The Uptake of Amino Acids by Carrot Slices

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(Received 16 February 1956)

The mechanism of the movement of organic and inorganic compounds in plants is as yet unknown. It has been established that certain inorganic ions are actively taken up by sections of plant tissues (see Lundegaardh, 1955). It has been shown by Arisz (1953) that amino acids are moved along the tentacles of *Drosera* by an active process; and by Mathieson & Catchside (1955) and Webster (1954) that several plant tissues will actively take up and accumulate amino acids. In contrast, much is known about the active uptake of metabolites by animal tissues and certain bacteria. As an approach to this problem in plant tissues we have investigated the conditions determining the uptake of amino acids by carrot slices.

MATERIALS AND METHODS

Methods of estimation

The amino acids used were all commercial samples. Histidine, arginine and lysine were used as the hydrochlorides. All experiments with these amino acids therefore contain an equivalent amount of chloride.

(a) Histidine was estimated by slight modifications of the colorimetric method of Macpherson (1946); these modifications included suggestions made in earlier papers of Koessler & Hanke (1919). For convenience the procedure is given here in full.

Diazotized sulphanilic acid was prepared at least 30 min. before use by mixing NaNO_2 (5%, w/v) and sulphanilic acid (1%, w/v, solution in N-HCl) in the ratio 2:1. The solution was kept at 0°.

The histidine samples (containing 0.1–0.5 μmole of histidine) were pipetted into 25 ml. volumetric flasks, and made to 5 ml. with distilled water. Sodium carbonate (anhydrous salt, 10%, w/v, 3 ml.) was added to each flask in turn, and the solutions were mixed thoroughly. At intervals of 1 min. the diazotized sulphanilic acid (3 ml.) was allowed to run into successive flasks from a rapid delivery pipette held just above the surface of the solution. The flask contents were swirled gently during this process. Twenty seconds after beginning the addition of the diazotized sulphanilic acid, ethanol (20%, v/v, 10 ml.) was run in over a period of 5 sec. from an automatic burette. The solution was shaken gently for a further 15 sec. When the colour had been developed in all the test flasks, the solutions were made to volume with distilled water, and the optical density at 497 $\text{m}\mu$. was read after 30 min. in a Beckman spectrophotometer (Model DU) against a distilled water blank. With close

attention to details of timing and procedure, the method has an accuracy of $\pm 1.5\%$.

(b) Arginine was estimated by the method of Macpherson (1946).

(c) Other amino acids were estimated by the use of chloramine T at pH 4.6 in the Warburg apparatus according to the method of Gibson & Wiseman (1951), with the exception that the side arm contained only 0.3 ml. of the chloramine T solution.

Tissue

Carrots obtained from the grower 1 day after being lifted were found to be satisfactory. They were stored at 0° in moist soil up to 1 week before use. No comparisons have been made between experiments with tissue from different carrots.

The carrots were peeled and cut into blocks which extended for 1 cm. on either side of the cambium. These were then cut with a microtome into a series of 1 mm. slices. After a preliminary washing to remove small fragments, the slices were circulated in distilled water through which air was drawn. When required for an experiment, the tissue was blotted dry between sheets of filter paper, and the slices (3 g. fresh wt.) were placed in the experimental solution (20 ml.) contained in a round-bottomed flask (50 ml.). The flasks were shaken at 150 oscillations/min. in a water bath at 25°, and suitable samples of the bathing solution were removed from time to time for analysis. In long (16 hr.) experiments, sulphanilamide (2.5 mm) was added to the external medium to inhibit growth of bacteria, a previous experiment having shown that the uptake of histidine was not affected under the conditions used. At the end of the experiment, the slices were removed, and dried in an oven at 110° for 5 hr. to determine the dry weight. The loss of weight has been taken as the water content of the tissue.

RESULTS

Preliminary experiments showed that the carrot slices removed L-histidine from a 20 mM solution in such an amount as to make the internal concentration greater than the external concentration. Consequently, the most suitable conditions for investigation of this process were determined.

Type of tissue

The behaviour of different types of tissue was examined by dividing the slices into three portions: an outer pigmented band, corresponding to the phloem; an inner band of xylem tissue; and a band

consisting of the cambium and adjacent phloem and xylem tissue, subsequently referred to as 'cambium'. It was found that at both higher (20 mM) and lower (0.5 mM) (Fig. 1) concentrations, the rate of removal of L-histidine from the medium was in the descending order 'cambium', phloem, xylem. It can be seen from the curves representing uptake by 'cambium' and phloem that all the histidine (estimated by the colorimetric method described) is removed from the external solution. Because of its greater activity, the tissue that included the 'cambium' was used in all subsequent experiments.

Fig. 1. Uptake of L-histidine by different carrot tissues: (a) 'cambium'; (b) phloem; (c) xylem. Tissue (3 g. fresh wt.) washed for 14 hr. before use, then incubated in 20 ml. of 0.5 mM solution. Initial pH, 4.5. Temp. of water bath, 25°.

Thickness of slices

The results given in Table 1 show that there was a rapid initial uptake by the thinnest slices (0.5 mm.) but little further uptake during the next 60 min. This rapid uptake is completed in 15 min., and probably represents the physical adsorption by the tissue (see below). The 1.5 and 1.0 mm. slices seem to behave similarly. As Turner (1938) has shown that the diffusion of oxygen is not limiting in slices of 1 mm. thickness, all subsequent experiments were done with 1 mm. slices.

Washing of tissue

Several workers (e.g. Turner & Hanly, 1949; Sutcliffe, 1954) have shown that the washing of tissue at first increases and later decreases its

Fig. 2. Effect of time of washing of tissue on the uptake of L-histidine; (a) washed for 98 hr.; (b) washed for 50 hr.; (c) washed for 215 hr. Tissue (3 g. fresh wt.) incubated in 20 ml. of 20 mM solution. Initial pH, 3.9. Temp. of water bath, 25°.

Table 1. Effect of thickness of slice on uptake

Uptake of L-histidine by carrot slices of different thicknesses. Tissue (3 g. fresh wt.) was washed for 12 hr. before use, and incubated in 20 ml. of L-histidine solution (5 mM). Initial pH, 4.0. Temp. of water bath, 25°.

Duration of uptake (min.)	Thickness of slice		
	1.5 mm.	1.0 mm.	0.5 mm.
	Concn. of external solution (mM)		
0	5.0	5.0	5.0
30	4.6	4.6	4.3
60	4.4	4.4	4.3
90	4.2	4.1	4.2
Total uptake (μmoles)	16	18	16

Table 2. Effect of pH on uptake

Uptake of L-histidine by carrot slices. Tissue (3 g. fresh wt.) was washed for 15 hr. before use and incubated in 5 mM histidine solution (20 ml.) buffered at different pH values with potassium phosphate buffer (20 mM). Temp. of water bath, 25°.

Duration of uptake (min.)	Concn. of external solution (mM)			
	4.9	5.9	7.0	8.0
Initial pH ...	...	...	...	...
Final pH ...	5.3	5.9	7.0	7.4
0	5.2	5.2	5.2	5.2
30	4.4	4.4	4.7	4.9
60	4.1	4.2	4.6	—
90	4.0	4.1	4.5	4.7
Total uptake (μmoles)	24	22	14	10

Table 3. *Recovery experiments*

Uptake of L- and D-histidine by carrot slices, and the estimation of histidine recovered from the slices. Tissue (3 g. fresh wt.) was washed for 14 hr. before use, then incubated in 20 ml. of 5 mM solution (L-histidine) or 0.5 mM solution (D-histidine). pH: L-histidine, 4.0; D-histidine, 4.5. Temp. of water bath, 25°.

Amino acid	Duration of uptake (hr.)	Initial amount present (μ moles)	Uptake (μ moles)	Recovery from tissue (μ moles)	Recovery from tissue (%)	Amount present in medium and washing fluid (μ moles)	Total recovery (μ moles)	Total recovery from tissue and medium (%)
L-Histidine	1	102	13.1	12.7	97	—	—	—
L-Histidine	1	102	13.1	12.5	96	—	—	—
D-Histidine	18	9.0	8.9	8.6	97	—	—	—
D-Histidine	18	10.0	9.8	9.5	97	—	—	—
L-Histidine	1	102	12.5	12.3	99	89.0	101	99
L-Histidine	18	98	67	69	103	28.0	97	99

activity, e.g. respiration, ion uptake. Similarly, the rate of removal of L-histidine from the solution was found to vary with the time of washing of the tissue after cutting. A batch of tissue was cut, samples were removed after different periods of washing, and the uptake of histidine was determined. Fig. 2 shows that the amount of histidine removed by the tissue increased progressively for periods of washing up to about 100 hr., then decreased slowly. As the tissue was quite active after washing for 12–16 hr., subsequent experiments were done with slices washed overnight—unless otherwise stated.

Effect of buffers and pH

The effect of buffering the histidine solutions was examined with a range of pH values, 20 mM phosphate buffer being used to give initial pH values of 5, 6, 7 and 8 (Table 2). It was found that the removal of histidine was greatest in the lowest pH range, decreasing markedly as the initial pH was raised from 5 to 7. (It may be of interest that the pH range 5–7 corresponds to the buffer range of the imidazole N of histidine pK 6.1.) However, as the rate of removal of L-histidine was much greater from solutions which were not buffered, in all subsequent experiments the solutions used contained histidine hydrochloride only, the initial pH being 3.9–4.0 (5 mM solution) or 4.5 (0.5 mM solution).

Recovery experiments

It was necessary to know whether the histidine taken up was metabolized or concentrated by the tissue.

Tissue samples were allowed to take up histidine, removed from the incubating solution, washed in 10 ml. of distilled water for 60 sec., and dispersed, first by a loose-fitting and then by a tight-fitting glass homogenizer, in about 20 ml. of distilled water. The dispersion was made up to a known volume with distilled water and trichloroacetic acid added to give a final concentration of 5% (w/v). It was then shaken, allowed to stand for 10 min., and centri-

fuged to remove solid matter. After two washings, each with 20 ml. of distilled water, the histidine content of the clear supernatant fluids was determined. Table 3 shows that the recoveries of both L- and D-histidine (by the colorimetric method used) were 96–103% of the amount disappearing from the medium, these figures being corrected for a small blank (0.5–1.3 μ moles) from the same amount of tissue not exposed to histidine solutions. The optical configuration of the histidine recovered after uptake was not investigated. Chromatography of tissue extracts after uptake showed a strong spot in the position of histidine. As a consequence of these experiments, the removal of the amino acid from the external solution could be equated with accumulation by the tissue.

Concentration ratios established by the tissue

Carrot slices were placed in solutions of L-histidine with concentrations varying from 50 to 0.5 mM. As shown in Table 4, the percentage of the total histidine disappearing after a given time (and, therefore, the quotient 'internal' concn./'external' concn.) increased as the concentration of histidine initially in the medium was decreased. Hence in subsequent experiments the removal of histidine was studied at the lower concentrations—usually 5 or 0.5 mM.

Effect of 2:4-dinitrophenol and cyanide

The effect of various concentrations of dinitrophenol was investigated. Dinitrophenol at 10^{-8} and 10^{-6} M produced some inhibition of uptake, but at 10^{-4} M a marked inhibition occurred; this concentration was used in all subsequent work.

Fig. 3 shows that the gross uptake consisted of two distinct processes. The first involved the rapid uptake of amino acid from the solution—at a concentration of 5 mM, 60–70% of the uptake over the first 30 min. occurred in the first 3 min.—and was not affected by dinitrophenol. Pre-incubation of the tissue in dinitrophenol for periods of 0–60 min.

Table 4. *Concentration gradients established by the tissue*

Uptake of L-histidine by carrot slices. Tissue (3 g. fresh wt.; water content, 2.76 g.) washed for 21 hr. before use, and incubated in solutions (20 ml.) of different concentrations. Temp. of water bath, 25°. The ratio of concentrations has been determined with allowance for the withdrawal of samples for analysis (0.5 ml. in Expts. 1-4 and 1.0 ml. in Expt. 5). All experiments were done with tissue from the same carrot.

Expt. no.	Initial concn. (mM)	Duration of uptake (hr.)	Concn. in medium (mM)	Uptake (μ moles)	Internal concn. / External concn.
1	49.0	0.5	47.5	30	0.2
		1.0	46.9	40	0.3
		6.0	45.5	69	0.6
2	25.0	0.5	23.3	34	0.5
		1.0	23.0	39	0.6
		6.0	21.6	66	1.1
3	10.0	0.5	9.0	20	0.8
		1.0	9.1	19	0.8
		6.0	8.0	39	1.8
4	5.0	0.5	4.4	12	1.0
		1.0	4.2	16	1.4
		6.0	3.2	35	4.0
5	0.5	0.5	0.34	3.2	3.4
		1.0	0.29	4.0	5.0
		6.0	0.02	9.0	163.0

Fig. 3. Effect of 2:4-dinitrophenol (10^{-4} M) on uptake of L-histidine; (a) control; (b) plus 2:4-dinitrophenol. Tissue (3 g. fresh wt.) washed for 12 hr. before use, then incubated in 20 ml. of the appropriate solution. Initial concn. of L-histidine, 5.0 mM. Initial pH, 4.0. Temp. of water bath, 25°.

Fig. 4. Release of L-histidine, previously accumulated by tissue, into (a) water and (b) dinitrophenol (10^{-4} M). Tissue (3 g. fresh wt.) washed for 18 hr. before use, then incubated in 20 ml. of the appropriate solution. Temp. of water bath, 25°.

did not affect the shape of this part of the uptake curve. The rate of uptake increased with the surface area of the tissue (see Table 1), and dinitrophenol had no effect on this first phase of uptake. It seems likely, therefore, that the initial rapid uptake is one of physical equilibration of the histidine in the medium with the intercellular fluids and with adsorption sites on the cell surfaces. Similar findings for the uptake of inorganic ions are reported by Robertson (1944), Robertson & Turner (1945) and Butler (1953).

After this initial rapid removal of histidine, a second slower process of 'active' uptake began in the system; in the presence of dinitrophenol this latter uptake was very markedly reduced (Fig. 3). With low concentrations of histidine and a long incubation period in the presence of dinitrophenol there was, however, some resumption of uptake. The addition of sodium cyanide (10^{-3} M, adjusted to pH 5) also completely inhibited the active phase of uptake.

The inhibition of uptake by dinitrophenol and cyanide suggests that provision of useful energy from respiration (oxidative phosphorylation) is necessary for active uptake to occur.

Further information about the energy requirements of the uptake process was obtained by a series of experiments on the release of L-histidine previously taken up by the slices. Tissue samples were allowed to take up L-histidine, removed from the incubating solution, blotted, washed for 1 min. in distilled water, blotted again and further incubated in 20 ml. of water or of dinitrophenol (10^{-4} M) (Fig. 4). Histidine released into water was taken up again, the rate of uptake exceeding the rate of release after about 30 min. In contrast to the results with water, histidine continued to be released into the dinitrophenol solution and was not taken up again.

Effect of inorganic ions and amino acids on the process of uptake

The uptake of L-histidine at 0.5 mM concentration was inhibited by the presence of 0.5 and 5.0 mM phenylalanine in the medium, the inhibition being 90% at the higher concentration. Fig. 5 shows the results of a series of experiments to investigate the effect of L- and D-phenylalanine and potassium chloride on the uptake of L-histidine. The initial

rapid uptake was not inhibited, but the inhibition of active uptake of L-histidine was greater in the presence of L-phenylalanine than with D-phenylalanine. Later work has shown that while potassium chloride always inhibits, the effect in some carrot tissue was confined to the initial rather than the final stage of uptake. L-Arginine and L-aspartic acid, at 5 mM concentration, inhibited the uptake of L-histidine (at the same concentration) by 50 and 60% respectively. The inhibition produced by phosphate buffers has been mentioned previously.

Histidine and arginine can be estimated independently by the methods used, and it was possible to investigate the uptake of these amino acids singly and together. The results from such an experiment are shown in Table 5. From the figures given it can be seen that L-histidine and L-arginine each inhibits the uptake of the other. Further, the sum of the amounts of L-histidine and L-arginine taken up from a mixed solution (5 mM with respect to each amino acid) was close to the mean of the amounts taken up from single solutions of these amino acids (both 10 mM). It seems likely, therefore, that competition for a common mechanism of uptake is responsible for the mutual inhibition by these two amino acids.

Uptake of D-histidine

In order to learn more of the mechanism involved, the uptake of D- and L-histidine was compared. It was found that: (i) The length of the period of washing of the tissue influenced the uptake of D-histidine in the same way as it did for the L-isomer, i.e. uptake was greatest after washing for about 100 hr. (ii) Quantitative recovery of D-histidine (by the colorimetric method used) from the tissues was found as for the L-isomer. (iii) Dinitrophenol reduced uptake in the same way (Fig. 6). (iv) The rate of uptake was less for D- than for L-histidine, although from 0.5 mM solutions of D-histidine the

Fig. 5. Effect of L- and D-phenylalanine and potassium chloride on the uptake of L-histidine. Initial concn. of all three amino acids and potassium chloride 5.0 mM; (a) L-histidine; (b) L-histidine plus D-phenylalanine; (c) L-histidine plus potassium chloride; (d) L-histidine plus L-phenylalanine. Tissue (3 g. fresh wt.) washed for 14 hr. before use and then incubated in 20 ml. of the appropriate solution. Initial pH of all solutions, 5.0. Temp. of water bath, 25°.

Table 5. Uptake of amino acids from single and mixed solutions

Uptake of L-arginine and L-histidine by carrot slices from single and mixed solutions. Tissue (3 g. fresh wt.) was washed for 14 hr., then incubated in 20 ml. of the appropriate solution. Initial pH, 5.0 (in all flasks). Duration of uptake, 300 min. Temp. of water bath, 25°.

Amino acid	Concn. of external solution (mM)		Total uptake (μmoles)
	Initial	Final	
L-Histidine	10.0	6.9	62
L-Arginine	10.0	7.8	44
L-Histidine plus L-Arginine	5.0	3.8	26
L-Arginine plus L-Histidine	5.0	3.8	26
L-Arginine	5.0	3.1	38
L-Histidine	5.0	2.6	48

Table 6. *Uptake of amino acids*

Uptake of all amino acids by 3 g. (fresh wt.) of tissue, incubated in 20 ml. of solution. Concn. of all solutions was 5 mM. Initial pH of solutions, 5.0. Temp. of water bath, 25°. Dinitrophenol (DNP), 10^{-4} M.

Amino acid	Duration of washing (hr.)	Duration of uptake (min.)	Uptake (μ moles)			
			L-Isomer		D-Isomer	
			Control	Control + DNP	Control	Control + DNP
Phenylalanine	250	350	33	6	—	—
	300	420	47	16	48	14
	400	320	37	7	36	4
Valine	30	300	41	8	44	1
	100	300	60	1	65	4
Leucine	70	300	24	3	16	—
	200	300	29	7	32	3
	350	300	34	9	47	5
Glutamic acid	200	350	30	-2	—	—
	250	260	28	-8	—	—
	200	540	60	14	—	—
Arginine	300	300	40	23	—	—

Fig. 6. Effect of 2:4-dinitrophenol (10^{-4} M) on the uptake of L- and D-histidine. Initial concn. of L- and D-histidine 0.5 mM; (a) L-histidine, (b) D-histidine, (c) L-histidine and dinitrophenol, (d) D-histidine and dinitrophenol. Tissue (3 g. fresh wt.) washed for 14 hr. before use and then incubated in 20 ml. of the appropriate solution. Initial pH, 4.5. Temp. of water bath, 25°.

concentration in the medium was again reduced to the limit at which it could be detected.

Uptake of other amino acids

Histidine and arginine were estimated by the specific methods discussed earlier. The other amino acids were estimated manometrically by the chloramine T method. Table 6 shows that dinitrophenol always markedly inhibited the uptake of both the L- and D-amino acids, and that the L-isomer was sometimes, but not always, taken up at a faster rate than the D-isomer.

Table 7 shows that, with the same sample of tissue, some amino acids were taken up at a similar rate (e.g. L-alanine and L-isoleucine), whereas others (e.g. L-aspartic acid and L-lysine) were taken up at quite different rates.

Table 7. *Comparison of uptake of pairs of amino acids*

Uptake of amino acids by 3 g. (fresh wt.) of tissue, incubated in 20 ml. of solution. Concn. of all solutions, 5 mM. Initial pH of solutions was adjusted to 5.0. Duration of uptake, 300 min. Temp. of water bath, 25°. Dinitrophenol (DNP), 10^{-4} M.

Tissue sample	Duration of washing (hr.)	Amino acid	Uptake (μ moles)	
			Control	Control + DNP
1	300	Alanine	34	2
		Isoleucine	37	3
2	96	Aspartic acid	32	2
		Lysine	21	6
3	216	Aspartic acid	47	-29
		Lysine	24	-1
4	300	Aspartic acid	47	-36
		Lysine	19	-18
5	50	Proline	44	2
		Hydroxyproline	41	1

DISCUSSION

The results presented above show that amino acids are taken up from a suitable medium by slices of carrot tissue, and that the process results in a considerable concentration of the amino acids inside the tissue. As it is inhibited by dinitrophenol and cyanide, it is likely that this process of uptake requires a supply of energy just as do similar systems in certain bacteria (Gale, 1954) and animal tissues (Christensen & Riggs, 1952; Agar, Hird & Sidhu, 1954).

It has been shown (Butler, 1953) that the uptake of chloride, potassium and phosphate by wheat

roots is inhibited by dinitrophenol; and that the uptake of chloride by carrot disks is inhibited by dinitrophenol and other respiratory inhibitors (Robertson, Wilkins & Weeks, 1951). In addition, these latter workers showed that the release of electrolyte accumulated by slices of carrot is increased by dinitrophenol. There are, therefore, close similarities between the uptake of inorganic ions in these systems and the uptake of amino acids by slices of carrot. Further, potassium chloride and potassium phosphate inhibit the uptake of L-histidine by this tissue. It is, therefore, possible that in carrot tissue inorganic ions and amino acids are taken up by the same mechanism. In contrast, Arisz (1953) found that phosphate does not interfere with the movement of asparagine along the tentacles of *Drosera*.

The carrot and bacterial systems seem to remove amino acids completely from the external medium, whereas the animal systems establish a concentration difference with a steady state between amino acids in the tissue and the medium. Further, the plant system studied above does not show the same high specificity for uptake of the L-isomers of the amino acids as do certain animal systems (Agar *et al.* 1954; Christensen, Riggs, Fischer & Palatine, 1952). For this reason there seems to be a difference between plant and animal systems.

SUMMARY

1. The conditions determining the uptake of amino acids by slices of carrot have been investigated.

2. Slices of carrot take up amino acids from the medium and establish a large concentration gradient in favour of the tissue. The process of uptake of both D- and L-isomers is inhibited by 2:4-dinitrophenol and by cyanide. The process, therefore, requires energy from respiration.

3. The rate of uptake of L-histidine was greater than that of D-histidine, although for phenylalanine, valine and leucine there was usually no significant difference between the amounts of the optical isomers taken up during 5 hr.

4. The inorganic salts potassium chloride and potassium phosphate (solutions at pH 6.0–8.0) inhibited the uptake of L-histidine. L-Histidine and L-arginine mutually inhibit the uptake of each other.

One of us (L.M.B.) held the Sir John and Lady Higgins Research Scholarship from the University of Melbourne during the course of this investigation.

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The Volatile Fatty Acids of Bovine-Muscle and -Liver Phospholipids

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(Received 3 February 1956)

It has long been known that C₄–C₁₂ volatile fatty acids with an even number of carbon atoms normally occur to the extent of approximately 6% by weight in the glycerides of milk fats (Hilditch, 1947), and more recently [James & Popják, unpublished observations quoted by Popják (1952)] this series has been extended to include acetic acid. Larger amounts of C₂–C₆ saturated volatile fatty acids

(12.9% by weight) occur in the triglycerides of the female reproductive organs of *Ascaris lumbricoides* (Fairbairn, 1955).

The development of a suitable micro-analytical technique for determining volatile fatty acids (James & Martin, 1952) has made possible the detection of smaller amounts of these acids in lipids from other sources, and it has already been shown