experiment was made up to 25 ml. This is termed the 'original' solution.

The entire process was repeated for tissue incubated in solutions of each of the amino acids used, and for a control tissue in water.

For each amino acid, equal volumes of the 'original' and 'final' solutions and of the pooled tissue extracts were combined thus:

(i) 'Original' solution plus extract of tissue incubated in water (to estimate total amino acid present in the solution before incubation, together with that in tissue which had not taken up amino acid).

(ii) 'Final' solution plus extract of tissue incubated in amino acid solution (to estimate amino acid present finally in solution and tissue after uptake).

Samples of these mixtures were analysed in two ways:

(1) Total ninhydrin-positive material was measured by direct estimation with the ninhydrin reagent.

(2) After chromatography, the recovery of the amino acid taken up by the tissue was determined. An allowance was made for the amino acid in the incubating solution (1 ml.) that remained in contact with the tissue at the end of the experiment.

The amino acid taken up by the tissue was determined directly from the ninhydrin-colour densities given by the 'original' and 'final' solutions.

It was found that appreciable amounts of lysine and ornithine remained adsorbed on the tissue at the end of the extraction procedure; however, more than 90% of similar amounts of these amino acids adsorbed on an equivalent sample of tissue was released by two further extractions with Na_2CO_3 (10%, w/v; anhydrous salt). After adjustment of pH, these solutions were analysed directly with the ninhydrin reagent.

(b) *Measurement of oxygen consumption.* The conventional Warburg apparatus was used. The main compartment contained 3 ml. of the amino acid solution or water, and 30–40 disks of tissue. The 'fluid' volume in the main compartment was determined by adding a similar number of disks to water in a measuring cylinder. The volume of the disks was always close to 0.5 ml., and this volume was included in the determination of the constant for the apparatus. N-NaOH (0.15 ml.) was used in the centre well. The bath temperature was 25°, and the manometers were shaken at 100 oscillations/min. Air was used as the gas phase. The first reading was taken 15 min. after the taps were closed. In experiments of long duration it was necessary to reset the manometers. At the end of the experiment, samples of the solution were withdrawn for amino acid estimation; the disks were removed, blotted lightly and the fresh weight was determined.

(c) *Collection and measurement of $^{14}\text{CO}_2$.* Collection of $^{14}\text{CO}_2$ was made in the Warburg apparatus; the initial procedure was the same as that described above. At the end of the experimental period, the filter-paper roll, which had been inserted in the centre well, and washings from

this well were transferred to a stoppered glass vessel. When CO_2 was collected in successive periods during the experiment, a new roll of filter paper was placed in the same vessel, the apparatus re-assembled and the experiment continued. The CO_2 was released from the alkali by acidification and collected in a saturated solution of baryta; the entire process was carried out in an all-glass apparatus, which was evacuated before the release of the CO_2 . The BaCO_3 was spun down in a centrifuge, the excess of baryta decanted and the precipitate washed twice with water and once with acetone. It was then suspended in ethanol (90%), plated out in a weighed planchet and dried by standing for some hours in a desiccator containing CaCl_2 ; this was followed by heating with an infrared lamp. The planchet was reweighed and the weight of BaCO_3 (10–20 mg.) calculated to permit a correction for self-absorption. Counting was done in a gas-flow counter (Tracerlab SC-16 windowless flow counter; Tracerlab autoscaler).

RESULTS

Recovery of amino acids after uptake by the tissue. It had already been established that histidine, as measured by the imidazole-colour reaction, was fully recoverable from the tissue after uptake (Birt & Hird, 1956). However, it was desirable to repeat this determination by a method specific for the intact amino acid. Accordingly, an experiment was performed in which the recovery of not only L- and D-histidine, but also of a number of other amino acids, was measured. Results are presented in Table 1. It is clear that metabolism of up to

Table 1. *Recovery of amino acids after uptake by carrot slices*

Tissue was washed for 140 hr. before use; 2.5 g. fresh wt. (water content 2.25 g.) was incubated in each of the appropriate solutions (16 ml.; amino acids 5 mM). Initial pH, 5.0; duration of uptake was 6–7 hr.

Amino acid	Total ninhydrin-positive material recovered (%)	Uptake (μmoles)	Recovered from tissue* (μmoles)	Recovery after uptake† (%)	Internal concn. External concn.
L-Glutamic acid	86	33.5	9.5	28	1.5
L-Aspartic acid	84	33.0	10.5	32	1.6
L-Alanine	88	25.0	6.8	27	0.9
D-Alanine	100	22.5	20.0	89	2.5
L-Phenylalanine	97	28.0	15.5	55	2.1
D-Phenylalanine	98	28.0	25.0	89	3.4
L-Leucine	103	25.5	21.5	84	2.8
D-Leucine	98	17.5	17.0	97	1.9
L-Isoleucine	104	21.5	18.5	86	2.3
D-Isoleucine	99	20.0	18.2	91	2.2
L-Valine	101	28.0	24.5	87	3.4
D-Valine	100	20.5	19.5	95	2.3
L-Methionine	103	20.0	16.8	84	2.0
D-Methionine	101	25.0	24.3	97	3.2
L-Histidine	100	50.5	49.5	98	12.2
D-Histidine	100	40.0	37.0	93	6.6
L-Lysine	97	32.0	29.0 (23.0)‡	91	4.3
L-Ornithine	98	25.5	23.5 (18.5)‡	92	3.0

* Tissue blank for total ninhydrin-positive material (after incubation) equivalent to 17 $\mu\text{moles}/2.5$ g. fresh wt.

† Variation in individual estimations was $\pm 2.5\%$; as the final results are determined by difference, the variation in these will be of the order of $\pm 15\%$.

‡ Figures in parentheses refer to recoveries obtained before the use of sodium carbonate as extractant; for details see text.

10% could not be detected with certainty; however, it can be seen that: (a) Within the limits of experimental accuracy the D-amino acids were fully recoverable. (b) With the exception of glutamic and aspartic acid, and alanine and phenylalanine, there was little metabolic destruction of the amino acids used. (c) As the total ninhydrin-positive material (which includes ammonia) is substantially higher than the corresponding recovery of the amino acids which are metabolized, it would seem that there is evidence for their transamination or deamination, or both. (d) All the amino acids except L-alanine were accumulated against a concentration gradient.

Oxygen consumption of tissue during amino acid uptake. When the oxygen consumption of tissue exposed to solutions of amino acids was measured, it was found that tissue washed for more than about 20 hr. before use showed a greater oxygen consumption in the presence of the amino acid, with the exception of one sample incubated in D-phenylalanine (Table 5). In all experiments of this

Fig. 1. Oxygen consumption of tissue during the uptake of L- and D-phenylalanine: ●, L-phenylalanine; ○, D-phenylalanine. Tissue (40 disks) was washed for 70 hr. before use, then incubated in 3 ml. of the appropriate solution (5 mM) in the Warburg apparatus.

kind, the rate of oxygen uptake, both in amino acid solution and water, did not diminish during the experimental period (Figs. 1 and 2, Table 2). Tissue whose oxygen consumption was enhanced by an amino acid was affected similarly by potassium chloride (Fig. 2), a salt shown by other workers to increase the respiration of washed carrot slices (see Sutcliffe, 1953). Sometimes, though not invariably, tissue washed for 20 hr. or less, while accumulating amino acid from the solution, did not exhibit an increased oxygen consumption (Table 3). In experiments with L-phenylalanine, L-leucine and glycine with longer periods of washing, it was found that the stimulation of respiration increased with increasing concentration of the amino acid but flattened off or fell at high concentrations. Results of experiments of this kind are given for phenylalanine in Tables 3 and 4. Similar results have been obtained with glycine (Table 2) and with L-leucine.

Fig. 2. Oxygen consumption of tissue in the presence of glycine and potassium chloride: ○, potassium chloride; □, glycine; ●, water. Tissue (40 disks) was washed for 60 hr. before use, then incubated in 3 ml. of the appropriate solution (20 mM) in the Warburg apparatus.

Table 2. *Effect of concentration of glycine in medium on oxygen consumption by carrot slices*

Tissue (40 disks) was washed for 25 hr. before use, then incubated in 3 ml. of the appropriate solution in the Warburg apparatus. Duration of uptake was 6 hr.

Concn. of amino acid (mM)	Oxygen consumption (μl./g. fresh wt.)				Oxygen consumption over 6 hr. (% of control)
	0-1 hr.	1-2 hr.	2-4 hr.	4-6 hr.	
0.0	135	144	230	273	100
0.5	142	135	291	292	110
5.0	144	152	308	340	121
10.0	153	149	317	310	119
25.0	140	156	300	324	118

Table 3. *Effect of concentration of L-phenylalanine on oxygen consumption by carrot slices*

Tissue (40 disks) was incubated in 3 ml. of the appropriate solution in the Warburg apparatus. Duration of uptake was 6 hr.

Duration of washing (hr.)	Initial concn. (mM)	Oxygen consumption (μ l./g. fresh wt./6 hr.)	Uptake of amino acid (μ moles/g. fresh wt./6 hr.)	Oxygen uptake (% of control)
20	0.0	903	—	100
20	0.5	915	—	101
20	5.0	917	16.0	101
20	25.0	909	—	101
20	50.0	915	42.0	101
168	0.0	1080	—	100
168	0.5	1160	—	107
168	5.0	1240	18.0	115
168	25.0	1290	—	119
168	50.0	1220	—	113

Table 4. *Effect of concentration of L-phenylalanine on its uptake and on oxygen consumption by carrot slices*

Tissue (40 disks) was incubated in 3 ml. of the appropriate solution in the Warburg apparatus. Duration of uptake was 6 hr.

Duration of washing (hr.)	Initial concn. (mM)	Oxygen consumption (μ l./g. fresh wt./6 hr.)	Uptake of amino acid (μ moles/g. fresh wt./6 hr.)	Oxygen consumption (% of control)
20	0.0	893	—	100
20	0.5	1125	3.1	126
20	5.0	1014	17.0	113
20	25.0	1101	31.0	124
20	50.0	996	42.0	112
83	0.0	1110	—	100
83	0.5	1212	3.0	109
83	5.0	1220	22.5	110
83	25.0	1319	44.0	119
83	50.0	1208	44.0	109
190	0.0	1531	—	100
190	0.5	1794	—	117
190	5.0	1936	10.5	126
190	25.0	1888	29.0	123
190	50.0	1939	—	126

Stimulation of oxygen uptake has been obtained with both the L- and D-isomers of phenylalanine and leucine, though it did not always occur with D-phenylalanine (Table 5; however, see also Fig. 1). It can be seen from Table 5 that the oxygen consumption in the presence of L-phenylalanine was usually greater than that in the presence of L- and D-leucine and D-phenylalanine. It has already been shown that the metabolism of L-phenylalanine by the tissue is greater than that of these other amino acids, and it was thought that this might be the reason for the observed differences in oxygen consumption. Accordingly, oxygen consumption in the presence of amino acids subject to considerable metabolic destruction was compared with that in the presence of amino acids which are not metabolized to the same extent by this tissue. The results, presented in Table 6, showed that the increase in oxygen consumption by the tissue is most marked in the presence of aspartic and glu-

tamic acids, less so with L-alanine and L-phenylalanine. The greatest stimulation, calculated per molecule of amino acid taken up, was obtained with aspartic acid. Thus the substantial metabolic destruction of certain amino acids previously described (Table 1) is accompanied by a considerable increase in the oxygen consumption of the tissue.

Production of $^{14}\text{CO}_2$ during the uptake of ^{14}C -amino acids. The appearance of radioactivity in the carbon dioxide collected in successive periods during an experiment with L- ^{14}C phenylalanine and DL- ^{14}C leucine is shown in Table 7. It can be seen that whereas there was some progressive increase in the consumption of oxygen by each batch of tissue exposed to L-phenylalanine, there was a much greater progressive increase in the activity of the carbon dioxide collected per hour during the course of the experiment; this difference was not found with DL-leucine. The final column can be

taken as a measure of the percentage destruction of the amino acid taken up. As the phenylalanine was uniformly labelled, there is an uncertainty in relating the $^{14}\text{CO}_2$ produced to the percentage metabolism. The minimum value for this percentage is given in the final column of Table 7, i.e. 2.3% of the amino acid taken up. The maximum value (assuming decarboxylation of C-1 only) would be 9×2.3 , i.e. 20.7%. With DL-[1- ^{14}C]-leucine, on the other hand, all of the $^{14}\text{CO}_2$ produced was drawn from C-1 of the amino acid. However, as it is probable that only the L-isomer is metabolized (see Table 1), the maximum value for metabolism of C-1 would be about 12%. The

maximum value is calculated on the assumption of an equal uptake of both isomers from solution.

Thus it would seem that these two amino acids, after uptake by the tissue, are drawn slowly into metabolic processes which lead to decarboxylation reactions; the total amount disappearing appeared to be greater for L-phenylalanine than for L-leucine (Table 7), a result in agreement with those obtained from experiments in which recovery and oxygen consumption were measured.

Saturation of the processes responsible for carbon dioxide production was shown in an experiment with DL-[1- ^{14}C]-leucine. It can be seen from Table 8 that, although the activity taken up in-

Table 5. *Oxygen consumption by carrot slices in the presence of D- and L-isomers of leucine and phenylalanine*

Tissue (40 disks) was incubated in 3 ml. of the appropriate solution in the Warburg apparatus; a separate batch of tissue was used for each experiment. All tissue samples were washed for at least 24 hr. before use, except in Expt. 2 where the duration of washing was 20 hr. Duration of uptake was 6 hr.

Amino acid	Initial concn. (mm)	Oxygen consumption over 6 hr. (% of control)				
		Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
L-Phenylalanine	5	116	127	126	126	113
D-Phenylalanine	5	98	96	109	—	—
L-Leucine	5	105	104	109	112	110
D-Leucine	5	107	106	110	—	—
L-Phenylalanine	25	139	121	124	123	124
D-Phenylalanine	25	97	96	112	—	—
L-Leucine	25	120	107	114	120	125
D-Leucine	25	115	107	119	—	—

Table 6. *Oxygen consumption by carrot slices in the presence of amino acids*

Tissue (40 disks) was incubated in 3 ml. of the appropriate solution in the Warburg apparatus; experiments marked with an asterisk were with a separate batch of tissue. Duration of uptake was 6 hr.

Amino acid	Duration of washing of tissue (hr.)	Oxygen consumption (% of control consumption)	Excess of oxygen consumption (μmoles)
			Amino acid taken up (μmoles)
Glutamic acid	24	169	4.0
	280*	197	2.4
	350	175	6.0
Aspartic acid	24	164	5.5
	280*	175	4.4
	350	155	12.0
L-Phenylalanine	24	129	0.8
	280*	147	0.9
	350	116	0.9
D-Phenylalanine	24	104	0.1
	280*	143	0.6
	350	116	0.8
L-Alanine	24	123	0.6
	280*	175	1.3
	350	126	1.5
D-Alanine	24	124	0.7
	280*	135	0.9
	350	111	0.5
L-Histidine	24	125	0.4
	280*	158	1.0
	350	114	0.5

creases by about 70% as the external concentration of the amino acid is raised from mM to 5 mM, there is an increase of only about 6% in the activity of the carbon dioxide produced. Further, as the amount of the amino acid taken up decreases, the percentage destruction increases.

Uptake and metabolism of D- and L-isomers of leucine and phenylalanine. It can be seen from Table 9 that dilution of either DL-[1-¹⁴C]leucine or L-[¹⁴C₉]phenylalanine with the corresponding non-radioactive L-isomer causes a relatively greater reduction in the activity of the carbon dioxide produced than does an identical dilution with the D-isomer. Two factors may operate to produce this result.

(i) If the L-isomer were metabolized more readily than the D-, such a change in activity would result from dilution with the non-radioactive L-amino acid.

(ii) If the D-isomer of DL- mixture were metabolized to produce ¹⁴CO₂, then any inhibition of the uptake of the D-isomer by the L-isomer would reduce the amount of ¹⁴CO₂ liberated. It will be shown subsequently that such inhibition could, in fact, be expected.

Results presented in Table 9 therefore do not necessarily reflect the relative rates of destruction of the isomers taken up by the tissue, for insufficient evidence was obtained in the experiments to permit a clear distinction of the importance of these two factors. For reliable interpretation of these points it would be necessary to use both [¹⁴C]isomers separately. However, the recovery experiments (Table 1) show that, within the limits of experimental error, there is no metabolism of D-isomers; this supports the first interpretation.

Metabolism of phenylalanine. Further information about the metabolism of phenylalanine was obtained by using DL-[2-¹⁴C]phenylalanine and DL-[3-¹⁴C]phenylalanine in addition to L-[¹⁴C₉]phenylalanine. Results of an experiment with these three compounds are given in Table 10. It can be seen that all three carbon atoms appear in the carbon dioxide produced; and that the percentage destruction of the amino acid increases with increasing duration of washing of the tissue before use. Assuming that D-phenylalanine does not give rise to ¹⁴CO₂, the following additional calculations may be made with the data given. If the uniformly labelled phenylalanine were subject to the removal of one carbon atom only, the percentage destruction of this type in the tissue washed for 280 hr. would be (9 × 3.9)%, i.e. approximately 36%. However, both C-2 and C-3 also give rise to ¹⁴CO₂ (5.2% C-2, 1.2% C-3). Therefore the maximum percentage decarboxylation of C-1 would be (36 - 5 - 1)%, i.e. 30%. With tissue washed for only 20 hr., the percentage decarboxylation would be of the order of only 10%.

Table 7. *Metabolism of [¹⁴C₉]phenylalanine and [1-¹⁴C]leucine by carrot slices*

Tissue was washed for 40 hr. before use, then samples of 40 disks were transferred to the Warburg apparatus and incubated in 3 ml. of the appropriate solution. Amino acid solutions were initially 5 mm; uptake of phenylalanine was 46%, of leucine, 40%. Duration of uptake was 6 hr.

Expt. no.	Amino acid	Oxygen consumption (μl./g. fresh wt.)				10 ⁻³ × ¹⁴ CO ₂ recovered (counts/min./g. fresh wt.)				Total counts/min./g. fresh wt. recovered in ¹⁴ CO ₂ (% of uptake)		
		0-1 hr.	1-2 hr.		2-3 hr.	3-6 hr.	0-1 hr.	1-2 hr.	2-3 hr.		3-6 hr.	Total
1	Control	145	150	161	488	—	—	—	—	—	—	
	L-Phenylalanine	164	171	198	608	—	—	—	—	—	—	
	L-[¹⁴ C ₉]Phenylalanine	174	178	193	615	1.8	2.2	3.6	24.9	32.5	2.3*	
2	Control	196	198	189	587	—	—	—	—	—	—	
	DL-Leucine	208	230	222	760	—	—	—	—	—	—	
	DL-[1- ¹⁴ C]Leucine	217	228	230	795	6.5	6.2	8.1	24.9	46.0	5.9†	

* Assuming that C-1 only is producing carbon dioxide, this figure becomes 9 × 2.3 = 20.7%.

† Calculated on the basis of metabolism of the L-isomer only, this figure becomes about 12%.

Tissue was washed for 40 hr. before use, then samples of 40 disks were transferred to the Warburg apparatus and incubated in 3 ml. of the appropriate solution. Amino acid solutions were initially 5 mM; uptake of phenylalanine was 46%, of leucine, 40%. Duration of uptake was 6 hr.

As is shown in Table 10, there was an increase in the metabolism of phenylalanine by tissue that had been washed for longer periods of time before use. This suggested the possibility that the altered metabolic behaviour of the tissue towards phenylalanine was due to a fall in the nutritional reserve of the tissue. (In the experimental procedure used, no exogenous substrate apart from the amino acid was added.) Accordingly, the effect of added glucose on the production of $^{14}\text{CO}_2$ from $[\text{C}_6]$ -

phenylalanine, and the effect of added phenylalanine on the production of $^{14}\text{CO}_2$ from $[\text{C}_6]$ -glucose, was studied, and the results are presented in Table 11. It can be seen that the increases in oxygen consumption produced by glucose and phenylalanine are additive and that each substance interferes with the metabolism of the other. However, under the experimental conditions used, the overall oxygen consumption is comprised of at least two factors, namely, the ground respiration

Table 8. *Metabolism of DL- $[\text{C}_6]$ leucine by carrot slices*

Tissue was washed for 44 hr. before use, then samples of 40 disks were transferred to the Warburg apparatus and incubated in 3 ml. of the appropriate solution. Duration of uptake was 6 hr.

Concn. of amino acid (mM)	Oxygen consumption ($\mu\text{l./g. fresh wt./6 hr.}$)	$10^{-4} \times ^{14}\text{C}$ added (counts/min./g. fresh wt.)	$10^{-4} \times ^{14}\text{C}$ taken up from solution (counts/min./g. fresh wt.)	$10^{-4} \times ^{14}\text{CO}_2$ recovered (counts/min./g. fresh wt.)	$^{14}\text{CO}_2$ recovered (% of ^{14}C taken up from solution)
Control	960	—	—	—	—
5.0	1080	270	76	3.5	4.6
1.0	980	53	44	3.3	7.5
0.5	960	27	23	2.8	12.2
0.1	950	5.8	3.1	0.91	29.4
0.05	960	2.9	—	0.47	—
0.01	950	0.57	—	0.083	—

Table 9. *Metabolism of leucine and phenylalanine by carrot slices*

Tissue was washed for (a) 20 hr., (b) 72 hr., (c) 20 hr., before use, then samples of 40 disks were transferred to the Warburg apparatus and incubated in the appropriate solution (3 ml.). Labelled amino acids were DL- $[\text{C}_6]$ leucine and L- $[\text{C}_6]$ phenylalanine. Concentration of L- $[\text{C}_6]$ phenylalanine was initially 2.5 mM in Expts. (b) and (c). Duration of uptake was 6 hr.

Amino acid	Ratio of isomers (L:D)	Total concn. (L + D-) (mM)	$10^{-6} \times ^{14}\text{C}$ added (counts/min./g. fresh wt.)	$10^{-4} \times ^{14}\text{CO}_2$ recovered (counts/min./g. fresh wt.)
(a) Leucine	1:4	5	1.2 ₂	0.95
	4:1	5	1.1 ₉	0.34
	1:4	25	5.6	2.5 ₃
	4:1	25	6.4	0.98
(b) Phenylalanine	1:0	12.5	2.8	0.05
	1:4	12.5	2.8	1.6 ₃
(c) Phenylalanine	1:0	2.5	1.6 ₆	1.2
	1:1	5.0	1.6 ₆	1.0 ₅

Table 10. *Metabolism of phenylalanine by carrot slices*

Tissue (40 disks) was incubated in 3 ml. of the appropriate solution in the Warburg apparatus. Amino acid solutions were initially 5 mM. Duration of uptake was 6 hr.

Amino acid	Duration of washing (hr.)	$10^{-6} \times ^{14}\text{C}$ added (counts/min./g. fresh wt.)	$10^{-6} \times ^{14}\text{C}$ taken up from solution (counts/min./g. fresh wt.)	$10^{-3} \times ^{14}\text{CO}_2$ recovered (counts/min./g. fresh wt.)	$^{14}\text{CO}_2$ recovered (% of ^{14}C taken up from solution)
L- $[\text{C}_6]$ Phenylalanine plus D-phenylalanine (unlabelled)	20	1.6 ₆	7.6 ₅	10 ₅	1.4
DL- $[\text{C}_6]$ Phenylalanine	11	9.3	3.4	3.9	1.1 ₅
DL- $[\text{C}_6]$ Phenylalanine	11	1.4 ₇	6.2	0.14 ₃	0.0 ₃
L- $[\text{C}_6]$ Phenylalanine plus D-phenylalanine (unlabelled)	280	1.5 ₈	8.4	33	3.9
DL- $[\text{C}_6]$ Phenylalanine	280	9.0	4.3	11	2.6
DL- $[\text{C}_6]$ Phenylalanine	280	1.3 ₁	6.3	3.8	0.6

Table 11. *Metabolism of phenylalanine and glucose by carrot slices*

Tissue was washed for 260 hr. before use, then samples of 40 disks were transferred to the Warburg apparatus and incubated in 3 ml. of the appropriate solution. Both amino acid and glucose initially were 5 mm. Duration of uptake was 6 hr.

System	Oxygen consumption (μ l./g. fresh wt./6 hr.)	Amino acid uptake (μ moles/g. fresh wt./6 hr.)	$10^{-6} \times ^{14}\text{C}$ added (counts/min./g. fresh wt.)	$10^{-5} \times ^{14}\text{C}$ lost from solution (counts/min./g. fresh wt.)	$10^{-4} \times ^{14}\text{CO}_2$ recovered (counts/min./g. fresh wt.)	$^{14}\text{CO}_2$ recovered (% of ^{14}C taken up from solution)
L-[$^{14}\text{C}_9$]Phenylalanine	1130	15	1.6 ₈	6.7	2.6	3.9
L-[$^{14}\text{C}_9$]Phenylalanine	1330	12	1.6 ₁	5.4	0.97	1.8
[$^{14}\text{C}_6$]Glucose	1110	—	—	—	24	—
L-Phenylalanine plus [$^{14}\text{C}_6$]glucose	1320	12	—	—	16	—
Control	885	—	—	—	—	—

and the stimulated respiration resulting from the presence of the amino acid; this complexity makes the precise significance of the observation obscure.

DISCUSSION

It has been shown that most of the amino acids studied are taken up by slices of carrot with little or no metabolism; that is, they are accumulated by the tissue. This is true for both D- and L-isomers of the amino acids. L-Glutamic acid, L-aspartic acid, L-alanine and L-phenylalanine, however, undergo considerable metabolic destruction. For the first three of these compounds this result was not surprising; but the considerable metabolism of L-phenylalanine was unexpected. It is of interest that the metabolism of L-phenylalanine increased with the duration of the prior washing. This increase is apparently due to depletion of endogenous reserves of metabolizable substrate as it is decreased when glucose is supplied to the tissue.

The production of $^{14}\text{CO}_2$ from DL-[1- ^{14}C]leucine, L-[$^{14}\text{C}_9$]phenylalanine, DL-[2- ^{14}C]phenylalanine and DL-[3- ^{14}C]phenylalanine gives clear evidence that C-1 of leucine, and C-1, -2 and -3 of phenylalanine, undergo conversion into CO_2 in carrot tissue. It is of some interest that such metabolism occurs in this tissue; for the removal of C-2 and C-3 of phenylalanine would involve hydroxylation and opening of the benzene ring if the steps involved were the same as those existing in mammalian tissue (Knox, 1955).

Stimulation of oxygen consumption accompanying the uptake of certain organic compounds has already been mentioned. In addition, many workers have shown that the uptake of certain inorganic ions by plant tissue results in an increase in oxygen consumption by the tissue (see reviews by Robertson, 1951; Sutcliffe, 1953; Lundegaardh, 1955; Epstein, 1956). To the present time, no generally acceptable explanation of this behaviour has been given. The results presented in this paper show that the uptake of the amino acids tested is

also accompanied by an increase in oxygen consumption. Comparison with the results for recovery experiments shows that this increase is most marked when the amino acid concerned is capable of yielding a keto acid which is readily metabolized; especially is this true of aspartate. With L-phenylalanine and L-leucine, part at least of the increase can be attributed to metabolism, but it is unlikely that the same explanation applies to the increased consumption of oxygen caused by the D-amino acids. While it is possible that the uptake process itself stimulates oxygen consumption, more experimental work is required to clarify this point.

Thus it is clear that, with four of the amino acids tested, disappearance from the external medium cannot be equated with accumulation in the tissue. An assessment of the importance of metabolic destruction of these amino acids would be necessary before a comparison of rates of uptake for these compounds, based solely on disappearance from the external medium, could be made. However, of the large number of amino acids tested, it was only with L-alanine that a concentration gradient in favour of the tissue was not obtained. It follows that carrot cells possess a mechanism which permits the uptake of many amino acids against a concentration gradient. Certain characteristics of this process of uptake are discussed in the next paper.

SUMMARY

1. The metabolic fate of a wide variety of L-amino acids and some D-amino acids, after uptake by slices of carrot, has been investigated.

2. Of the L-amino acids tested, only alanine, aspartic acid, glutamic acid and phenylalanine underwent substantial metabolic destruction. There was no evidence of metabolic destruction of the D-amino acids used. All the amino acids tested, with the exception of L-alanine, were concentrated against a gradient.

3. Under certain conditions the addition of D- and L-amino acids caused an increase in the oxygen consumption of the tissue. With the [^{14}C]amino acids DL-leucine, DL-phenylalanine and L-phenylalanine it has been shown that carrot tissue metabolizes C-1 of leucine and C-1, -2 and -3 of phenylalanine to carbon dioxide. By use of [$^{14}\text{C}_6$]glucose and L-[$^{14}\text{C}_9$]phenylalanine it has been shown that glucose and L-phenylalanine inhibit the production of carbon dioxide from each other. This effect is more pronounced after prolonged pre-washing of the tissue, which lowers the nutritional reserves of the tissue.

4. The significance of the above observations is discussed.

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Kinetic Aspects of the Uptake of Amino Acids by Carrot Tissue

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In previous investigations, a number of features of the process of uptake of amino acids by carrot tissues have been described (Birt & Hird, 1956, 1958). Thus it has been shown that the D- and L-isomers of many amino acids are taken up against a concentration gradient, and that such uptake is inhibited by certain metabolic inhibitors. Further, it has been shown that the rate of uptake is increased by 'washing' the tissue in water for about 20 hr. or more, and is accompanied by an increased consumption of oxygen by the tissue. The present paper extends certain of the above observations to a wider range of amino acids, and attempts a further characterization of the mechanism of uptake by examination of the kinetics of the system.

MATERIALS AND METHODS

The amino acids used were all commercial samples. All solutions used for incubation, including those containing inhibitors, were unbuffered, but adjusted to pH 5.0. The temperature during incubation was 25°.

Methods of estimation

Histidine was estimated by the method of Macpherson (1946), as modified by Birt & Hird (1956). Other amino acids, including glutamine and asparagine, were estimated with the ninhydrin reagent of Connell, Dixon & Hanes (1955) as described by Birt & Hird (1958).

Tissue

The treatment of the tissue did not differ from that previously described (Birt & Hird, 1956), but experiments were carried out in conical flasks (50 ml.) shaken at 150 oscillations/min. in a water bath at 25° with air as the gas phase. The volume of each experimental solution was initially 20 ml., and the fresh weight of the tissue was 3 g. Uptake of amino acid was followed by withdrawing samples of the incubating solution for analysis at the times indicated. As the water content of the tissue did not alter during the incubation period a change in the concentration in the external medium was used to follow uptake.

RESULTS

Comparison of rates of uptake of amino acids.

A comparative study of the rate of uptake of 13 L-amino acids was made. The amino acids chosen were of three types:

(a) Those with no net charge on the side chain (e.g. phenylalanine, leucine, glycine).

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