The Dependence of Auxin-induced Growth on Auxin-independent Metabolic Changes in Slices of Storage Tissue [1]

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Water absorption by slices of storage organ tissue is markedly enhanced in the presence of auxin (1, 3, 4, 5). A lag period that varies from hours to days, depending on the type of tissue and its storage history, precedes auxin-induced enlargement (1, 2, 3).

When bulky storage organs are sliced, there is a general quickening of metabolic activity which increases with time (1, 6, 7, 10). In potato, for example, the respiration rises approximately fourfold within a day, and starch degradation, protein synthesis and salt absorbing capacity increase sharply (10, 15, 18). In slices of both potato (16) and red beet (15) the onset of vigorous salt absorption is preceded by a period roughly equal in duration to the growth lag phase, although the enhancement of salt absorbing capacity is independent of auxin.

There is considerable evidence that the induced respiration in storage organ slices is largely different in kind from the initial, or basal, respiration (6, 7, 9, 13). In particular, slicing is followed by a dramatic enhancement of oxidative phosphorylation which far exceeds expectations based simply on the gross increase of respiratory activity (11). It is well established that auxin-induced water uptake is respiration dependent (3). The question which is raised herein is whether auxin-induced growth of storage organ slices is dependent upon the respiratory changes which follow slicing. The hypothesis which underlies this investigation is that the onset of active phosphorylation associated with the induced respiration is causative to the many physiological changes noted in aging tissue slices—including the development of auxin responsiveness.

In most of the experiments which have dealt with auxin induced enlargement in storage tissue, slices were routinely brought to a fully turgid condition in a preliminary 24-hour incubation period in water in an air atmosphere (2, 3, 4). The reported respiration rates at the beginning of the experimental period for both potato and artichoke slices indicate that the respiratory rise has largely taken place by that time. As will be more fully discussed below, Adamson has recently recognized that the preliminary period is in fact of special importance, and his observations have led him to conclusions similar to ours (1). In any event slices must experience a period of exposure to auxin before auxin-induced growth is manifested, whether the auxin dependent lag period is concomitant with, or follows, the respiratory rise. The basic question remains whether the respiratory transformation which occurs in slices in the first day independently of the presence of auxin controls subsequent auxin mediated growth.

In studies dealing with the nature and control of the induced respiration it was discovered that *inter alia*, lithium ion (8) and acetaldehyde (6, 7) effectively respress the normal respiratory changes which attend aging, without inhibiting respiration per se. Upon removal of either lithium or acetaldehyde the respiratory rise occurs in the usual way. Acetaldehyde is but one of several aldehydes which effectively suppress respiratory development. Chloral (CCl_3CHO) has proved most convenient for experimental purposes.

In the experiments which follow slices were incubated in auxin (NAA) together with one of the above-mentioned inhibitors. Subsequently the tissue was removed to auxin alone and the time-course of growth was observed to determine whether the onset of growth was delayed for a period equivalent to that required for the induced respiration to develop. It will be shown that although lithium or chloral prove to have an independent effect on water absorption whenever provided, the influence of these inhibitors in the first 24 hours is of special significance and distinguishable from subsequent effects.

Materials and Methods

Potato tubers of the variety Russet Burbank, and Jerusalem artichoke (*Helianthus tuberosum*) were purchased locally and stored at 7° and 1°, respectively. Disks were prepared and maintained aerobically in a shaker at room temperature as previously described (7). Water uptake was calculated as the percent increase in fresh weight of blotted disks over the initial fresh weight. Oxygen uptake was determined with groups of disks (approximately 1.0 g fr wt), using constant volume Warburg respirometers. Solutions of 10^{-2} M chloral, 5×10^{-2} M LiCl and 40 mg/liter streptomycin were prepared at pH 6.0 to 6.2, and contained 10 mg/liter naphthalene acetic acid in 10^{-4} M $CaSO_4$. All measurements were in duplicate, each series being repeated 2 or more times.

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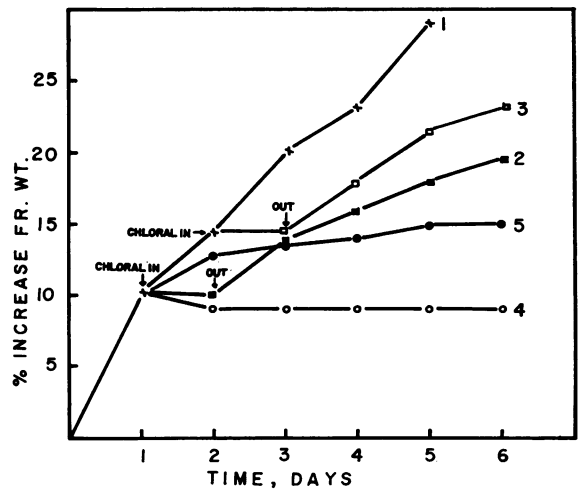
Results

Potato. Figure 1 describes the effect of chloral on water absorption by potato slices. Enlargement in the first 24 hours reflects the attainment of osmotic equilibrium, and is the same whether incubation occurs in dilute $CaSO_4$, NAA, chloral, or a combination thereof. In the pertinent experiments cited in the introduction, osmotic equilibration was regularly attained in a preliminary period so that the weight increase noted in the first day in figure 1 was completed by the beginning of the experimental period. It is evident from the figure that when auxin and chloral are added together from the beginning auxin-induced water uptake is completely repressed. It is clear as well, however, that chloral inhibits water absorption when provided at any time following the onset of auxin-induced growth, and that the inhibition under the latter circumstances is released as soon as the tissue is removed from chloral and returned to auxin alone. When growth is restrained by chloral even for a day, maximal enlargement (i.e. by comparison with the auxin treated control) is no longer attainable. The foregoing observation resembles the restriction imposed on maximal growth in Jerusalem artichoke slices by the temporary withholding of auxin (see below). Insofar as chloral also prevents the limited enlargement which occurs in the absence of auxin, it is suggested that growth in the latter instance depends on endogenous auxin and is similar to that evoked by NAA.

Since short term respiratory measurements show no effect of chloral, a direct effect of chloral on respiration per se cannot account for its influence on growth. In potato slices the developed respiration declines in the course of a day in response to chloral (fig 2), but remains well above the initial or basal rate. In Jerusalem artichoke slices (see below) the decline in the space of a day is negligible. By contrast growth in both cases is inhibited at once by chloral. It is evident that NAA evokes a respiration rate in excess of the control [fig 2, 4; cf. (4)]. Whether auxin indeed causes a stimulation of respi-



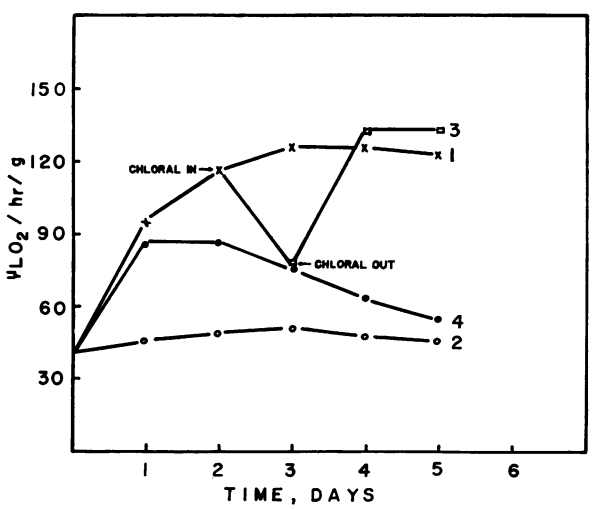


FIG. 1 (*upper*). Effects of chloral on auxin-induced water uptake of potato disks. 1. Disks in NAA (10 mg/liter) from the beginning. 2. Disks in NAA for 24 hours, followed by a day in NAA plus chloral (10^{-2} M) and return to NAA. 3. NAA for 48 hours, followed by chloral plus NAA for 24 hours, and return to NAA alone. 4 Disks in chloral with or without NAA from the beginning. 5. Control disks without auxin. 10^{-4} M $CaSO_4$ in all cases.

FIG. 2 (*lower*). Effect of chloral on the respiration of potato disks. 1. Disks in NAA (10 mg/liter) from the beginning. 2. Disks in NAA and chloral (10^{-2} M) from the beginning. 3. Disks in NAA for 48 hours, followed by a day in chloral plus NAA, and return to NAA. 4. Control disks in auxin-free solution.

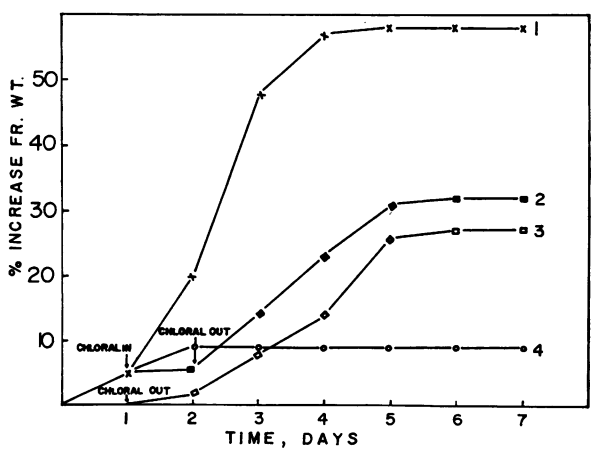


FIG. 3. Effect of chloral on auxin-induced water uptake of Jerusalem artichoke disks from stored tubers. 1. Disks in NAA (10 mg/liter) from the beginning. 2. Auxin for 24 hours, followed by chloral (10^{-2} M) plus auxin for a day, and return to auxin. 3. Chloral and NAA for a day, followed by auxin alone. 4. Control disks in auxin-free solution. Tubers approximately 10 months from harvest.

ration above the control, or serves to maintain the respiration at an elevated level while the control respiration declines [cf. Sacher (14)], is beside the point, since the chloral sensitive respiratory changes at issue regularly take place in the absence of auxin.

Lithium ion (5×10^{-2} M) represses respiratory development in storage organ slices much as does chloral (8). However, again as with chloral, lithium represses auxin-induced growth at any time during the growth period. Streptomycin (40 mg/liter), which prevents the respiratory rise in slices of old potato tubers but not in new (B. C. Loughman, personal communication), completely repressed water uptake in our experiments without stemming the increase in respiration.

Jerusalem Artichoke. The above indicated growth responses of potato slices to auxin, though essentially similar to those first described by Reinders (12), fell well short of the pronounced growth stimulation observed in potato by Hackett and Thimann (3). Experiments were therefore continued with slices of Jerusalem artichoke, a tissue showing

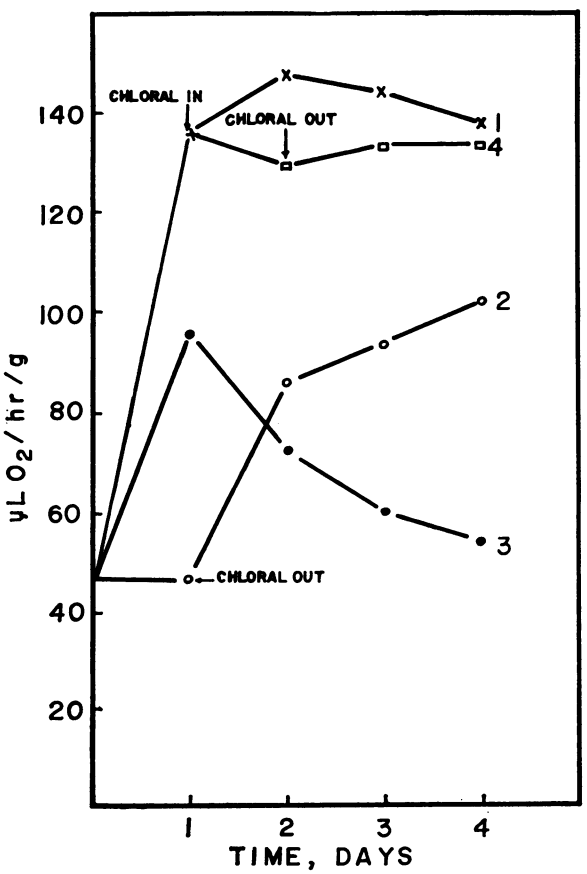


FIG. 4. Effect of chloral on the respiration of Jerusalem artichoke disks from stored tubers. 1. Disks in auxin from the beginning. 2. Disks in auxin plus chloral for a day followed by auxin alone. 3. Control disks in auxin-free medium. 4. One day in NAA followed by 24 hours in chloral plus NAA, and return to auxin. Tubers 10 months old.

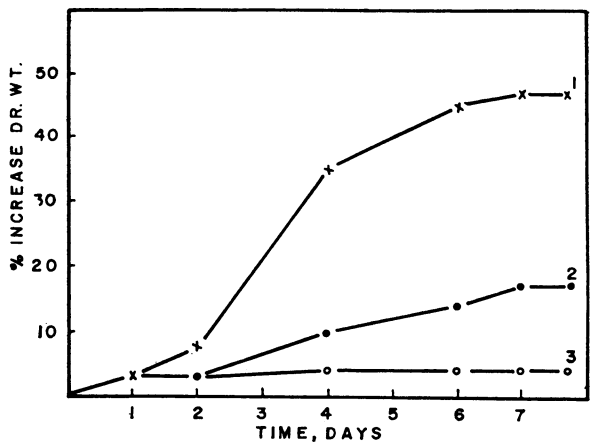


FIG. 5. The effect of delayed auxin application on auxin-induced water uptake of Jerusalem artichoke disks. Tubers 4 months from harvest. 1. Disks in NAA (10 mg/liter) from the beginning. 2. 24 hour delay before presentation of auxin. 3. 48 hour delay.

a more rigorous dependence upon, and a greater response to, auxin (1, 4, 5). Preliminary experiments indicated that in Jerusalem artichoke slices, as in potato, chloral not only prevents the respiratory rise which occurs in the first day (fig 4) but also promptly inhibits auxin-induced water uptake when provided at any time during the growth period. Attention was therefore turned to the question of whether the effect of chloral when presented for the first 24 hours is distinguishable from its effect when presented at any time thereafter. Figure 3 suggests that such is the case. When chloral and NAA are administered for the first 24 hours and the disks subsequently removed to NAA alone, there is an additional 24 hour lag period in which growth is minimal, after which the growth rate increases markedly. Alternatively, when chloral is presented in the second 24-hour period and the disks then returned to NAA alone, growth during the following day is maximal for the particular treatment. An examination of figures 3 and 4 indicates that auxin-induced enlargement in each case occurs primarily after the respiratory rise has largely taken place. Chloral is seen to have little effect on the respiration when presented after the first 24 hours.

It is noteworthy that both the growth rate and the maximum growth attained are always less following exposure to chloral at any time than in its total absence (fig 3). An explanation is conceivably inherent in the observations depicted in figures 5 and 6 wherein it is demonstrated that when growth is temporarily precluded in aerobically incubated artichoke slices by withholding auxin, the subsequent rate and maximum level of growth in auxin are sharply diminished. The implication is that an irreversible reduction in growth potential takes place when growth is temporarily prevented whether by withholding auxin or by inhibition with chloral [cf. (2)].

The ability to respond to auxin following a period in its absence is a function of tuber age, increasing from several months after digging to incipient sprouting. Growth without added auxin similarly increases with the age of the tuber. The growth lag period, though increasing during the first 3 to 4 months after harvest (2), subsequently diminishes with time [fig 5, 6; cf. (5)]. The foregoing observations are consistent with, and may reflect, an increase in endogenous auxin in the later stages of tuber maturation. In this view the duration of the period of responsiveness in the absence of added auxin may represent the time in which endogenous auxin is depleted.

Discussion

A circumstantial case has been made to support the proposition that auxin-induced growth in storage organ slices depends upon the induced respiration which is manifested within the first day after slicing. Prevention of the respiratory rise invariably curtails auxin-induced enlargement, but since it has turned out that inhibitors which repress the rise in respiration independently stop water-uptake at any time during the growth period, an unambiguous answer to the problem has not been possible. Nevertheless, inhibitor action in the first 24 hours, when auxin-induced enlargement is not taking place in any event, is distinguishable from the direct inhibition of water uptake at a later time. In the first instance an additional day's incubation in auxin is required upon removal of the inhibitor before significant growth begins. In the second case growth resumes following removal of disks from chloral at a maximal rate for the given experimental treatment.

A preparatory period, or lag, in auxin is normally a preliminary to auxin induced growth, although the lag may be relatively short in old tubers. As evidenced by a comparison of the foregoing experiments with others (1, 5) the auxin dependent lag may accompany or follow the respiratory changes which are elicited by slicing. In our experiments where auxin was provided from the beginning the growth lag and the period of respiratory change were concomitant. The experimental procedure was dictated by the fact that withholding auxin for a day normally resulted in a severe loss of responsiveness to auxin provided subsequently. Thus although in view of the demonstrable effect of chloral on respiratory development it is suggested that the particular effect of chloral on growth when given in the first 24 hours is attributable to the suppression of the induced respiration, it can be argued that chloral directly inhibits preparatory growth reactions in the lag phase [cf. Hanson and Bonner (5)]. The same uncertainty would apply to any inhibitor of both growth and respiratory development when the precise mode and locus of action are unknown.

Observations made by Adamson (1) strongly support the contention that the induced respiration implements auxin-mediated growth in artichoke slices. Adamson noted that the maximal growth rate ultimately achieved in response to auxin was found in slices preincubated for periods up to 24 hours in air, at room temperature, in the absence of auxin. Incubation in auxin from the moment of cutting markedly diminished the ultimate growth response. In each case the preincubation period resulting in greatest growth corresponded to the time required for the respiratory rise to be completed. Preincubation in the absence of auxin for periods longer than 24 hours caused a subsequent diminution in auxin induced growth (cf. fig 5). It appears that with respect to preincubation history, growth reflects opposing trends. On the one hand the development of the induced respiration is a preliminary to growth; on the other hand irreversible loss of auxin responsiveness attends aging in the absence of auxin. In most of our experiments the tendency towards loss of responsiveness proved overriding, and auxin was therefore provided from the beginning. We failed to note the above-mentioned inhibitory effect of auxin which may perhaps be manifested only in relatively newly harvested tubers. With tissue slices having the characteristics of those in Adamson's experiments it should prove possible to obtain a direct answer to the question at issue by noting the consequences of the inhibition of the respiratory rise in the total absence of auxin.

Table I

The effect of incubation in the absence of auxin on subsequent auxin response of Jerusalem artichoke disks.

Time Till NAA Provided	Growth					
	Fresh*	1	2	3	4	5 days
hours	% increase fresh weight					
Control (No NAA)	0	5	9	9	9	9
0	0	3	16	42	50	52
24	0	4	18	41	48	51
48	0	4	7	10	16	22
60	0	4	7	7	10	16

* Average initial fresh weight, 1.93 g. Tubers at incipient sprouting. NAA 10 mg/liter; 10^{-4} M $CaSO_4$.

The varying behavior of artichoke slices depends on the physiological condition of the tubers, which in turn reflects their age and storage conditions. Tuber age determines not only the duration of the growth lag phase in slices (2), but also the maximum growth rate attainable (1), and, as has been shown above (fig 5, 6; table I), the extent of persistence of responsiveness when auxin is withheld. On the basis of the foregoing it is possible to reconcile instances where little or no preincubation in the absence of auxin is tolerable (fig 5), with the numerous examples cited where preincubation for a day is the usual procedure.

The auxin-dependent growth lag varies from almost no lag at all (4) to a day or more (2). As

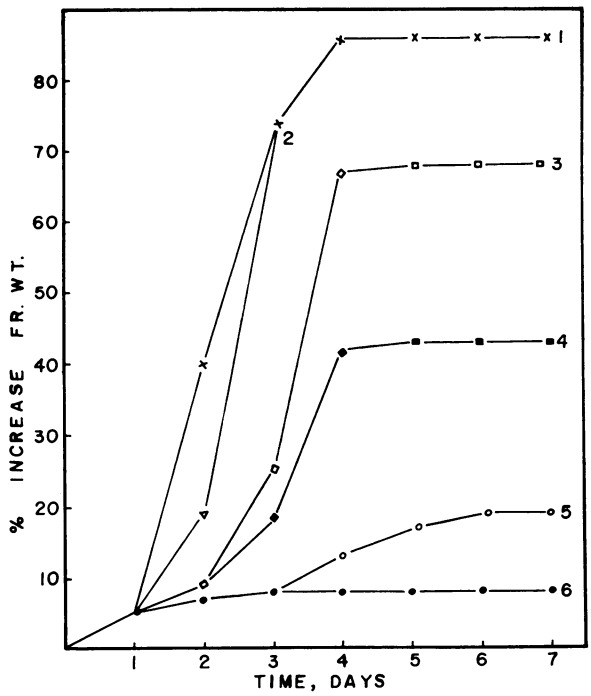


FIG. 6. The effect of delayed auxin application on auxin-induced water uptake of Jerusalem artichoke disks. Tubers 10 months from harvest. 1. NAA (10 mg/liter) from the beginning. 2. 24 hour delay before presentation of auxin. 3. 28 hour delay. 4. 40 hour delay. 5. 48 hour delay. 6. Control, auxin-free medium. 10^{-4} M $CaSO_4$ in all cases.

has been pointed out, the auxin-depedent lag has most often been described following a preincubation period in the absence of auxin. It may prove informative to compare the duration of the growth lag period with the time-course of respiratory development in cases where the lag periods differ widely.

Summary

Potato and Jerusalem artichoke slices were incubated in auxin (NAA) from the time of cutting together with lithium ion or chloral, either of which suppresses the respiratory changes which normally follow slicing. Auxin-induced growth was thereby prevented. Lithium ion or chloral were found also to inhibit enlargement independently of their effect on respiratory development when administered after the completion of the respiratory rise. When chloral was added with auxin in the first 24 hours and the slices then removed to auxin alone, an additional incubation period, equivalent in duration to the time required for the development of the induced respiration, was necessary before significant auxin induced growth occurred. When disks in auxin were treated with chloral for 24 hours after the completion of the respiratory rise, growth resumed with no observable delay when the tissue was transferred to auxin. On the basis of the foregoing observations and related experiments (1) it was concluded that auxin-mediated growth depends upon the induced respiration.

Acknowledgments

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