THE ACTION OF INHIBITORS ON WATER UPTAKE BY POTATO TISSUE

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(WITH ONE FIGURE)

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Introduction

The potato tuber and other storage tissues have been used for more than 30 years in the study of permeability and salt accumulation. However, until recently, less attention was paid to the uptake of water by these tissues, though both Stiles and Steward noted that when thin slices are used the water uptake is considerable. In 1938 REINDERS (**5**) showed that the prolonged uptake of water is a strictly aerobic process, and concluded (**6**) that the energy of respiration "creates the condition" causing water uptake. She also found that the uptake is promoted by low concentrations of auxin. This observation, which has been confirmed by several workers, suggested that a more careful study might shed some light on the mechanism of auxin action. The work reported here, in summary form, deals only with the relation between water uptake and metabolism as shown by means of several metabolic inhibitors.

Methods

A cylinder of tissue is cut from a potato (var. Katahdin) previously stored at about 14° C. Discs 1 mm. thick and 1 cm. in diameter are sliced from this with a hand microtome. The discs are washed for 15 minutes in running tap-water, allowed to stand in shallow water for 24 hr., blotted and weighed in groups of ten discs, and then transferred to the experimental apparatus. In the past the usual procedure has been to place the discs in a relatively large volume of water, through which air was bubbled (**7, 6**). However, in studies of the growth of sections of Avena coleoptiles (**10**) it was found that when the sections were mounted so as just to break the surface of the liquid they grew as rapidly as if they were submerged and vigorously aerated. Accordingly, we have mounted the potato discs so as to break the surface of a shallow unaerated solution. The apparatus consists of a fine net stretched over a large glass ring, 8 cm. in diameter, resting in a Petri dish. After weighing, the discs are placed on the net, the solution added up to the appropriate level (40 to 50 cc.); and the dishes transferred to a dark room at 25° C. Discs are usually reweighed at two-day intervals.

The principal justification for this simplified method is the fact that the phenomena described in the literature with the more complex method can be duplicated. The absolute values of the water uptake obtained by

this method are within the range of values (which vary widely) reported by other investigators.

Results

Results of a typical experiment are given in table I. A duplicate experiment gave almost identical results. The uptake of water, as measured by the increase in fresh weight, continues for six days in those discs which are breaking surface. In comparable experiments the percentage increase

TABLE I

THE UPTAKE OF WATER BY POTATO TISSUE AS MEASURED BY INCREASE IN FRESH WEIGHT. BLOTTED FRESH WEIGHTS IN GRAMS OF TEN DISCS (1 CM. × 1 MM.) IN EACH SERIES

DAYS FROM START*	0	2		4		6	
Solution	WEIGHT	WEIGHT	PER CENT. INCREASE	WEIGHT	PER CENT. INCREASE	WEIGHT	PER CENT. INCREASE
Water, breaking surface	1.23	1.30	*5.7*	1.335	*8.5*	1.345	*9.4*
Water, submerged	1.14	1.16	*1.8*	1.17	*2.6*	1.18	*3.5*
IAA (10 mgm./l.)	1.20	1.275	*6.3*	1.345	*12.1*	1.38	*15.0*
NaN_3 (10^{-4} M, pH 6.0)	1.14	1.15	*0.9*	1.155	*1.3*	1.17	*2.6*

* The experiment starts when the discs are put into the test solution, *i.e.* after 24 hours' washing.

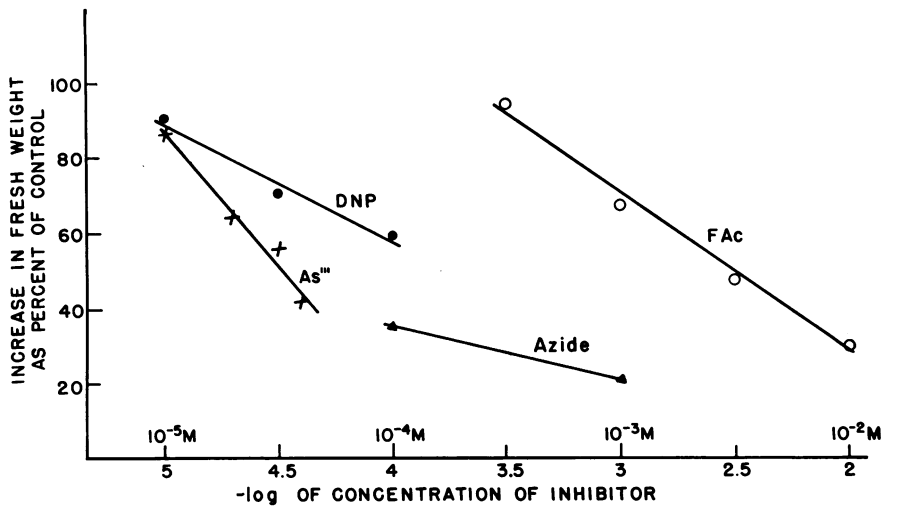

FIG. 1. The increase in fresh weight, as per cent. of that of controls in distilled water, of 1 cm. × 1 mm. potato discs after four days in solutions of enzyme inhibitors. Each point represents the weight of 10 discs and in addition is the mean of the following number of repetitions: for three to five dinitrophenol (DNP), four; for arsenite, four; for azide, four to six; for fluoroacetate, four. (January–April 1950). pH of solutions 6.0–6.2, except DNP 5.5.

after six days has varied from 7 to 13%. Discs submerged in the same volume of water, under a depth of about 5 mm., show less than half the water absorption. Indole-acetic acid in a concentration of 10 mgm./l. increases the water uptake considerably. These facts are in accord with Reinders' findings on the effect of anaerobic conditions and auxin. On the other hand, a 10^{-4} M solution of sodium azide very strongly reduces the water absorption throughout the six-day period. The discs remain in excellent condition, although slightly paler than the controls. In none of the cases reported here did any of the discs show signs of injury or bacterial decomposition.

The results of a large number of similar experiments with four different inhibitors are combined in summary form in figure 1. The increase in fresh weight, as per cent. of that of the control, is plotted against the negative logarithm of the concentration of inhibitor (in distilled water). The expression of fresh weight as per cent. of that of the control allows the comparison of experiments in which the absolute water uptake of the sections varies. Although the range of effective concentrations differs considerably, in each case there appears to be a roughly linear relationship between water uptake and the log of the inhibitor concentration. Clearly, the aerobic uptake of water by potato discs can be prevented by a variety of enzyme inhibitors.

Discussion

The inhibitors used have been shown to act on a number of different processes: azide acts on cytochrome oxidase, arsenite inhibits enzymes containing essential –SH groups, fluoroacetate acts as an antagonist of acetate and blocks its metabolism via the Krebs cycle, while dinitrophenol apparently acts by uncoupling phosphorylation from respiration. The fact that all these compounds inhibit the increase in fresh weight suggests that the general respiratory metabolism of the cell underlies the process of aerobic water-uptake.* Specifically, the data indicate that cytochrome oxidase, one or more –SH enzymes, the oxidation of acetate, and a phosphorylating mechanism are linked in some way to the uptake of water.

The mechanism whereby oxidative metabolism can control water movement is not clear. The work of Brauner *et al.* (**1**) suggests that an osmotic gradient may be the ultimate controlling factor, and the effect of auxin in promoting water uptake was interpreted in this way by Reinders (**6**) and by Commoner *et al.* (**2**). However, it seems not improbable that there is a metabolically controlled system which accumulates water in a manner analogous to the way ions are accumulated. Indeed, suggestions of this sort, under the name of "active" water uptake, have been made by several authors (**8, 4**; cf. also (**3**)).

* In Levitt's researches (Plant Physiol. 23: 505–515. 1948) on auxin-induced water uptake it may be seen that cyanide does not inhibit even the uptake in the absence of auxin. Since this is in clear conflict with the above data obtained with four different inhibitors, it is evident that the case of cyanide is a special one which calls for more detailed consideration.

All of the compounds used have been shown to act as inhibitors of the growth of sections of Avena coleoptiles and Pisum stems, in presence of auxin (9). Iodoacetate, which has been extensively used in that connection, was less satisfactory in the present experiments because of apparent injury accompanying moderate inhibition. A comparison of the concentrations of the various inhibitors which produce a 50% inhibition of both growth and water uptake shows a striking similarity, as shown in table II.

TABLE II

CONCENTRATIONS OF INHIBITORS CAUSING 50 PER CENT. INHIBITION OF GROWTH AND WATER UPTAKE

	CONCENTRATION X 10^{-5} M OF:—			
	ARSENITE	DINITROPHENOL	FLUOROACETATE	IODOACETATE
Growth of coleoptiles	1	4	300	4
Growth of peas	10	20	600	60
Water uptake by potatoes	3	25	300	(30)*

* The figure is approximate owing to varying degrees of injury.

It may be suggested that those metabolic processes which are intimately linked with growth are the same as those linked with aerobic water uptake. Both, indeed, are phenomena of cell enlargement.

Summary

A simplified method for the measurement of the aerobic water uptake by discs of storage tissue is described.

The water uptake can be inhibited quantitatively by azide, dinitrophenol, arsenite and fluoroacetate, all of which are inhibitors of oxidative enzyme systems and also of growth.

Implications of the results with respect to the link between respiration and water absorption are briefly discussed.

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