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Phases of Water Absorption in Germinating Raphanus Embryo

By

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When dry seeds are supplied with water under proper conditions, their tissues absorb water and start various physiological activities. Goo (1) discriminated three phases in the water absorbing process when dried pine seeds are made to germinate. OOTA (2, 3) and STANLEY (4) found the same phenomenon in the seeds of *Vigna sesquipedalis* and *Pinus*, respectively, and they studied also some concomitant biochemical changes.

It is worth while to study what happens in the cells when they shift from the latent state to biochemically active state. As a first step of research into this problem, germination process of decotyledonized embryo of *Raphanus* was studied chiefly in connection with respiration. Some experimental results are reported here.

Material and Methods

Seeds of *Raphanus sativus* '*Kurobaminowase*' were used. Cotyledons were cut off with razor blade. Decotyledonized embryos were laid on moistened filter paper in a Petri-dish, which was covered and kept at 28°, unless otherwise described. Water uptake was expressed by fresh weight increase in percentage of the weight of unmoistened embryos at the start of the experiments. For fresh weight determination, ten to twenty embryos were sampled at intervals, blotted with filter paper, and weighed by means of a torsion balance.

Effects of inhibitors on the water absorption were observed by infiltrating the filter paper with a solution of inhibitor and laying unmoistened embryos on it.

Respiration was measured by the "direct method" using conventional WARBURG's respirometer. In the main room of respirometer vessel, 20 to 40 embryos were introduced suspended in 1.2 ml of phosphate buffer of pH 6.4 supplemented with 0.3 ml of an inhibitor solution or water as control. In the case of observing the effect of KCN, a mixture of KCN and KOH was used

as carbon dioxide absorbent.

Dehydrogenase activity was estimated by methylene blue decolouration. Enzyme solution was prepared by grinding about 1 g dry weight of embryos in 0.02 M phosphate buffer at pH 7.4 in a chilled mortar, and rejecting the pulp by filtering with flannel.

Results

Water absorption —— Embryos laid on wet filter paper absorbed water very rapidly at first. This phase was followed by a period of no apparent water absorption, and then the water absorption began to proceed at a steady rate. These phases of water absorption will be denoted as phases A, B, and C in the order. It is shown in Fig. 1 that the period of phase B was shorter, and the rate of water absorption in phase C was higher at 30° than at 25°, while phase A was not influenced by temperature so apparently. It seems that phases B and C are controlled by physiological processes while the water absorption in phase A is rather physical in nature.

Effect of respiratory inhibitors on the water absorption —— When dried

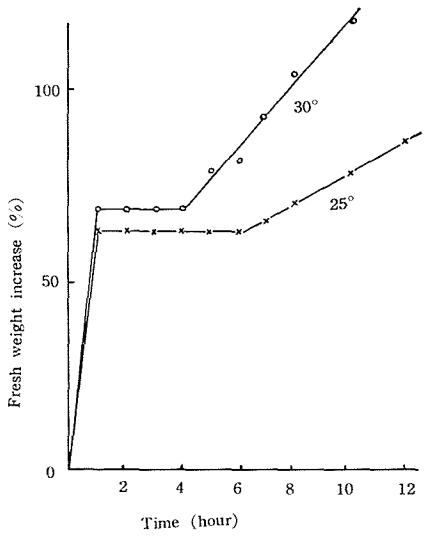

Fig. 1

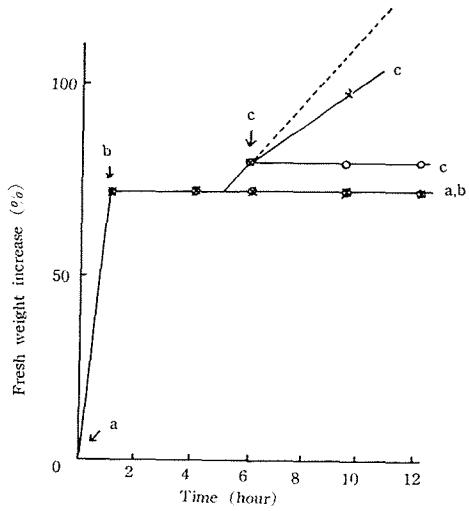

Fig. 2

Fig. 1. Effect of temperature on the water uptake pattern.

Fig. 2. Effect of respiratory inhibitors on the water uptake pattern. ----, Control; -○-, 10^{-3}M NaN_3; and -×-, 2×10^{-3}M salicylaldoxime; Arrows indicate the time of inhibitor application. Curves a, b and c are those obtained by inhibitor applications at arrows a, b and c, respectively.

embryos were laid on filter paper moistened with a solution of 10^{-3} M azide or 2×10^{-3} M salicylaldoxime, phase A proceeded just as in control, but further water absorption, i. e. phase C, did not begin (a in Fig. 2). Phase C did not appear also when embryos were transferred from distilled water to an inhibitor solution at the beginning of phase B (b in Fig. 2). If embryos were transferred to the inhibitors when phase C was already in progress, water absorption was inhibited completely by the azide solution, but only partially by the salicylaldoxime solution (c in Fig. 2). These results suggest that the water uptake in phase A does not depend on respiration, while that in phase C and probably also the completion of phase B do.

Effects of inhibitors on the respiration and water uptake in phase C were as represented in Table 1. There appears to be parallelism between the relative inhibition of water uptake and that of oxygen uptake at phase C.

Fig. 3 shows changes in the gas exchange and in the respiratory quotient, accompanying the progress of germination process. The rate of oxygen uptake began to increase at an early period in phase B to reach, before entering into phase C, the same rate of raspiration as in the latter phase. The respiratory quotient, which was high at first, fell conspicuously during phase B and reached a value which was kept in phase C at least until 15 hours.

Effects of inhibitors on respiration of embryos at different ages of germination are shown in Fig. 4. Inhibition by diethyldithiocarbamate (DIECA) and salicylaldoxime was stronger at a stage in phase B than before and after it. On the other hand, inhibition by hydroxylamine and α, α'- dipyridyl was weak at the beginning of phase B but grew stronger later on. The level of inhibition by cyanide and azide did not change significantly during the period of observation.

Table 1. Relation between inhibition of respiration and of water uptake at phase C

Inhibitors	Concn. (M)	Inhibition of oxygen uptake (%)	Inhibition of water uptake (%)
NaN_3	10^{-6}	7	25
	10^{-5}	11	52
	10^{-4}	56	100
	10^{-3}	73	100
α, α'-dipyridyl	10^{-5}	4	4
	2×10^{-5}	8	21
	2×10^{-4}	13	52
	2×10^{-3}	29	89
Salicylaldoxime	2×10^{-3}	8	21

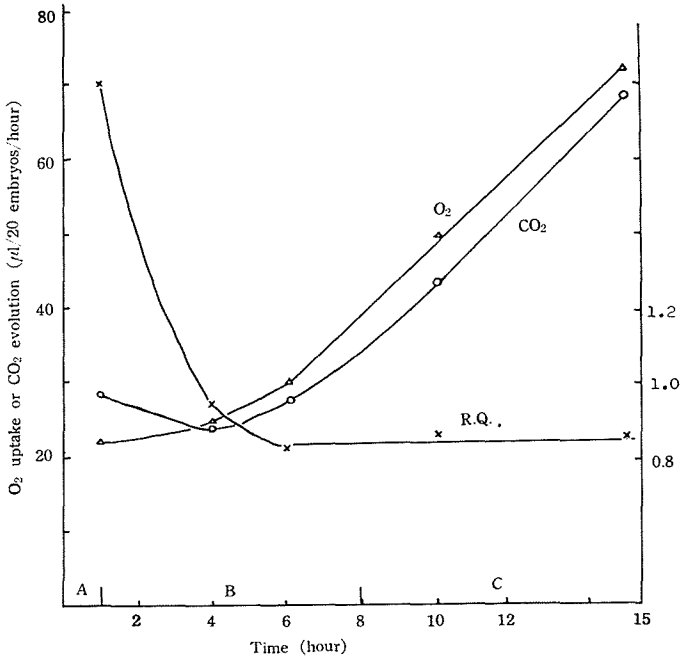

Fig. 3. Respiratory rate and respiratory quotient of decotyledonized embryos. Water was given at zero time. Phases A, B and C of water absorption are indicated along abscissa.

These facts may suggest that the respiration via copper enzymes increases relatively in a special period and the respiration via iron enzymes is more predominating in the rest of periods. Since, however, this shift in the main respiratory system did not correspond to the transition from phase C, some factor(s) other than the respiratory metal enzymes must be responsible for the transition.

Dehydrogenase activity —— Since respiration is inhibited by malonate only weakly in the early stage of germination as seen in Fig. 4, respiration seems to depend only little upon the TCA cycle in this stage. Activities of three dehydrogenases of the cycle were measured by means of the THUNBERG technique, using filtrates of mashed embryos at 1 hour of age, namely in phase B, and at 13 hours of age, namely in phase C.

As shown in Fig. 5, activities of succinic, malic and citric dehydrogenases were lower in the 1-hour sample than in the later sample when no cofactor was supplemented. But addition of coenzymes revealed that the activities of malic and citric dehydrogenases were extremely limited by the amount of coenzymes in the preparation at each stage.

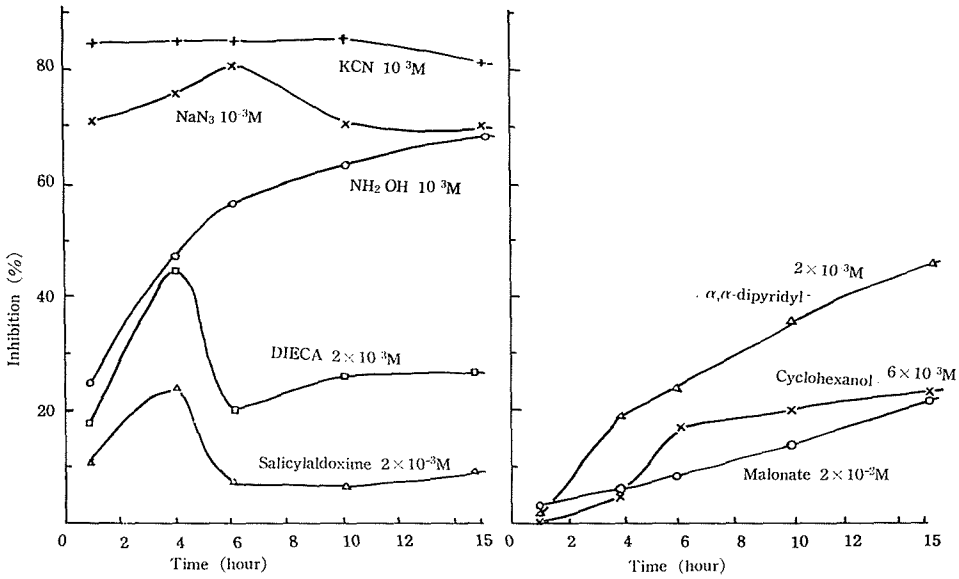

Fig. 4. Inhibition of oxygen uptake by inhibitors given at various periods after moistening of embryos at zero time.

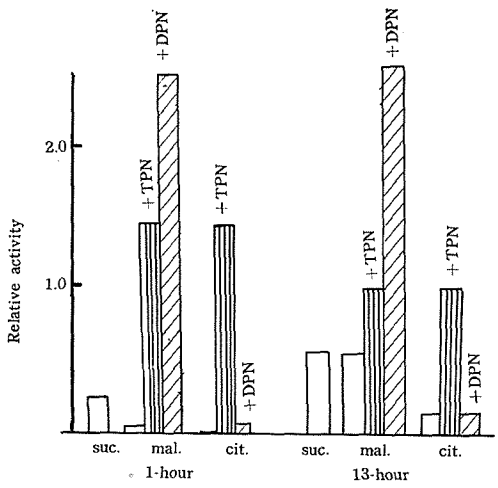

Fig. 5. Activities of succinic, malic and citric dehydrogenases of extract from embryos at 1- and 13-hour of age, with and without addition of TPN and DPN.

Effect of osmotic pressure and respiratory inhibitor on water absorption —— When embryos which had been wetted with distilled water for 6 hours were transferred to 0.5 M mannitol solution, the water uptake in phase C beginning at 8 hours proceeded at a lower rate than control as shown in Fig. 6 A. And

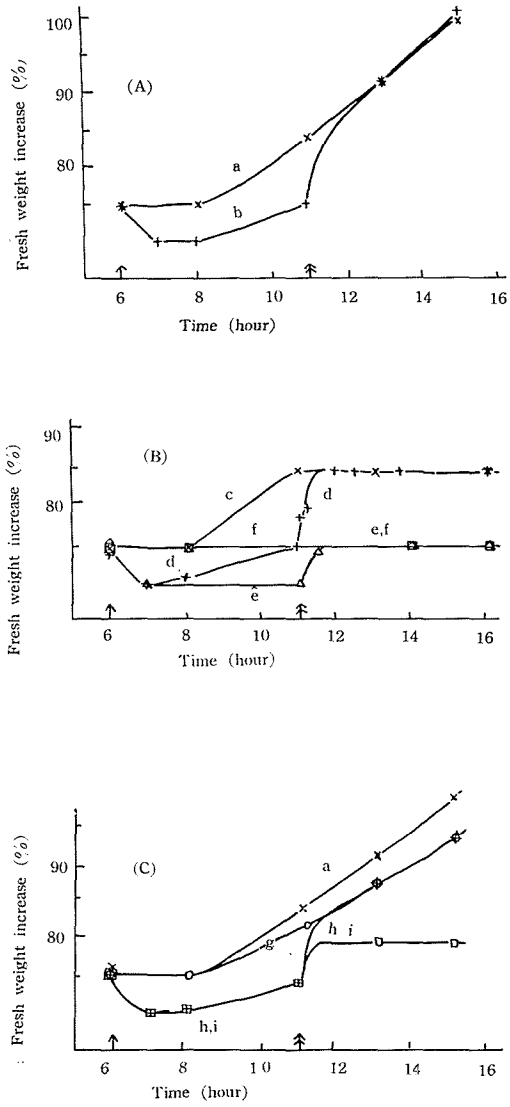

Fig. 6. *Effect of environmental osmotic pressure on the water uptake pattern of embryos soaked previously in distilled water for 6 hours.*

(A) : a, control (in distilled water) ; b, transferred from water to 0.5M mannitol at ↑ and returned to water at. ↕

(B) : c, transferred from water to 10^{-3}M azide at ↑ ; d, transferred from water to 0.5M mannitol at ↑ and from this to 10^{-3} M azide at ↕ ; e, transferred from water to 0.5 M mannitol + 10^{-3}M azide at ↑ and trom this to 10^{-3} M azide at ↕ ; f, transferred from water to 10^{-3} M azide at ↑ and returned to water at ↕.

(C) : g, transferred from water to 10^{-5} M azide at ↑ ; h, transferred from water to 10^{-5} M azide + 0.5 M mannitol at ↑ and from this to 10^{-5} M azide at ↕ ; i,transferred from water to 10^{-5}M azide + 0.5M mannitol at ↑ and to 10^{-3}M azide at ↕.

when they were returned to distilled water, they absorbed water so rapidly that the fresh weight soon came up with that of control.

When embryos in phase C were transferred from water to 10^{-3} M azide at 11 hours, water uptake was inhibited immediately (curve c in Fig. 6 B). When embryos were transferred to 0.5 M mannitol solution at 6 hours and then to 10^{-3} M azide solution without mannitol at 11 hours, they absorbed

water up to the level of curve c and no more (curve d). And when embryos were transferred at 6 hours to a solution containing both 10^{-3} M azide and 0.5 M mannitol and then at 11 hours to a pure azide solution, water was absorbed (curve e) just to the level of phase B, which was maintained under the inhibition by azide (curve f). Some water uptake occurred when 10^{-5} M azide was used (curve g in Fig. 6 C). When embryos were transferred to a solution containing both 10^{-5} M azide and 0.5 M mannitol at 6 hours and then to 10^{-5} M azide at 11 hours, rapid water absorption occurred (curve h) to catch up with curve g. Thus, the enviromental osmotic pressure and azide seemed to affect the water absorption independently of each other.

Discussion

Three distinct phases were discriminated in the process of water absorption when the decotyledonized embryo of *Raphanus* seed was laid on wet filter paper. This pattern of water absorption at incipient germination seems to be common to many plants, since essentially the same has been observed with pine (4) and *Vigna* (2, 3).

Temperature and respiratory inhibitors did not affect the rapid water absorption in phase A. The embryos which were killed by boiling in water and dried in a desiccator absorbed water again in the same way as dried living embryos did in phase A. Hence the water absorption in this phase is considered to be physical in nature.

No net water absorption occurred in phase B. However, since the length of this phase was temperature dependent ($Q_{10} = 2.7$ between 25° and 30°), some temperature dependent process must be going on in this phase to bring the embryo into the phase of active growth. If embryos which have finished phase B and have just begun the water uptake of phase C are dried in a desiccator, phase C directly follows phase A when they are returned to water (unpublished). Thus, once the embryo has completed phase B, it becomes equipped with the mechanism which is necessary for the physiological water absorption and which is not easily destroyed by drying. The internal condition essential to the growth must be established during phase B, which follows the physical absorption. The process occurring during phase B is of interest.

The respiratory system sensitive to copper chelators seems to decrease in its activity as phase B proceeds, and, in place of it, the system sensitive to iron chelators increases in activity. A similar trend has been reported for *Vigna* (3) and lettuce seeds (5). The master iron enzyme in phase C of *Raphanus* embryo appears to be cytochrome oxidase, since the embryo oxidized *p*-phenylenediamine but not catechol. However, cytochrome oxidase activity as measured by *p*-phenylenediamine was high already in an early part of phase B when respiration was low. Hence, the dehydrogenase system linked to the cytochrome system may be limiting the rate of respiration. This

assumption is compatible with the facts that some dehydrogenases which mediate the TCA cycle are less active in the early period of phase B than in the later period, probably limited by the amount of coenzymes. The internal change which goes on in phase B to make ready for phase C involves the setting up of efficient energy-yielding machinery including the TCA cycle and the cytochrome system. The establishment of energy-producing systems is essential for the intiation of phase C.

The processes to go on in phase B and C do not seem to be seriously disturbed by a hypertonic condition, because the embryo which had been in contact with a hypertonic mannitol solution was ready to absorb as much water as the embryo which had been under hypotonic environment (curves b and d in Fig. 6). This fact may be explained if it is assumed that the cell wall extensibility is decreasing in phase C even under the hypertonic condition. It is known that the effect of auxin decreasing the wall pressure is separable from the actual water uptake (7). The water uptake in phase C seems to involve auxin action, because the former is inhibited by an anti-auxin, 2,4,6-triiodobenzoic acid, and by excess of indoleacetic acid (unpublished).

Thus, the process of incipient germination of decotyledonized *Raphanus* embryo may be sketched as follows; when a dry embryo is given water, it absorbs water as a physical process (phase A). In the moistened embryo, various enzymes become activated, and the normal respiratory system and the internal conditions for auxin action are gradually elaborated (phase B). And when the cell wall is made extensible by auxin, the net increase in wet weight begins (phase C).

The present paper is concerned with the incipient germination process as viewed mainly from the respiratory system. Studies on phase B in relation to protein and nucleic acid metabolism will be reported shortly.

Summary

1. The process of incipient germination was studied with the decotyledonized embryo of *Raphanus* seed.
2. When dry embryos are put on wet filter paper, they soon absorb an amount of water as a physical process (phase A). Net increase in their fresh weight is suspended for a period (phase B) the length of which depends on temperature, then to begin physiological water absorption (phase C).
3. Respiration increases steadily from the middle of phase B to phase C. Respiratory quotient is 1.4 at the beginning of phase B, but falls during this phase to 0.83, the value in phase C.
4. Inhibition of oxygen uptake by copper chelators is remarkable in a short period prior to the middle of phase B.
5. Inhibition of oxygen uptake by malonate is small at first and grows steadily larger in and after phase B.
6. Activities of malic and citric dehydrogenases of tissue extract are lower

in phase B than in phase C. Amounts of cofactors seem to be limiting these dehydrogenase activities in phase B.

7. By respiratory inhibitors, water uptake is inhibited more strongly than oxygen uptake.
8. Embryos seem to finish phase B even under a hypertonic condition.

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