Increase in the Process of Accumulation of Amino Acids in Carrot Slices with Prolonged Aerobic Washing

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The ability of slices of storage tissue of certain plants to accumulate inorganic ions (Asprey, 1936-37; Stiles & Dent, 1946; Rees, 1949; Sutcliffe, 1952) and amino acids (Birt & Hird, 1956, 1958*a*) is markedly stimulated by a prior period of aerobic circulation in water. Related phenomena are the development of salt-stimulated respiration (Robertson, 1941) and malonate-sensitivity of respiration (Hanly, Rowan & Turner, 1952; Laties, 1959) on aerobic washing of plant storage tissue. The factors underlying the development of these apparently changed physiological states are unknown.

The results reported here are an attempt to assess the importance of some of the processes occurring during the washing procedure. They indicate that during aerobic circulation of the tissue, removal of competitors (endogenous material) for the mechanism of accumulation is associated with increased accumulation of amino acids and salt-stimulated respiration.

MATERIALS AND METHODS

Materials

The amino acids and sulphanilamide used were commercial samples. Extracts and solutions of amino acids were adjusted to pH 5.0 with HCl before use. Commercial oxygen-free nitrogen was used as the means of circulating carrot slices anaerobically.

Estimation of amino acids

Amino acids were measured by direct development of the colour in samples of the solution, with 2 ml. of the ninhydrin reagent of Connell, Dixon & Hanes (1955). The samples (40 μ l.) were pipetted from a semi-automatic pipette on to pieces of Whatman no. 3 paper (1 cm.²) prepared by the method of Connell *et al.* (1955). This method was also used to determine the concentration of ninhydrin-reacting material (amino acids plus ammonia) in the carrot extracts obtained, and in assessing leakage from the carrot tissue into the external medium.

Histidine was estimated by the method of Macpherson (1946) with the modifications of Birt & Hird (1956).

Tissue extracts

Ethanolic extraction. Carrots were peeled and cut into slices 1 mm. thick; 500 g. of slices was added to 2 l. of ethanol (80%, v/v). The extraction proceeded, with occa-

sional shaking, for 16 hr. at room temperature. The extract was then poured off and replaced by fresh 80% ethanol (1 l.) and this was allowed to stand, with frequent shaking, for a further 2 hr. The total extract (3 l.) was then filtered to remove suspended material and concentrated under reduced pressure at 35°. In order to remove the last traces of ethanol, the residue was taken up in 100 ml. of water which was dried under reduced pressure. The process was repeated with 50 ml. of water. The residue was dissolved in water to give the final extract, which was adjusted to pH 5.0 and made up to give a final concentration of 50 mM with respect to ninhydrin-reacting material. Undissolved material was removed by centrifuging. The concentrated extract was analysed for material reacting as histidine (histidine, tyrosine and phenols) by the method of Macpherson and found to be 0.46 mM with respect to a histidine standard. The final volume was 150 ml.

Boiling water extraction. Slices (500 g.) (1 mm.) were added, over a period of 2 min., to 3 l. of boiling water and boiled for 5 min. The container was then rapidly cooled with water and allowed to stand for 20 hr. The extract was then filtered and concentrated under reduced pressure as before and made up to give a final concentration of 50 mM with respect to ninhydrin-reacting material. The concentrated water extract was analysed for histidine-reacting material and found to give a colour yield equivalent to 0.37 mM-histidine. The final volume was 152 ml.

Both extracts were treated with 1 g. of activated carbon/100 ml., being briefly brought to 60° as the carbon was added, and cooled rapidly immediately after the addition. This treatment decreased the turbidity of both extracts and diminished the orange colour of the ethanolic extract.

Tissue

Carrots were obtained not more than 24 hr. after harvest, and sliced on the day they were obtained. Slices (1 mm.) were prepared by the method given by Birt & Hird (1956) and were taken from the cambial zone. For uptake of amino acids the slices were cut into pieces (20-60 mm.²). In order to obtain greater reproducibility in sampling for respiration experiments, disks (0.4 cm. in diameter) rich in cambial tissue were prepared from the slices with the aid of a stainless-steel cork borer.

The freshly cut slices or disks were given several preliminary washings to remove cell and surface debris and to mix the pieces of tissue. In experiments where some tissue was to be circulated aerobically and some anaerobically, the pieces of tissue were always mixed before being divided.

Circulation was carried out in 1 l. round-bottomed flasks through which air was drawn and which were kept at 25° in a constant-temperature room. Sulphanilamide (mM) was

present in the circulating water to inhibit the growth of bacteria. A previous experiment had shown that the tissue exhibited salt-stimulated respiration and active uptake of amino acids after circulation under these conditions. When simultaneous circulation under anaerobic and aerobic conditions was used, gas flows of air and nitrogen were regulated until the rates of circulation of the pieces of tissue were approximately equal. After circulation for 0.5–1 hr. the fluid was replaced several times with water, and finally water containing sulphanilamide was added. This procedure removed substrates for bacterial multiplication, which are released by the tissue after it is cut. After this initial replacement, the circulating medium (water containing sulphanilamide) was changed every 24 hr. Sulphanilamide was not used in the final incubation medium except in one experiment, which was of 24 hr. duration. Here mm-sulphanilamide was also used.

Before being used in an experiment the pieces of tissue were washed in water several times and blotted lightly with hardened filter paper.

Experimental procedure

Measurement of oxygen consumption. A circular Warburg apparatus was used. About 40 disks were placed in 2 ml. of incubation fluid in the main compartment. The total 'fluid volume' in the main compartment was determined by taking the specific volume of the carrot disks to be 1 and making a correction for the volume obtained from the fresh weights of the disks (approx. 0.4 ml.). The centre well contained NaOH. The bath temperature was 25° and the manometers were shaken at 160 oscillations/min. The gas phase was air.

The tissue was allowed to pre-incubate for 15 min. before the first reading. Experiments generally lasted 2–2.5 hr., at the end of which the disks were removed, blotted lightly and weighed. Dry weights were determined after drying overnight at 105°.

Measurement of amino acid uptake. Uptake of amino acids by the tissue was followed by determination of the change in concentration of ninhydrin-reacting material in the medium. Tissue during the experimental period did not exhibit a net uptake or release of water.

The tissue slices (about 2 g.) were weighed and placed in 10 ml. of the incubating solution at pH 5.0 in a stoppered conical flask (50 ml.) and shaken at 150 oscillations/min. in a water bath at 25°. The stopper was removed twice for a few minutes during the experiment to allow gas exchange. Samples were removed for analysis at appropriate intervals.

Chromatography of boiling-water extract. Descending paper chromatography was used with Whatman no. 1 paper. The moving phase was butanol-acetic acid-water (4:1:1, by vol.) and also phenol saturated with water.

RESULTS

Effect of alternate aerobic and anaerobic circulation on salt-stimulated and control respiration. Fig. 1 shows salt-stimulated respiration expressed as a percentage of control respiration. It is seen that in unwashed tissue potassium chloride (50 mM) slightly depresses respiration. After 64 hr. of aerobic circulation, the presence of potassium chloride stimulates respiration by 43%; these

results are in keeping with earlier work (see Robertson, 1941). Tissue having developed this salt-stimulated respiration is shown to lose it after a further 70 hr. circulation under anaerobic conditions, and its respiration is actually inhibited in the presence of potassium chloride. On being recirculated under aerobic conditions for a further 68 hr. the salt-stimulated respiration is restored and increases to 82% above the control respiration.

Tissue from the same batch, when initially circulated anaerobically, does not show salt-stimulation. With changes from aerobic to anaerobic circulation, there is the same stimulation and inhibition of respiration by potassium chloride as occurs in tissue initially circulated aerobically.

Experiments were not continued beyond 200 hr., which was in the region of the life of the tissue under the conditions used. The results are expressed as percentages of controls receiving similar aerobic and anaerobic treatment. The control respiration generally increased after aerobic circulation and decreased after anaerobic circulation.

Increased uptake for some D- and L-amino acids after circulation. Birt & Hird (1958a) showed that isoleucine and valine were rapidly accumulated by tissue after circulation and that this accumulation was not complicated by metabolism of these amino acids. They were therefore chosen here for experiments designed to follow the development of the uptake process. Further it had been shown in the above-mentioned paper that the L-isomers of amino acids had a greater affinity for the uptake process than the D-isomers.

Fig. 1. Effect of alternate aerobic and anaerobic circulation on salt-stimulation of respiration. The tissue (0.4 g.) was in 2 ml. of water or 2 ml. of 50 mM-potassium chloride. Incubation was carried out at 25° for 2 hr. Experiments were performed in triplicate and means were plotted. ---○, Period of aerobic circulation; —●, period of anaerobic circulation. Change in respiration is expressed as percentage of control respiration on a fresh-weight basis. Maximum deviation of Q_{O_2} values from mean is 13%.

Birt & Hird (1956, 1958*b*) showed that 0.1 mM-2:4-dinitrophenol markedly inhibited the uptake of amino acids. Although some work has been done on the dinitrophenol-insensitive uptake it was felt that dinitrophenol-sensitive uptake, being considerably greater, would be a useful fraction of active uptake for study.

Fig. 2 shows that in tissue freshly cut there was substantial dinitrophenol-sensitive uptake of L-isoleucine but considerably less of the D-isomer. Further it is shown that the percentage increase after a period of aerobic circulation was larger with the D-isomer than with the L-isomer. The graph shows that after 21 hr. circulation under aerobic conditions, no further increase in dinitrophenol-sensitive uptake for either D- or L-isoleucine occurs. Additional experiments showed that occasionally there was a small increase after 21 hr. Similar results were obtained with D- and L-valine. About 60 hr. was used as the circulating period in subsequent experiments, as this period represented termination of the development of uptake.

Fig. 2. Effect of aerobic circulation on development of accumulation of D- and L-isoleucine: ○, D-isoleucine; ●, L-isoleucine. Tissue (2 g.) was incubated in 10 ml. of appropriate solution (5 mM) with and without dinitrophenol (0.1 mM). The initial pH was 5.0 and duration of uptake 6 hr.

Table 1. Effect of washing on dinitrophenol-insensitive uptake of D- and L-isoleucine

Tissue (2 g. fresh weight) was incubated for 6 hr. in 10 ml. of solution at 25°. Initially, the pH was 5.0, the concentration of D- and L-isoleucine was 5.0 mM and of dinitrophenol 0.1 mM. The uptake period was 0–6 hr.

Time of washing (hr.)	Uptake (μmoles)	
	D-Isoleucine	L-Isoleucine
1	0.8	0.6
22	2.1	4.5
45	2.3	5.1
65	7.6	12.5

Table 1 shows that dinitrophenol-insensitive uptake of both D- and L-isoleucine also increases during circulation. After circulation dinitrophenol-insensitive uptake of L-isoleucine is at all times greater than the similar uptake of D-isoleucine. Similar results were also obtained with D- and L-valine and suggest the operation of a carrier mechanism with an energy requirement insensitive to dinitrophenol (? glycolysis). The results again show the increase in the process of uptake although with this minor component there is no evidence for the differential increase of uptake of the D-isomer.

Dinitrophenol-sensitive uptake of some D- and L-amino acids after alternate aerobic and anaerobic circulation. With the D- and L-isomers of valine and isoleucine, an increase in dinitrophenol-sensitive uptake during the first 45 hr. of circulation always occurred under aerobic conditions. This increase was considerably greater with the D-isomer than with the L-isomer. During initial anaerobic circulation there was often a fall in dinitrophenol-sensitive uptake, but after the first circulation period a marked and uniform response has been repeatedly obtained, i.e. dinitrophenol-sensitive uptake increased after aerobic circulation and decreased after anaerobic circulation. Results obtained with D-isoleucine are shown in Fig. 3.

A similar result was obtained with L-isoleucine, L-valine and D-valine. Once again changes in active uptake were always less marked for the L-isomers than for the D-isomers.

Salt-stimulated respiration and amino acid uptake from the same batch of tissue. With the same batch of tissue, the effect of alternate aerobic and anaerobic circulation on unstimulated respiration, salt-stimulated respiration Q_{O_2} of the tissue and dinitrophenol-sensitive uptake of D-isoleucine was tested simultaneously. These all increased after aerobic circulation and all decreased after anaerobic circulation, as in previous experiments in which batches of different tissue were used. The results obtained

Fig. 3. Effect of alternate aerobic and anaerobic circulation on dinitrophenol-sensitive uptake of D-isoleucine. The tissue (2 g.) was in 10 ml. of D-isoleucine (5 mM) and 10 ml. of D-isoleucine plus dinitrophenol (0.1 mM). Initial pH was 5.0 and duration of uptake 6 hr. ---○, Period of aerobic circulation; —●, period of anaerobic circulation.

therefore describe a common behaviour of carrot tissue under the conditions used.

Ninhydrin-reacting material in the tissue. It had been observed that ninhydrin-reacting material is released from freshly cut carrot tissue, and in order to obtain more quantitative information about this material the amount extractable by 80% ethanol has been determined. The results for eight carrots from the same batch indicate that surprisingly high concentrations (35–61 mM) of ninhydrin-reacting material are found in the tissue.

These results were felt to be of interest in relation to the observed competition for the carrier system between externally supplied amino acids (Birt & Hird, 1958b). It was decided therefore to investigate the process of leakage of ninhydrin-reacting material from the tissue, as it was thought that migration of endogenous amino acids during the circulation process might be related to the development of active uptake.

Release of ninhydrin-reacting material during initial aerobic circulation. Release from freshly cut tissue into water, and water containing 0.1 mM-dinitrophenol, was studied. The particular tissue had been determined on the basis of its water content to be 50 mM with respect to ninhydrin-reacting material. Results over 6 hr. are shown in Fig. 4. In the presence of water and water containing dinitrophenol, 3 and 8% respectively of the endogenous amino acids were lost after 6 hr. In water a maximum concentration was obtained at 3 hr. incubation, i.e. after 3 hr. the rate of reabsorption exceeded the rate of release. In another experiment reabsorption had removed almost all the amino acids from solution after 12 hr. In the

Fig. 4. Release of ninhydrin-reacting material from freshly cut tissue. The tissue (2 g.) was in 10 ml. of water or 0.1 mM-dinitrophenol. The estimated concentration of ninhydrin-reacting material in the tissue was 50 mM. Incubation was in a shaking water bath for 6 hr. at 25°. ●, Water; ○, dinitrophenol. Experiments were performed in duplicate; vertical lines indicate the spread of results (where these exceeded the diameter of the circle).

presence of dinitrophenol the rate of leakage exceeds the rate of any reabsorption which may occur.

Release of ninhydrin-reacting material after prolonged aerobic and anaerobic circulation. Release into water was extremely small after prolonged circulation whether the tissue had received either aerobic or anaerobic treatment.

Release into 0.1 mM-dinitrophenol was much greater, and showed marked fluctuations with change of circulation procedure. After the initial aerobic or anaerobic circulation period of 60 hr., release into dinitrophenol is variable but comparatively low (Fig. 5). After this period prior anaerobic circulation causes a marked stimulation of release, whereas prior aerobic circulation either causes no change or reverses the effect of anaerobic circulation.

The processes of leakage of ninhydrin-reacting material into dinitrophenol solution after aerobic and anaerobic circulation thus show similar responses, but opposite in sign, as do salt-stimulated respiration and amino uptake under the same conditions.

The reversal of the process of leakage, the development of salt-stimulated respiration and active uptake of amino acids are thus associated with aerobic circulation; anaerobic circulation reverses these processes. These effects are likely to have a common basis.

Effect of cold-water extract on uptake of L-histidine. It was decided to determine whether material released into cold water by freshly cut tissue was responsible for the inhibition of uptake of amino acids in unwashed tissue. Because of its relatively specific method of assay, L-histidine was chosen for this aspect of the investigation. A cold-water extract (3 hr.) was prepared and its effect on the uptake of L-histidine was tested with aerobically

Fig. 5. Effect of alternate aerobic and anaerobic circulation on release of ninhydrin-reacting material. The tissue (2 g.) was in 10 ml. of water or 0.1 mM-dinitrophenol. Incubation under aerobic conditions was for 6 hr. at 25°. Correction was made for small loss into water alone. ---○, Period of aerobic circulation; —●, period of anaerobic circulation.

circulated tissue, i.e. tissue with a well-developed active uptake. An extract of 3 hr. was chosen, as this represents the mid-point of the 6 hr. period used to follow uptake. The concentration present after 3 hr. (0.2 mM in ninhydrin-reacting material) would therefore represent the 'average' concentration in the medium resulting from leakage during an uptake experiment with freshly cut tissue.

The results showed that such an extract only slightly inhibited uptake (less than 10%) by both circulated and freshly cut tissue from the same batch of carrots from which the extract was prepared. This small inhibition of uptake by circulated tissue therefore could not account for the low uptake observed in freshly cut tissue.

These experiments have shown therefore that only a small proportion of the endogenous amino acids leak out of freshly cut tissue, and that the amount released does not account for the low rates of uptake in freshly cut tissue. As can be seen from the previous section the concentration of ninhydrin-reacting material in the water of the tissue tested varied from 30 to 70 mM. It therefore seemed more likely that it was the high internal concentration which was responsible for the inhibition of uptake of amino acids in freshly cut tissue.

Accordingly, 80% ethanolic extracts and boiling-water extracts of tissue were prepared. These were adjusted to give concentrations of the required order (50 mM with respect to ninhydrin-reacting material) as described in the Materials and Methods section.

Analysis of boiling-water extract and 80% ethanolic extract. Chromatography of these extracts (concentrated to 50 mM in ninhydrin-reacting material) and freshly expressed carrot juice showed the same amino acids to be present in the same relative amounts; in this respect the extracts were therefore substantially representative of the fresh

tissue. The major amino acids were glutamine, glutamate, asparagine, aspartate and alanine. These amino acids accounted for most of the ninhydrin-reacting material in the extracts.

The effect of aerobic circulation for 150 hr. on the concentrations of these amino acids in the tissue was studied by visual comparison on paper chromatograms. In one experiment asparagine and the glutamine-aspartate spot increased, whereas glutamate and alanine decreased considerably during the circulation period. In another experiment of 150 hr. all the amino acids decreased to low amounts.

Analysis of the boiling-water extract (50 mM with respect to ninhydrin-reacting material) gave the following results for inorganic ions: Na^+ , 47 mM; K^+ , 98 mM; PO_4^{3-} , 23 mM.

Effect of extracts on accumulation of D- and L-histidine. Uptake of histidine has been determined from 0.5 to 6.0 hr. to eliminate the substantial initial inactive uptake from 0 to 0.5 hr. (Birt & Hird, 1956). In several cases, which are noted in the text, the small uptake which is insensitive to dinitrophenol during this period has been subtracted.

Table 2 (Expt. 3) shows that at concentrations of 2.5 and 5.0 mM, the water extract markedly inhibited the accumulation of the two isomers of histidine. It is significant that the percentage inhibition of the D-isomer was very much greater than that of the L-isomer. In comparative experiments shown in Table 2 (Expts. 1 and 2), boiling-water and ethanolic extracts are shown to give quantitatively similar inhibitions of the uptake of L-histidine.

Table 3 shows the active uptake of L-histidine found by using as competitors two amino acids of relatively low and high affinity for the carrier system (glutamine and methionine respectively; Birt & Hird, 1958b). Ethanolic and boiling-water

Table 2. *Effect of extracts of carrot tissue on uptake of D- and L-histidine by aerobically circulated carrot tissue*

Tissue (2 g. fresh weight) was incubated for 6 hr. in 10 ml. of solution at 25°. The initial pH was 5.0 and concentration of D- and L-histidine 5.0 mM. The tissue was circulated aerobically for 46 hr. The uptake period for the extract was 0.6 hr. and, for D- and L-histidine, 0.5–6.0 hr. Different batches of tissue were used in each experiment. At 5 mM, the ninhydrin-reacting material in the ethanolic and boiling-water extracts had a dinitrophenol-sensitive uptake of 19.3 and 14.9 μmoles respectively in the absence of histidine.

Concn. of ninhydrin-reacting material in extract (mM)	Dinitrophenol-sensitive uptake (μmoles)			
	L-Histidine			D-Histidine
	Expt. 1. Ethanolic extract	Expt. 2. Boiling-water extract	Expt. 3. Boiling-water extract	Expt. 3. Boiling-water extract
Nil	19.9	20.2	28.5	17.2
1.0	19.7	17.8	—	—
2.5	15.6	15.9	15.4	2.5
5.0	12.7	12.4	11.4	0.4
10.0	6.9	5.1	—	—

Table 3. *Effect of glutamine, methionine and extracts on uptake of L-histidine by aerobically circulated tissue*

Tissue (2 g. fresh weight) was incubated for 6 hr. in 10 ml. of solution at 25°. The initial pH was 5.0 and concentration of L-histidine was 5.0 mM. The tissue was circulated aerobically for 45 hr. and the uptake period of L-histidine was 0.5–6.0 hr.

Competitor	Concn. of ninhydrin- reacting material (mM)	Dinitrophenol- sensitive uptake of L-histidine (μ moles)
None	—	15.1
Ethanol extract	2.5	11.7
Ethanol extract	5.0	7.7
Boiling-water extract	2.5	10.2
Boiling-water extract	5.0	7.4
L-Glutamine	5.0	7.4
L-Methionine	2.5	2.1

extracts are included for comparison of effects on the same tissue. The results show that similar inhibitions of L-histidine uptake are produced by 5 mM-glutamine, boiling-water extract, 5 mM with respect to ninhydrin-reactive material, and ethanolic extract, 5 mM with respect to ninhydrin-reactive material. 2 mM-Methionine (with a high affinity) gives greater inhibition than extracts or 5 mM-glutamine.

It has been found that the uptake of ninhydrin-reacting material in these extracts has a large dinitrophenol-sensitive component and that the total uptake is of the order of magnitude obtained with amino acids. It is concluded that the endogenous ninhydrin-reacting material mostly consists of assorted amino acids and this is confirmed by chromatography. As these amino acids would be of the L-configuration, the greater inhibition of uptake of D-histidine as opposed to L-histidine in fresh tissue can be explained in terms of affinity for the carrier.

Birt & Hird (1956) showed that potassium chloride and potassium phosphate buffer decreased the uptake of L-histidine by washed carrot tissue, and the possibility is not excluded that the high concentrations of inorganic ions in the extract inhibit to some extent the uptake of externally supplied amino acids.

DISCUSSION

Birt & Hird (1958*b*), using carrot tissue previously circulated aerobically, showed that uptake of the various amino acids involved a common carrier, and that when these were added together they competed for this carrier. The nature of the side chain was shown to be important in determin-

ing the affinity for the carrier, and the L-isomers were shown to have much greater affinity than the corresponding D-isomers. It has also been shown that active uptake increased considerably on circulating the tissue aerobically (Birt & Hird, 1956, 1958*b*).

In the present investigation we have found high concentrations, from 35 to 61 mM, of free amino acids (presumably the L-isomers) in the tissue. During the course of aerobic circulation the uptake of D-amino acids increases from a lower initial value, and to a greater extent than the uptake of the L-amino acids; it has also been shown that the rate of leakage of endogenous amino acids into a medium containing dinitrophenol is lowered on aerobic circulation. Tissue treated in this way when circulated anaerobically shows the converse of these changes. The results obtained are thus consistent with removal of endogenous and competing L-amino acids from the carrier during aerobic circulation and their return during anaerobic circulation. This disappearance and appearance of amino acids at the carrier site is possibly connected with fluxes into and out of the vacuole, but metabolism (Birt & Hird, 1958*a*) of these amino acids will also play a part in their disappearance. Extracts of tissue and comparable amino acid solutions produced inhibitions of the expected magnitude in the uptake of D- and L-histidine by aerobically circulated tissue. It seems likely therefore that the process leading to an increase in the active uptake of amino acids during aerobic circulation is the removal of endogenous and competing material from the carrier site.

The increase in salt-stimulated respiration after aerobic circulation and its loss after anaerobic circulation follows a similar pattern to the accumulation of amino acids under the same conditions. With salt-stimulated respiration, however, the situation is more complex; the reasons for a stimulation of respiration in the presence of potassium chloride are unknown, and the various substrates used will probably make a different quantitative contribution to respiration after aerobic and anaerobic treatments. It is likely therefore that not only might endogenous anions and cations inhibit uptake of the salt in freshly cut and anaerobically circulated tissue, but also that salt-stimulated respiration might only occur with special substrates dominating after aerobic circulation. Consequently it is possible that the development of accumulation of amino acids and of salt-stimulated respiration have in common only the removal of endogenous substances from the region of the carrier (cytoplasm).

There is an extensive literature concerning the accumulation of inorganic ions by plant tissues after aerobic circulation (see review by Sutcliffe,

1959), and a carrier mechanism is generally accepted as being implicated in the accumulation process (see reviews by Epstein, 1956; Sutcliffe, 1959). Rees (1949) has shown that after anaerobic circulation, uptake, by red-beet tissue, of manganese and chloride ions is less than in aerobically treated tissue. Skelding & Rees (1952) and Dale & Sutcliffe (1956) have also shown that extracts of the tissue inhibit the uptake of inorganic ions. There are thus many similarities between this system and the one studied in the present work. These observations on the accumulation of salts after aerobic circulation and the decreased accumulation after anaerobic circulation could similarly have an explanation in terms of the removal or non-removal of competing endogenous anions and cations from the region of the carrier.

The increase in malonate-sensitive respiration by slices of plant tissue after aerobic circulation has obvious parallels with the increase in uptake of amino acids and inorganic ions, and with the increase in salt-stimulated respiration. It has commonly been assumed that the increase in malonate-sensitivity of respiration (Hanly *et al.* 1952; Laties, 1959) is associated with an increase in a qualitatively different component of respiration. However, the problem may have a simpler explanation in terms of the removal, during aerobic circulation, of competitors which prevent the entry of inhibitors, such as malonate, to their sites of action.

SUMMARY

1. The increase in salt-stimulated respiration and of the active accumulation of amino acids in slices of carrot has been investigated during washing by circulation aerobically and anaerobically.

2. Salt-stimulated respiration and amino acid accumulation which increase on aerobic circulation can be reversed by anaerobic circulation of carrot slices.

3. When the rate of dinitrophenol-sensitive accumulation of D- and L-amino acids is compared

in freshly cut slices and in aerobically circulated slices, it has been found that the increased rate of accumulation after circulation is much greater with the D-isomer than with the L-isomer.

4. High concentrations (30–70 mM) of free amino acids are found in the tissue fluid, and extracts of the tissue containing these amino acids have been shown to compete with other externally supplied amino acids for the accumulation process.

5. It has been concluded that the increase in salt-stimulated respiration, and in the accumulation of amino acids when the tissue is aerobically circulated, is related to the removal during this process of endogenous substances from the cytoplasm or carrier sites or from both.

6. The results obtained are discussed in relation to the increase in the active accumulation of inorganic ions and of the so called malonate-sensitive respiration.

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