THE GROWTH PHYSICS AND WATER RELATIONS OF RED LIGHT-INDUCED GERMINATION IN LETTUCE SEEDS

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ABSTRACT

THE GROWTH PHYSICS AND WATER RELATIONS OF RED LIGHT-INDUCED GERMINATION IN LETTUCE SEEDS

By

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Certain varieties of lettuce seed require red light (660 nm) as a prerequisite to germination. The effect of red is reversed by far-red (735 nm); germination is under the control of the phytochrome pigment system. Scheibe and Lang (1965) have found that the effect of red light is to induce an increased growth potential in the embryo, enabling it to overcome osmotic resistance and presumably likewise mechanical restriction imposed by the outer seed layers.

This thesis concentrates on a study of the growth physics of lettuce seed germination. The increased growth potential induced by red light was quantified by incubating naked embryos in osmotica (mannitol or polyethylene glycol 4000) which act as artificial, physical barriers to germination. The water potentials of the osmotic solutions were determined with a vapor pressure osmometer. Growth potential of embryos was measured as lowered water potential

(increased potential for water uptake). Modifications of the gravimetric technique were used to eliminate errors introduced by penetration of osmoticum into the tissue. Specifically, the osmotic concentration preventing growth was measured during the course of germination in osmoticum. The graph of water potential of the embryos versus time was then extrapolated to zero hours in osmoticum for each osmoticum used. The difference between water potential of red light-treated embryos and that of dark-treated embryos was equivalent to the potential of 0.30 molal mannitol. Methods for the determination of osmotic potential in embryos grown in osmoticum are discussed. It is not known if the decreased water potential of light-treated embryos germinated in osmoticum is due to decreased osmotic potential in the cells or to decreased pressure potential.

The force necessary to penetrate external layers of the seed was measured by a technique using glass rods to simulate embryo tips and was found to be less than or equal to that developed by red light-treated embryos in osmoticum. The effect of red light in inducing germination of photodormant lettuce seeds is thus to cause development of a lowered water potential which allows uptake of water and generation of the force necessary for the embryo to penetrate the outer seed layers.

When embryos are germinated in water as opposed to osmoticum, the lowered water potential of red light-treated

seeds is rapidly transformed into increased growth. Thus, red light-treated embryos grow more rapidly in water than dark-treated ones, but do not acquire a greater water potential. The dark- and red-treated embryos both have a water potential equal to that of 0.0-0.1 molal mannitol.

The osmotic potential of the contents within water-incubated embryos was measured using two new methods, one of which relied on the penetration rate of D_2O before and after osmotic stress, and the other depending on the penetration rate of ^{14}C-labeled osmoticum. Both rely on the fact that as plasmolysis begins both D_2O and osmoticum enter cells more rapidly. Using these methods, light- and dark-treated embryos germinating in water were found to have the same osmotic potential, equivalent to the potential of 0.34-0.41 molal mannitol. During growth in water, turgor (the difference between cell water potential and the potential of cell contents) is thus equivalent to 0.24-0.41 molal mannitol. A reduction in this level of turgor will prevent growth, and is caused by external osmotic stresses greater than that of 0.1 molal mannitol. Such stresses raise the net water potential to a value equivalent to or higher than 0.0 molal. Maximal turgor would occur if the osmotic potential in the embryos were exactly balanced by the pressure potential of the wall.

There is general agreement in recent literature that growth in plant cells does not occur unless a minimal turgor pressure, considerably above zero, is maintained. Neither an increase in cell wall extensibility nor a decrease in osmotic potential of the cell contents (and therefore of the over-all water potential of the cell) can at present be held uniquely responsible for plant cell growth. However, the observations reported in this thesis suggest that generation of osmotic potential must continue to produce near maximal levels of turgor if growth is to occur.

REFERENCE

J. Scheibe and A. Lang, 1965. Lettuce seed germination: Evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temperature effect. Plant Physiology 40: 485-492.

THE GROWTH PHYSICS AND WATER RELATIONS OF RED LIGHT-INDUCED GERMINATION IN LETTUCE SEEDS

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I. INTRODUCTION

General

The lettuce "seed" is the achene of *Lactuca sativa* L. and consists of an embryo surrounded, from inside to out, by an endosperm 2 or 3 cell layers thick, a seed coat derived from the integument and consisting of several layers, and a pericarp or fruit coat (Borthwick and Robbins, 1928). Germination, which is defined here as in most seeds as protrusion of the radicle through the external layers, occurs around 14 hours after the seeds are placed in water or on moistened filter paper at 20° C. This thesis is concerned with the germination of light-requiring (photodormant) lettuce seeds. The subjects of seed dormancy in general and photodormancy in particular have been reviewed in recent years by Evenari (1965); Koller *et al*. (1963); Mayer and Poljakoff-Mayber (1963); and Scheibe (1966).

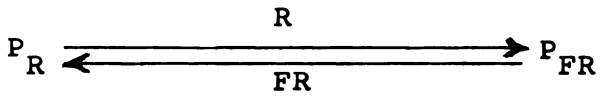
Light Requirement in Lettuce Seeds

In certain varieties of lettuce, notably Grand Rapids, the seeds may require exposure to red light (maximal effectiveness at 660 nm) as a prerequisite to germination; the red light effect is reversible by far-red light (735

nm) given immediately or at least within a certain time period after the red. The effect of alternating exposures to red and to far-red is equal to that of the final exposure given alone (Borthwick et al., 1952). If too long a time is interspaced between red and far-red, the latter becomes ineffective in reversing the effect of red, indicating that red light promotion of germination has passed beyond control of the phytochrome photosystem.

Red light is generally most effective in breaking dormancy when given after several hours of imbibition. An excessive length of imbibition (3 to 4 days) before the red light treatment results in a secondary dormancy--skotodormancy--which is not broken by red light (Evenari, 1965). The decline in responsiveness to red light is at least in part related to the length of red irradiation. After 24 hours imbibition, dormancy was not ended by 30 seconds red but was broken by 120 seconds irradiation (Mayer and Poljakoff-Mayber, 1963).

The receptive pigment in light induction of germination has an action spectrum like the absorption spectrum of phytochrome, a blue biliprotein, which has been extracted from many plant tissues, including lettuce seeds (Boisard et al., 1968), and identified in vivo by spectrophotometric means (Hillman, 1967). In red light, phytochrome is found in the far-red absorbing form (P_{FR}); far-red light transforms it to the red absorbing form (P_R).



Phytochrome is implicated as the physiologically active pigment in a large number of metabolic, tropic, and developmental responses of plants (Hillman, 1967; Hendricks and Borthwick, 1967). At least 2 long-lived forms of phytochrome are involved in the transformation diagrammed above (Briggs and Fork, 1969a, 1969b). The intermediates of the forward and reverse reactions appear to be different (Linschitz and Kasche, 1967).

Origin of Photodormancy

The extent of the red light requirement in lettuce seeds varies from lot to lot. Several workers have linked development of red sensitivity to factors affecting the parent plant. Seeds from plants well supplied with mineral fertilizer are less dormant than those from plants which received no fertilizer (Thompson, 1937); seeds harvested late in the season are less dormant than those harvested early in the season (Thompson, 1937); and a high positive correlation is found between the temperature in the last 30 days before harvest and percentage of the seeds which germinate in the dark (Harrington and Thompson, 1952). Also, 24-hour photoperiods during seed maturation result in fewer dormant seeds than do 8-hour photoperiods (Koller, 1962). The metabolic basis of these effects is obscure.

The degree of dormancy in the seeds seems related to the P_{FR}/P_R ratio and to the presence of physiologically active phytochrome in the seeds. The metabolism of the parent plant might affect the state and amount of phytochrome in the seeds. Also, the production and accumulation in the seeds of promoters or inhibitors of germination could be affected by the parent plants. Reasonably, any metabolic change in the parent plant could change the level of dormancy in the seeds.

The total concentration of P_{FR} present in the seeds at harvest is probably not the direct determinant of photodormancy. Analysis of the P_{FR} and P_R content in other plants in relation to their physiological responses to red or far-red light has shown that the bulk of phytochrome is apparently inactive physiologically, since irradiations which are physiologically effective may produce no detectable changes in levels of P_{FR} or P_R (Hillman, 1967). Furthermore, the ratio of P_{FR} to P_R, rather than the absolute amount of P_{FR}, determines physiological activity: Morphologically similar pea plants having five-fold differences in measurable phytochrome respond similarly, in terms of growth, to equal P_{FR}/P_R ratios (Fox and Hillman, 1968a, b). For these plants, constant ratios are established and measured for bulk (physiologically inactive) phytochrome, and the assumption is made that constant ratios must also be established in the physiologically active fraction.

Possibly, then, the ratio of P_{FR} to P_R established in the active phytochrome fraction during seed development determines the basic light sensitivity of the seed at room temperature. However, dormancy of the stored dry seeds is lost more rapidly in light than in darkness (Evenari and Newmann, 1953). Also, Scheibe (1966) found that loss of dormancy with dry storage (after-ripening) occurred less rapidly at 5° than at 23°. After-ripening did not increase the growth potential of the embryo; thus the endosperm was indirectly shown to be the site at which changes occur (see the following section). Storage conditions after harvest can thus influence the light requirement of freshly harvested seeds. Furthermore, the temperature of dark imbibition appears to be the major environmental factor determining presence or absence of photodormancy in the seed, and, by inference, influencing the P_{FR} to P_R ratio (see p. 7).

Role of the External Seed Layers in Photodormancy

The light requirement for lettuce seed germination is lost if the layers surrounding the embryo are removed (Evenari and Neumann, 1953). Removal of the fruit and seed coats alone does not abolish the light requirement (Evenari and Neumann, 1952; Ikuma and Thimann, 1959). This fact as well as experiments using depth-controlled deuteron irradiation of seeds supported the idea that the endosperm

is the seed layer which prevents germination in the dormant seed (Klein and Preiss, 1958; Preiss and Klein, 1958). The mechanism of endosperm restraint could be purely mechanical, or could involve a restriction of O_2 exchange or the production of inhibitors. Although the latter two factors have not been completely eliminated as contributors to endosperm imposed dormancy (Poljakoff-Mayber et al., 1957; Wareing and Foda, 1956), the main factor has been conclusively demonstrated to be mechanical restraint. Scheibe and Lang (1965, 1967), Scheibe (1966) found that the light requirement could be reimposed on naked half and whole embryos if they were incubated in an osmoticum such as mannitol. By using half embryos (the lower 40% of seed length, including the radicle) they cast doubt on the possibility that the cotyledons produce an inhibitor which is responsible for the light requirement.

Location of Phytochrome in the Seed

The site of light action is known to be in the radicle half of the seed (Ikuma and Thimann, 1959). Furthermore, the red light promotion is reversed equally well by far-red whether applied to the red-irradiated side or to the opposite side of a seed (Poljakoff-Mayber, 1958). This result seemed to indicate that the active photoreceptor is located in the embryo. The experiments of Scheibe and Lang (1965), showing that the naked embryo responds to red light, have demonstrated this fact implicitly but directly.

Other Means of Inducing and Breaking Dormancy

Light-requiring lettuce seeds can often be stimulated to germinate by factors other than light (Table 1); conversely, several factors can induce dormancy in non-dormant seeds (Table 2).

1. Temperature. Breakage of dormancy by low temperature is at least indirectly related to the phytochrome system. (a) The promotive effect of low temperature (0°) incubation on subsequent germination at 30° (at which germination is normally minimal) is less if far-red is given before 0° and somewhat less if far-red is given after 0° and before 30° (Roth-Bejerano et al., 1966). (b) After a 37° treatment (which induces dormancy in imbibed seeds by presumably reducing the P_{FR} to P_R ratio to a low level), far-red promotes rather than inhibits subsequent germination at 11° (Scheibe and Lang, 1965). The interpretation given to this result is that far-red, which usually contains some red light, actually raises the P_{FR} to P_R ratio when given after a 37° treatment. A subsequent 11° incubation preserves this ratio. From this interpretation it follows that photodormancy is not an absolute feature of a given seed lot, but depends on the temperature at which the seeds are imbibed. Thus, no seed is dormant at 11°, which preserves a high P_{FR}/P_R ratio; some seeds are dormant at 20° because during imbibition the P_{FR}/P_R ratio falls below a critical level.

The induction of dormancy by high temperatures during imbibition can be understood in a similar manner. It was found that seeds given a red treatment required a longer period of 37° incubation to induce dormancy than seeds given no red treatment (Toole et al., 1953). In general, then, the rate of decay of an in vivo phytochrome state favorable for subsequent germination seems to vary directly with imbibition temperature. Or, conversely, the rate of production of an in vivo phytochrome state favoring subsequent germination seems to vary inversely with imbibition temperature. So far, no direct evidence has been presented in support of either possibility, and while they seem to offer the most direct explanation, other formulations have appeared in the literature (Berrie, 1966).

2. Growth Hormones and Regulators. Gibberellins have long been known to promote dark germination of photodormant lettuce seeds (Evenari, 1965). Moreover, gibberellin-induced germination may bear some relationship to the actual mechanism of phytochrome potentiation since gibberellins are found in dry lettuce seeds (Blumenthal-Goldschmidt and Lang, 1960), and an increase in the level of gibberellin-like compounds has been reported in lettuce seeds soon after treatment with red light (Köhler, 1966). On the other hand, experiments of Bewley, Black and Negbi (1967, 1968) have demonstrated that if P_{FR} is allowed to act for as few as five minutes (after which the seeds are given a far-red

treatment) seeds will germinate if treated with 5 μg/ml gibberellin while neither the short duration P_{FR} action nor this concentration of gibberellin alone will induce such levels of germination. Thus, the effect of P_{FR} action is not due to production of gibberellins in the seed. By similar experiments and reasoning, it has been shown that interactions between P_{FR} and kinetin, thiourea, or chloramphenicol are not due to production of these compounds in the seeds.

Kinetin seems to stimulate germination only if the seeds are also given small amounts of light (Miller, 1958). Ikuma and Thimann (1963) have shown that kinetin induces expansion of the cotyledons and has no effect on the radicle; thus, kinetin promotes so-called atypical germination only. Scheibe and Lang (1965) have confirmed this finding.

In some plant systems, abscisic acid reduces the response to gibberellin; this effect can be overcome by increasing the gibberellin concentration (e.g., Chrispeels and Varner, 1967). However, in lettuce seed germination, the inhibition induced by abscisic acid is not overcome by gibberellins but is overcome by kinetin (Khan, 1968). Coumarin and xanthatin induce a dormancy in lettuce seeds which can be broken by red light plus kinetin but not by red light alone or kinetin alone (Khan and Tolbert, 1965). Coumarin-induced dormancy is also reversible by 3-chlorethyltrimethylammonium chloride (CCC) plus light but not by CCC or light alone (Khan and Tolbert, 1966a).

Indole-acetic acid (IAA) creates a dormant state in lettuce seeds which can be broken by CCC but not by 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate (AMO-1618), kinetin, or gibberellins (Khan and Tolbert, 1966b). In most plant systems CCC and AMO-1618 appear to be specific inhibitors of gibberellin biosynthesis (Kende et al., 1963; Dennis et al., 1965); thus the interpretation of the lettuce seed observations is rather difficult.

Ethylene induces dark germination of light-requiring lettuce seeds, and germinating seeds produce the gas (Abeles and Lonski, 1969; Stewart and Freebairn, 1969). In a number of plant systems IAA has been shown to cause production of ethylene, which is itself responsible for the observed physiological response to IAA (Burg and Burg, 1966). The possible relation of ethylene production to IAA in lettuce seeds is obscure at present since IAA itself induces dormancy. In some plant systems higher levels of IAA can produce metabolic inhibition which is unrelated to ethylene production (Burg and Burg, 1966). Possible relations between the effects of ethylene and those of IAA in lettuce seeds need further investigation.

3. Antimetabolites. Actinomycin D and chloramphenicol, inhibitors of DNA-dependent RNA synthesis and of protein synthesis, respectively, have been found to induce dark germination of photodormant lettuce seeds at temperatures

between 20^{o} and 30^{o} (Black and Richardson, 1967). The promotion by chloramphenicol was highest at the lower temperatures. The action of these compounds has been shown to be located in the embryos themselves since naked embryos in an osmoticum which inhibits dark germination will germinate if supplied with chloramphenicol (Frankland and Smith, 1967).

Attempts to inhibit lettuce seed germination with various inhibitors of DNA, RNA, or protein synthesis yield confusing results which are difficult to interpret meaningfully (Khan, 1967a, b). For instance, in intact seeds and half-seeds cycloheximide inhibits germination whereas puromycin does not; and 6-azauracil and 2-thiouracil inhibit germination whereas actinomycin-D does not (Khan, 1967b). The fact that some of these inhibitors can themselves promote germination of photodormant seeds seems to explain some of the data. However, it remains to be demonstrated that all ineffective antimetabolites are actually, for unknown reasons, promotors of germination. In the case of 5-fluorodeoxyuridine (FUDR), an inhibitor of thymidine production and therefore of DNA synthesis, inhibition of the germination of whole or half seeds was reported <u>not</u> to occur (Khan, 1967b). If, however, naked embryos are incubated in an osmoticum, which slows germination, FUDR inhibits germination, and the inhibition is reversed by thymidine (Table 3). Under no conditions

investigated did FUDR promote germination. The fact that FUDR is effective when the rate of germination is slowed suggests that the penetration rate of the inhibitor may determine its effectiveness.

4. Other Inhibitors and Promoters of Germination. Thiourea and a number of similar compounds promote dark germination of lettuce seeds (Evenari, 1965; Kefford et al., 1965). Similarly, germination is induced by high (20-80%) CO_2; the level of CO_2 required rises with higher incubation temperature (Thornton, 1936). Nothing is known about the possible mechanism of action of either of these promoters.

Low levels of gamma radiation (250-1000 kR) induce dormancy in lettuce seeds; this dormancy can be broken by light, kinetin, GA, or thiourea, although certain other effects of the radiation--lower respiration rates, inhibited growth--are not reversed (Haber and Luippold, 1959). Germination is induced by GA at higher levels of gamma radiation (1300 kR) and occurs without the cell division and nuclear DNA synthesis which normally accompany germination (Haber et al., 1969). In seeds irradiated with 1300 kR, abscisic acid will inhibit GA-induced germination.

Haber has demonstrated that DNA synthesis and cell division are not necessary for radicle protrusion in germination, although both accompany it under normal conditions. However, these facts do not substantiate the conclusion that FUDR fails to inhibit germination for a

similar reason (Khan, 1967b). First, it must be shown whether or not FUDR is actually entering the seeds and inhibiting cell division when germination occurs; and second, under certain conditions (Table 3) FUDR does inhibit germination.

II. OBJECTIVES OF THIS THESIS AND SOME METHODOLOGICAL PROBLEMS

Objectives of This Thesis

The findings of Scheibe and Lang (1965) are basic to an understanding of the growth physics and the physiology of red light-induced germination of lettuce seeds. It had long been known that photodormant seeds do not germinate in the dark unless they are pre-irradiated in red light or unless the seed coat and endosperm are removed. Scheibe and Lang found that the red light requirement can be restored to naked embryos if they are incubated in an osmoticum such as 0.46 molar mannitol. Seed coats and osmoticum are apparently acting as alternative, external physical resistances to radicle elongation. Red light thus induces an increased "growth potential" in the embryo which enables the radicle to overcome such physical barriers. In photodormant lots, dark-treated[1] seeds are unable to overcome the force imposed by the external layers and thus do not germinate. Dark-treated naked embryos germinate in water since all physical barriers to growth have been removed. Light-treated naked embryos are found to germinate

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[1]In this thesis, "dark-treated" is used literally to mean "not light-treated."

more rapidly in water than dark-treated ones (Figure 6); the increased growth rate is an expression of the light-induced increase in growth potential.

The term "growth potential" is necessarily vague in that it says nothing about the physical magnitude, mechanical origin, or metabolic determinants of the forces leading to light-induced germination. In an attempt to understand germination in these more exact terms, this thesis concentrates on a study of the growth physics of red light- and dark-treated lettuce seeds and embryos. The red light-induced increase in growth potential will be measured--in terms of water potential--for naked embryos germinating in osmoticum. Further, the expression of the increased potential will be measured in embryos germinating in water. Finally, the resistance imposed by layers external to the embryo will be determined. Once the forces causing germination are understood quantitatively, an approach can be made to a determination of their metabolic origin.

Water Relations of Plant Cells and Plant Growth

For the non-photosynthetic embryo germinating in distilled water, growth is an increase in fresh weight due solely to water uptake. The basic equation describing water relations in plants has a standard form with wide variations in terminology (Kozlowski, 1964). Two of the more common variations are given below:

1. Net water potential of the cell equals the osmotic potential of the cell contents plus the potential due to inward pressure of the wall on the contents.

water potential = osmotic potential + pressure potential

$$\psi_w = \psi_o + \psi_p$$

In this thesis the above terminology will be used. However, it should be understood that the equation does not basically differ from the older alternative.

2. diffusion pressure = osmotic pressure - wall pressure deficit

$$DPD = OP - WP$$

When ψ_w or DPD is zero, no water uptake into the cell occurs. As the capacity of the plant to take up water increases, ψ_w becomes more negative and DPD more positive. Thus a higher osmotic concentration in the cell is recorded as a more negative ψ_o and as a more positive OP. Similarly, in a turgid cell ψ_p is positive, as is WP. At incipient plasmolysis ψ_p and WP are zero. Turgor pressure is exactly equal but opposite in direction to ψ_p or WP and is the force with which cell contents press against the cell wall. The terminology based on water potential is directly derived from thermodynamics and is more commonly used in recent work (Slatyer and Taylor, 1960).

Growth, for embryos or seeds germinated in distilled water, consists exclusively of water uptake. Regardless of

the physiological determinants of water uptake, the process itself is a physical one and can occur only if the water potential within the tissue is negative in relation to that in the medium. In light of the water relations equation, then, growth can occur in one of two ways (Lockhart, 1965): (1) the osmotic potential may decrease, that is, the cell's osmotic concentration may rise; or (2) the wall pressure may decrease, which is to say that the resistance of the cell wall may lessen.

In most plant systems, the relationship of cell elongation to cell wall loosening or increased osmotic concentration is unknown. For Avena coleoptiles, Nitella, and green leaves, however, relevant data have appeared in the literature. These three examples are discussed briefly since growth in these tissues or cells has much in common with growth of lettuce embryos as discussed in this thesis. The importance of auxin in coleoptile elongation seems quite unrelated to lettuce embryo growth. However, coleoptile growth is an instance in which elongation can be related to both cell wall loosening and to increased osmotic concentration; therefore, a discussion of the system seems pertinent.

In many plant organs and/or tissues, cell elongation is dependent on the presence of auxin, which has been demonstrated to increase cell wall extensibility (Heyn, 1940). In Avena coleoptiles, the increased extensibility occurs

only if turgor is maintained (ψ_p greater than zero). Cell elongation, however, does not occur at any ψ_p greater than zero, but only if ψ_p is greater than a critical value (Cleland, 1967). In Avena coleoptiles no marked elongation occurs in the absence of auxin; this implies that cell wall loosening is necessary for growth. On the other hand, elongation does not occur in the presence of auxin either, unless a high enough osmotic concentration is present to generate at least a minimal pressure potential. These findings give neither mode of cell elongation the primary role in causing growth.

In the alga Nitella (Green, 1968), and in green leaves (Boyer, 1968), no direct factor causing increased cell wall extensibility is known; but as in Avena, a minimal pressure potential, considerably greater than zero, must be present for elongation to occur.

While growth in plants cannot be related primarily to either increased osmotic concentration or decreased wall pressure, one can say that in all cases carefully studied so far, the osmotic potential (which produces turgor) must be maintained as growth occurs. The specific manner by which most plants control osmotic potential is not understood. In Valonia, a marine alga, internally perfused cells respond to reduced turgor by increasing their uptake of potassium (Gutknecht, 1968). In other plants the breakdown of storage compounds and the production of osmotically

active substances are certainly involved. The guard cells of stomata have the best-understood system for regulating turgor in higher plants. Breakdown and synthesis of starch may be critical for turgor control in these cells (Levitt, 1967), although some workers doubt that starch metabolism is significant (Zelitch, 1969; Sawhney and Zelitch, 1969). Potassium uptake has also been suggested as playing an important role in regulating turgor in the guard cells (Fischer and Hsiao, 1968; Humble and Hsiao, 1969; Zelitch, 1969; Sawhney and Zelitch, 1969).

The increased growth potential which red light induces in lettuce embryos can be understood and quantified as an increased capacity for water uptake--a lowered water potential. Before investigation into the origins of the potential can begin, it must first be measured accurately.

Methods for Determining Water Potential

Four commonly used methods can provide accurate determinations of water potential. These are the gravimetric technique, the Shardakov method, use of a pressure chamber, and use of a thermocouple psychrometer. An excellent recent review of these and other methods of determining water potential is available (Barrs, 1968).

1. The gravimetric method involves subjecting tissue to external osmotic stress for a length of time sufficient to allow for attainment of water equilibrium. Different concentrations of an osmoticum are used; the tissue is

weighed before and after stress. In working with plant material such as Avena coleoptiles, changes in length rather than changes in weight are recorded. The osmotic potential at which no water uptake (weight increase) occurs equals the water potential of the tissue.

Two errors are possible with this method. One is introduced in the measurements as a result of the fact that the long incubation times--hours--which must be used in order for measurable growth of the tissue to occur allow osmoticum to penetrate into the tissue (Slatyer, 1966). One can of course shorten the necessary incubation period by using more tissue or more accurate instruments. During any finite incubation however, osmoticum may enter the cells, lowering the osmotic potential of the tissue and thus the water potential (Slatyer, 1966). The second error may occur in air grown material when liquid may enter air-filled spaces to increase the weight of the tissue even if no growth has taken place (Ashby and Wolf, 1947). Both sources of error lead to an overestimation of the osmotic concentration required to prevent water uptake by the tissue; that is, the water potential measured may be lower than the actual potential.

The second source of error should not occur in lettuce embryos since they are incubated in water continually. The first error can be eliminated in two ways. (1) Various lengths of incubation are utilized and the resulting osmotic concentrations preventing growth are plotted against

length of osmotic incubation. The resulting plot is extrapolated back to zero hours in osmoticum. (2) Several osmotica with different penetration rates are used. If the water potential values for "zero time in osmoticum" are equivalent, one can assume that penetration of the osmoticum has been eliminated as a source of error.

2. In the Shardakov method, osmotic solutions of varying concentrations are made up, and one portion of each is colored with a dye which does not appreciably change the osmotic potential. The tissue is incubated in the non-colored portion; then a drop of the incubation solution is put on top of its colored counterpart. If the non-colored drop sinks, the tissue has taken up water and has a water potential less than that of the osmoticum. The lowest concentration of incubation solution producing a stationary drop is taken to have a water potential equal to that of the tissue.

3. Water potential in some tissues such as leaves can be accurately determined by use of a pressure chamber (Boyer, 1967). The tissue is put in the chamber with its cut end extending outside. Pressure is applied until sap appears at the cut end. The water potential is equal to the sum of applied pressure potential and the osmotic potential of the sap.

4. The most reliable, error-free values for water potentials of plant tissues are obtained with a thermocouple psychrometer. Several types of psychrometers,

varying somewhat in construction of the thermocouple and principle of operation, are available (Barrs, 1968). In the Richards and Ogata model two wires of different metallic compositions form a junction at a silver ring which is filled with water. The other junction of the wires remains dry. As water evaporates from the silver ring the wet junction is cooled, and a measurable electric current flows between the wet and dry junctions according to the Seebeck effect. Thermocouple and tissue are placed in a closed, constant-temperature chamber.

One method of collecting data is to record thermocouple output with water on the junction and tissue in the chamber. This value is compared with several reference values obtained with water on the junction and solutions of known concentration, i.e., known water potential, in the chamber. Leaf resistance to vapor transfer may introduce errors to such determinations (Boyer and Knipling, 1965). In another method, the first thermocouple is removed and a second with an osmotic solution (negative water potential) on the junction is inserted in its place with the tissue still in the chamber. The output of the thermocouple is recorded after stabilization, and the outputs from the two thermocouples are plotted against the water potential of the relevant solution. The graph is extrapolated to zero output; water potential at this point--the isopiestic point--is considered to represent that of the tissue. Since no vapor transfer occurs at this point, leaf resistance

does not affect the water potential. An additional, dry thermocouple can be used to record any temperature changes produced by respiration.

Adaptation of a psychrometer system for use in seed germination presents several problems. (1) Radicle elongation occurs rapidly. The long equilibration periods required in psychrometer measurements would not allow the method to accurately portray changes in water potential over short time periods. In whole seeds the embryos appear to undergo a rapid buildup in water potential before penetrating the outer layers of the seed; as will be discussed later, this buildup occurs within an hour or less. A psychrometer would be unable to accurately record such a rapid change unless the equilibration time could be considerably shortened. (2) In the naked embryo, growth potential is accumulated only if incubation is carried out in an osmoticum, which acts as an artificial force preventing elongation. If embryos in an osmoticum were put into the psychrometer chamber, the measurements would record only the osmotic potential of the osmoticum.

The pressure chamber method of determination might be applicable to lettuce embryos if a micro-pressure chamber could be devised. The embryo would be sectioned, and the cut surface positioned so it protruded out of the pressure chamber. Lettuce embryos have nothing comparable to xylem space and probably have little intercellular space to

interfere with direct measurement of the water potential. However, the ratio between the surface area of the observed surface of the embryo and the total embryo volume is much smaller than the ratio of the cross sectional area of the petiole to the volume of a leaf. Therefore the pressure method would be inherently less accurate for embryos than for leaves.

The Shardakov method is probably adaptable to studies of seed germination, but does not appear to have any advantages over the gravimetric method. In addition, the latter technique routinely provides data on the exact water content of the embryo. Water content data give a measure of the extent of germination and are necessary for concentration determinations if chemical analyses are performed.

The gravimetric method used in the experiments reported in this thesis is subject to the first objection leveled against psychrometer measurements: Short term changes in water potential cannot be measured. However, gravimetric data are easily collected from embryos grown in osmoticum whereas psychrometric data are not. If the appropriate methods are used to eliminate error due to osmotic penetration, the embryos are in fact measuring their own water potential as they grow.

Methods for Determining Osmotic Potential

A commonly used method of obtaining values for osmotic potential is to examine the tissue under the microscope after

osmotic stress. The concentration of osmoticum producing plasmolysis in 50% of the cells is taken to represent the osmotic potential. A correction can be made for the volume change which has occurred as the tissue has proceeded from the fully turgid condition to one of incipient plasmolysis. Sources of error might be (1) evaporation between time of cutting and time of observation, (2) injury to tissues and loss of sap during sectioning, and (3) adhesion of cytoplasm to cell walls. The other well-known method of determining osmotic potential of tissue is based on finding the freezing point of expressed sap. It is impossible, however, to determine whether or not the extracted solution bears any direct relationship to the solution in the osmotically active regions of the tissue.

For estimations of the embryo's osmotic potential, two new methods were developed which utilized large sections of radicle tissue and required little tissue manipulation.

1. Penetration Rate of Deuterated Water. The first technique measures the penetration rate of deuterated water (D_2O) into embryo tissue which has been subjected to a previous or simultaneous osmotic stress. Theoretically, if a gradient is set up which varies between 0 and 100% D_2O and 0.0 and 1.0 molal osmoticum, the rate of fall of plant tissue in the gradient can be divided into three stages (Quail and Varner, unpublished):

(1) Following Stokes law for each increment of the gradient, the rate of fall will exponentially decrease until tissue density is equal to the density of the suspending medium at that point in the gradient. Stokes law states that a sphere of radius R, falling in a viscous medium, will have a velocity of fall, v, equal to $\frac{2/9\ (D - d)\ g\ R^2}{\eta}$. D and d are the densities of the sphere and the medium, respectively; g is the gravitational constant; η is the viscosity of the medium.

(2) Then rate of fall will depend on the rate of D_2O exchange and will theoretically be constant for a small section of tissue. In practice, the rate of fall will decrease as the tissue moves down the gradient in the hypotonic region. At least four factors are involved in the decreasing rate of fall. (i) As brought out in Table 4, D_2O penetration must occur farther into the tissue for it to move down the gradient. (ii) In a combination D_2O and osmoticum gradient the concentration of water decreases as the osmolality of the gradient increases. This occurs because as more and more osmoticum is added to a standard volume of water, solution volume increases; yet each increment of the gradient has an identical volume, and therefore the concentration of water per increment decreases and the rate of D_2O exchange is reduced. The effect is independent of the osmotic potential of the osmoticum and has a magnitude which can vary greatly between two osmotic solutions of equal potential. For instance 0.1 mole PEG 4000 in 1000

gm H_2O has a solution volume of 1300 ml, whereas 0.5 mole mannitol in 1000 gm H_2O has a solution volume of 1060 ml. Both solutions have the same osmotic potential. (iii) The rate of fall slows because as the tissue falls through the gradient, turgor pressure drops as a result of the increasing osmotic potential of the gradient. As turgor pressure is lowered the cells decrease slightly in volume, causing a net efflux of water from the cells and thus an interference with D_2O exchange. (iv) As the osmotic potential of the gradient increases so does the density of the osmoticum itself. Since the tissue does not equilibrate with osmoticum as rapidly as with D_2O, the rate at which the tissue falls in the gradient is reduced.

(3) When the osmotic potential of the gradient becomes less than that of the tissue, plasmolysis will begin. Since D_2O can penetrate the cell wall much more rapidly than the cell membrane, the volume of tissue available to D_2O penetration per unit time will increase. As plasmolysis begins, the tissue will begin to fall more rapidly through the gradient.

Combination D_2O-osmoticum gradients thus yield values for the point of incipient plasmolysis (Figure 22) if the following conditions are met:

(1) Gradient density near the point of incipient plasmolysis must be greater than tissue density. This condition is necessary so that D_2O exchange rather than Stokes law is governing rate of descent.

(2) The rate of fall of the tissue must be slow enough so that plasmolysis can occur at the actual position in the osmotic gradient at which the osmotic potential of the tissue equals that of the gradient.

In the case of advanced stages of radicle protrusion and of root growth, the second condition is met and a point of incipient plasmolysis (i.p.) is observed (Figure 22). In early stages of germination, however, it is not met (Figure 22) and the tissue falls to the bottom of the gradient before significant plasmolysis can occur. These results are explained by the fact that tissue density decreases as germination proceeds; and as the density is lowered the tissue must exchange a greater volume of water in order to fall through each increment of the gradient (Table 6). Therefore it falls more slowly. The use of smaller tissue sections would tend to alleviate effects of initial density on the rate of fall; but in the case of easily visible sections the problem is not eliminated. An additional problem associated with gradients is that only one or a few embryo sections may be observed at any one time. In a population of embryos a wide variation is observed in the germination stages of individual embryos at any given time. Therefore a large number of sections must be dropped through the gradient to obtain an accurate estimate of average behavior.

A modified method for studying D_2O penetration under various osmotic stresses allowed for complete equilibration

before D_2O entry and for the observation of a number of embryo sections simultaneously. A short D_2O column (no gradient) was poured; embryos were pretreated for 10 minutes in various concentrations of osmoticum (2-5 minutes in a hypertonic concentration was sufficient for full plasmolysis), then a small section of radicle was cut with a razor blade and placed on top of the column. A shaking, vertically placed glass rod continually pushed the sections beneath the surface and eliminated adhesion of embryos to the air-water interface. The time required for them to fall 6 cm down the column was recorded and was found to rapidly decrease as plasmolysis occurred.

The tissue was subject to osmotic stress only before exposure to D_2O. For plasmolyzed cells, then, deplasmolysis would begin in the D_2O column and the point of incipient plasmolysis might be shifted to somewhat higher osmotic concentrations. This is not, however, a serious difficulty with the method since the time required for plasmolysis--and presumably deplasmolysis--is on the order of three times that required for plasmolyzed tissue to fall through the column. The source of error can be completely eliminated by including osmoticum in the D_2O column. However, a separate column must then be made for each osmotic concentration used, and in the cases of mannitol or PEG 4000 the density of the osmoticum itself markedly slows the rate of fall of the embryo.

2. Penetration Rate of Labeled Osmoticum. A second set of values for points of i.p. is obtained from uptake studies of labeled osmoticum (Figure 24). At 19.5 hours after dark or light treatment, embryos were placed in galactose solution with a standard amount of ^{14}C-galactose. After one hour shaking at 24^{o} the embryos were rinsed for 30 seconds in water; they were then placed directly in Bray's solution and counted each day until a plateau value was reached. Factors influencing the shape of the curve may be many and complex; but the presence of a break at 0.39 molal seems likely to be caused by plasmolysis. A satisfactory hypothetical replica of the data is produced if one draws an isotope dilution curve for the experiment (Figure 24) and adds to this a concentration-dependent uptake curve. At plasmolysis the osmoticum becomes much more easily available to interior cells: this may account for the sudden leveling of the slope at the point of i.p. Of course, other explanations of the curve are possible, including some which would not consider the occurrence of plasmolysis.

III. MATERIALS AND METHODS

1. Seed Source. Grand Rapids Waldmann's lettuce seeds were purchased from Pieters-Wheeler Seed Company, Gilroy, California, and stored in the dark in a freezer. Lot number 164-G-2 of 1966 gives a dark germination of 0-4% at 20°.

2. Light Sources. The red source consisted of General Electric F40WW Warm White fluorescent lamps underlaid with a 3 mm thickness of Rohm and Haas 2444 red plexiglas. Irradiation was for 10 minutes at an intensity of 3770 ergs sec^{-1} cm^{-2} with a peak wave length of 625 nm. The far-red source consisted of General Electric 300 W heat resistant reflector flood lamps underlaid with a 3 mm thickness of Rohm and Haas V-58015 "black" plexiglas. Irradiation was for 10 minutes at an intensity of 11,400 ergs sec^{-1} cm^{-2} with wave lengths of 665-775 nm.

3. Osmotica. Mannitol, galactose, KNO_3, and polyethylene glycol 4000 (PEG 4000) were used as osmotica in the experiments reported in this thesis. PEG 4000 in particular was chosen because it is easily miscible with water; its high molecular weight should reduce or slow down penetration into cells; it is available in labeled form. The concentrations of galactose, KNO_3, and PEG 4000 are

expressed in terms of mannitol solutions yielding equivalent osmotic pressures. The comparisons of mannitol and KNO_3 are based on the osmosity figures in the Handbook of Chemistry and Physics (Weast, ed., 1965). Galactose and mannitol solutions of equal concentration have equal osmotic pressures. Osmotic pressure of mannitol and PEG 4000 were compared by using a vapor pressure osmometer (Figure 3). The osmotic pressure of PEG varies not only with molality, but also with the molecular weight of the particular PEG used (Manohar, 1966; Table 5). As the molecular weight of PEG is increased, the osmotic potential developed by a 1.0 molal solution becomes higher. Thus higher molecular weight PEG's are more efficient osmotica on a molal basis. In the past, failure to consider this fact has led to misinterpretation of the experimental data (Thimann et al., 1960).

4. Removal of Seed Coat and Endosperm from the Embryo. Most experiments were done with naked embryos. To remove endosperm and seed coat, seeds were allowed to imbibe in a petri dish on filter paper soaked with excess double distilled water in the dark at 20° for 3.5 hours; then a 10 minute light or dark treatment was given, and the seeds were slit longitudinally with a razor blade from the cotyledon end to about midway down under a green fluorescent bulb wrapped in blue, yellow, and green cellophane. An exposure of up to 50 minutes of light from this source did not increase subsequent germination. The slit seeds were soaked

an additional 1.5 hours; then the embryos were pushed out of the slit by a slight pressure on the radicle end. In this thesis, "embryo" refers always to a naked embryo with all seed layers removed.

5. Incubation of Embryos. Except where noted, embryos were placed in 50 ml flasks with 5 or 10 ml of water or solution of an osmoticum. The flasks were wrapped in foil and placed in a shaking water bath at 20 ± 0.25°, at 80 opm.

6. Determination of the Water Content of the Embryo. The incubation medium was removed from the flasks and the embryos were rinsed in 10 ml water for several minutes. They were then blotted on filter paper; placed on filter paper in a centrifuge tube; spun 2 minutes in a clinical centrifuge; and finally transferred to pre-weighed, 2-dram vials which were immediately sealed to prevent water loss. After weighing, the vials were unsealed, placed in a boiling water bath for 5 minutes, then air dried for 48 hours at 105°. A typical vial contained 60 embryos with an embryo fresh weight of 0.04375 gm, an embryo dry weight of 0.04120 gm, and a water content of 0.00255 gm. Fresh weight in the sealed vial remained unchanged for 20 minutes or more. The embryo water contents were converted to milliliters water per gram dry weight. The balance used was accurate to ± 0.00005 gm.

7. D_2O-Osmoticum Gradients. These gradients were prepared in 11 steps, usually 50% D_2O with 0.0 molal osmoticum (mannitol equivalents) to 100% D_2O with 1.0 molal osmoticum. Individual steps were of 5% D_2O and 0.1 molal osmoticum, and were of equal volume. The gradient was made by layering each step, with a pipette, into a 25 or 100 ml graduated cylinder. Occasionally a 2-chambered "gradient maker" was used. In these instances 100% D_2O and 1.0 molal osmoticum was put in the proximal (mixing) chamber and 100% D_2O and 0.0 molal osmoticum was put in the distal chamber. Flow rate from the gradient maker to the gradient was about 4 ml per minute.

IV. CHARACTERIZATION OF THE SEED LOT AND SOME TECHNICAL REFINEMENTS

Time Course of Far-Red Reversibility of Germination

If the seeds are given a red light treatment after several hours of imbibition, radicle protrusion will be at the 50% level at around 14.5 hours after red light. A far-red treatment immediately after red will reimpose dormancy, and even if an interval of 3 hours is left between red and far-red, dormancy will be complete in the seed lot used in these experiments. However, if the interval between red and far-red is increased further, far-red becomes less and less effective in restoring dormancy. If far-red is given 8.5 hours after red, 50% of the seeds will germinate, and 17 hours after red the far-red has completely lost its effectiveness (Figure 1). Thus, the first irreversible event in phytochrome-controlled germination is complete in 50% of the embryos after 8.5 hours. A 6 hour "silent" period then ensues before the effect of P_{FR} on the growth potential of the embryo becomes evident as radicle protrusion. It should be noted that the absolute timing of far-red reversibility varies widely from lot to lot of seeds and according to experimental conditions. In general, the

seeds used in the experiments reported in this thesis maintain far-red reversibility for a much longer time than seeds of lots used by other authors.

Water Potential of Embryos Germinating in Osmoticum

Scheibe and Lang found that if naked half embryos were incubated on filter paper in 0.46 molar mannitol their light requirement for germination, lost upon removal of the endosperm, was restored. If the concentration of mannitol is varied, this method can be used to determine the water potential difference between light-treated embryos and dark-treated ones.

Embryos placed on filter paper saturated with water or various solutions of mannitol show 72 hour germination percentages inversely related to the osmotic pressure of their incubation medium (Figure 2). Any visually apparent lengthening or curvature of the radicle was recorded as germination. At 72 hours germination percentages are final; that is, no additional germination occurs if the embryos are incubated for longer periods. In a similar experiment using liquid shaking culture, total inhibition of germination was attained at a higher concentration of mannitol (Figure 4). Since liquid incubation in a shaker places the embryos in maximum contact with their aqueous environment, this method was used in all further experiments. Figure 4 and Table 6 indicate that at 72 hours PEG 4000 prevents

germination at lower osmotic concentrations than does mannitol. Probably this result is due to the relation between the molecular weight of an osmoticum and the rate of its penetration into a tissue. It should be noted that the light-dark osmotic difference of 0.18 molal is the same for both osmotica. Figure 5 shows that KNO_3 inhibits germination at concentrations below those at which light-dark differences appear. Thus, over long periods of incubation KNO_3 does not have a strictly osmotic effect. Osmotica slow the rate of germination considerably; it is possible therefore that the measured water potentials are not those of normal germination. In order to allow for termination of these experiments during the initial stages of radicle protrusion a more accurate method for measuring the extent of germination was introduced.

A New Method of Recording Germination

Since germination percentage is rather qualitative and yields no quantitative information "below" 0% and "above" 100% germination, embryo water content (fresh weight minus dry weight/1.0 dry weight) was substituted for radicle protrusion as a more meaningful measure of "germination" insofar as it involves water uptake. The course of water uptake by light- and dark-treated embryos is shown in Figure 6. When the difference between the light and dark curves is plotted against time, two phases can be clearly

distinguished (Figure 7). The first phase (10-26 hours after light treatment) corresponds to radicle elongation and the second (27 hours on) to root development and elongation. In both phases light-treated embryos are taking up water more rapidly than their dark-treated counterparts. During transformation to a root-like structure the radicle becomes slightly swollen in a region several millimeters back of the tip. Root hairs then form in this region and below it.

V. WATER POTENTIAL OF EMBRYOS GERMINATING IN OSMOTICUM

As before, embryos were incubated in various concentrations of mannitol or PEG 4000 after light or dark treatment. In these experiments however the incubations were ended after varying intervals (Figures 8, 9, 10, 11), whereas before all were terminated at 72 hours. For each incubation period, the concentration of osmoticum which prevented net water uptake was recorded. A graph of these values versus extent of germination was extrapolated back to the time at which elongation begins in water-incubated embryos (Figures 12 and 13).

Embryos were incubated in osmotica from 2 to 3 hours after light or dark treatment. Probably no significant penetration of osmoticum occurs until new osmotic space is created as germination begins (at around 12 hours). This assumption is substantiated by experiments in which embryos incubated in water until just before elongation (8 hours) and then put in osmoticum were compared to embryos incubated only in osmoticum (Figures 8, 11, 12, 13). In one instance (PEG 4000) the osmotic concentration preventing elongation was the same for both cases, indicating no

significant penetration of osmoticum prior to 8 hours after light or dark treatment. In another instance (mannitol) the difference was small and in the "wrong" direction (i.e., water uptake of embryos in osmoticum for 8 hours was shut off by a lower concentration of osmoticum), and occurred in water controls also; this difference is most likely related to some aspect of the transfer operation (from water to osmoticum) such as temperature change, and not to osmoticum penetration.

Further support for the postulate that no significant osmotic penetration occurs before creation of new osmotic space appears in the curve for dark, PEG 4000-treated embryos (Figure 12): here osmotic shut-off of growth occurs at around 0.0 to 0.1 molal (mannitol equivalent). This value corresponds favorably to that of Figure 14 (discussed later), in which experimental design eliminates any possibility of osmotic penetration at "zero hours in osmoticum."

Light-dark differences in water potential of -0.15 to -0.30 molal (mannitol equivalent) are observed, depending on the incubation time (Figure 15). The differences of -0.30 molal which are found early in germination seem more correct than those of more than -0.30 molal later in germination since the red light effect is less pronounced as time goes on (Figure 7).

In general, the PEG data seems more reliable since the higher penetration rate of mannitol makes a reading of

the osmotic shut-off values difficult. Figures 12 and 13 are plots of the osmotic concentration which allows for no water uptake above 0.7 mls water per gram dry weight--the value attained by embryos which are fully imbibed but have not started to grow. The PEG 4000 data plot quite linearly and therefore an osmotic value preventing water uptake is easily obtained. Mannitol data, however, becomes hyperbolic near the 0.70 ml value. This situation probably is related to the penetration rate of mannitol. In any case, for the mannitol data two values for osmotic shut-off are plotted: a value which follows the data plot exactly; and a value which is extrapolated to 0.70 mls from the more linear portion of the curve.

When the osmotic concentration permitting water uptake is plotted against time in osmoticum, a change in slope is observed at around 24 hours (Figures 12 and 13). The decreased slope is related to a growth rate change independent of light treatment or osmoticum. Either the penetration rate of the osmoticum has declined for some reason, or even after the osmotic barrier to germination has been lowered the embryos take up water more slowly due to a smaller (less negative) water potential. Whether these observations relate to the development of a state of secondary dormancy after prolonged osmotic incubation was not investigated. Osmotic volume increases during germination; and the rate of water uptake of groups of embryos increases until around

20 hours after light or dark treatment, when it becomes relatively constant. Either of these phenomena could result in a progressive slowing down in growth recovery by osmotically stressed embryos, as germination proceeded; and thus either could explain the changing slopes of Figures 12 and 13.

Two lines of evidence indicate that the positive slopes of Figures 12 and 13 are due to penetration of osmoticum. Attention can be focused on the PEG 4000 data since the difference between these and the mannitol data is definitely due to the higher penetration rate of mannitol, which is related to its lower molecular weight.

1. If the positive slope does not represent osmoticum penetration, it must be caused by an osmotic build-up by the embryo. The build-up must occur in both light- and dark-treated embryos. To test this possibility, dark-treated embryos were germinated in 0.07 molal PEG 4000 for 19 hours to allow for development of any potential. Then they were transferred to water to allow for its expression in growth. The growth rate of the transferred embryos at no time exceeded that of dark-treated embryos grown in water (Figure 16). Water uptake of transferred embryos is exactly like that of water-incubated embryos as they begin to germinate. This indicates that the slopes of Figures 12 and 13 are probably not due to a build-up of an embryo-produced water potential. The transferred embryos

might be thought to have a more negative osmotic potential--due to uptake of osmotica alone. However, as discussed earlier, significant osmotic penetration prior to germination does not appear to occur.

2. Penetration rates of PEG 4000, mannitol, and galactose were measured using labeled osmotica and appear in Table 7 and Figure 17. The penetration values are uncorrected for uptake into non-osmotically active regions of the embryos and assume that osmotically active and inactive aqueous fractions absorb at equal rates. In any event the data are probably an accurate reflection of the fact that osmoticum uptake is rapid enough to account for the slopes of Figures 12 and 13. In the case of 0.05 molal galactose, for instance (Figure 17), 60-70% of the embryos' water has equilibrated with the osmoticum at the end of 1 hour. This figure undoubtedly represents penetration of osmoticum into osmotically active space as well as free space.

VI. WATER POTENTIAL OF EMBRYOS GERMINATING IN WATER

If embryos are germinated in osmotica, differences between the water potentials developed by light- and dark-treated embryos can be seen. A high enough concentration of osmoticum will inhibit dark germination and permit germination of light-treated embryos. In water-incubated embryos, however, the light requirement is apparently lost, and it is observed that light-treated embryos germinate more rapidly than dark-treated ones (Figure 6).

The increased rate of water uptake by light-treated embryos is a reflection of the decreased water potential they can develop. Their greater capacity for water uptake is in fact being utilized; one would thus expect to find a decreased light-dark difference in the potential, or perhaps none at all, during growth of embryos in water.

The expectation was investigated by use of the modified technique for gravimetric determination of water potential described earlier. Embryos germinating in water were tested 15.5 hours after light treatment and 18.5 hours after dark treatment, when both groups had the same water content/gm dry weight, and groups of embryos were

subjected to various osmotic stresses in PEG 4000 or mannitol for varying lengths of time. In each experiment one group was weighed before the osmotic incubation as a "no growth" control. Osmotic shut-off values were determined from Figures 18, 19, 20, and 21 and plotted against time in osmoticum (Figure 14).

One can see that at "zero hours in osmoticum" 0.0 to 0.1 molal mannitol equivalents of either mannitol or PEG 4000 prevented water uptake of both light- and dark-treated embryos. Thus 0.0 to -0.1 molal represents the water potential of embryos germinating in water. The additional water potential which light-treated seed can build up is evidently promptly and totally utilized in producing the higher growth rate of light-treated embryos, compared to the dark-treated ones. Furthermore there is no consistent indication that light-treated embryos attempt to develop an increased growth potential while under osmotic stress, although the PEG data seem to show such a buildup. It is perhaps appropriate to point out that the osmotic volume of the embryos at 15.5 hours light and 18.5 hours dark is considerably more than that of embryos not germinating. If the added growth potential of light-treated embryos is produced internally by generation of a lower osmotic potential, considerable time might be required to build up a measurable potential difference in later stages of germination.

Dark germination in water is prevented by 0.0 to 0.1 molal mannitol equivalents of PEG 4000. When dark-treated embryos are placed in osmoticum, the early stages of radicle elongation are again prevented by 0.0 to 0.1 mannitol equivalents of PEG 4000. This agreement in water potential values confirms the hypothesis that no significent penetration of osmoticum occurs before cell elongation begins in embryos germinated in osmoticum.

VII. OSMOTIC POTENTIAL OF EMBRYOS GERMINATING IN WATER

Since the water potential of embryos germinating in water is known, it would be appropriate to determine the osmotic potential of the tissue, thus permitting a discussion of the complete water relations equation. Utilizing the two new methods for determining osmotic potential (discussed in Chapter II), data were obtained (Figures 23 and 24 and Table 8) using KNO_3 and mannitol as the osmotica. Small embryo sections (generally the elongating part of the radicle) collected at various times after light or dark treatment were used for the determinations (for short incubations KNO_3 appeared not to have the previously noted adverse effects which occurred in long term incubations). The point of i.p. is about the same for light- and dark-treated embryos throughout the initial hours of germination. This is the expected result if any increased osmotic concentration in light-treated embryos is rapidly transformed into increased growth. The difference between i.p. values labeled "#1" and "#2" will be discussed later. The osmotic range for the point of i.p. is taken as that covered by either set of values. Corrections were not made for volume

change as the cells approached plasmolysis; they would be minimal since even under prolonged hypertonic osmotic stress tissue volume decreases very little.

The osmotic potential of both light- and dark-treated embryos germinating in water is between -0.34 and -0.41 molal. Since the water potential was found to be between 0.00 and -0.10 molal, the complete water relations equation for the embryos can be solved:

$$\psi_w = \psi_o + \psi_p$$

$$(0.00 \text{ to } -0.10) = (-0.34 \text{ to } -0.41) + (0.24 \text{ to } 0.41)$$

Although the point of incipient plasmolysis can be said to approximate zero pressure potential, it is not always true that it is the point at which the growth rate becomes zero. In fact, pressure potential must be considerably above zero (0.24 to 0.41 molal) for growth to occur. This situation is true for germinating lettuce embryos and for several other plant systems which have been carefully investigated (Cleland, 1967; Boyer, 1968; Green, 1968; see Chapter II).

VIII. STRENGTH OF THE SEED COATS SURROUNDING THE EMBRYO

It has been demonstrated that light-treated embryos in an osmoticum are capable of generating a water potential of around -0.30 molal, whereas under conditions of normal growth--as naked embryos or seeds which have penetrated the external seed layers--their water potential is between 0.00 and -0.10 molal. It is now pertinent to consider the precise effect of the seed coat in light-treated seeds and the quantitative value of the resistance imposed by the external layers of the seed.

The force needed by the embryo to break through the layers covering it was measured directly. Embryos were removed from seeds 18-20 hours after dark treatment (after a light treatment would have been given). Two glass rods with hemispherically-shaped tips 0.25 mm and 0.40 mm in diameter were made. These diameters were chosen because they approximate diameters of the radicle at and slightly above the tip. One of these rods was inserted into the empty seed coat-endosperm while the distal end of the rod rested on the pan of a Mettler balance. The layers were then pulled slowly onto the rod until the rod protruded, after several seconds elapsed time. The grams force at this point was recorded

and transformed into osmotic equivalents which could be directly compared with the osmotic parameters of the embryo determined before (Table 9). If only downward force vectors (those directed toward the radicle tip) were effective in protrusion, the molal values would be halved. But since it is likely that lateral force vectors (those directed to the sides of the radicle) are at least partially effective, the resistance of the seed coat-endosperm layer is placed between 0.16 and 0.38 molal (mannitol equivalent) by this method. A lesser force applied over a longer time (minutes to hours) might be equally effective in penetration.

The natural course of water uptake by light-treated whole seeds is presented in Figure 25. Whole seed data are corrected for the water content of the seed coat and endosperm. Presence of these layers delays radicle elongation of whole seeds by as much as 6 hours and more, and lowers the water content values to those of dark-treated embryos. Since light-treated, osmotically restrained embryos can develop at least the water potential needed by embryos restrained by coats to break through the latter, one can say that seed coat pressure is equal to or less than 0.30 molal. The water uptake rate of light-treated whole seeds parallels closely that of dark-treated embryos, so the light-dark difference in embryo pressure potential would seem to approximate the resistance of external layers over time as well.

These layers continue to retard water uptake even after 100% germination (initial penetration) has occurred, at around 16 hours.

IX. DISCUSSION

What Has Been Accomplished

Scheibe and Lang (1965) showed that photodormant lettuce seeds do not germinate in the dark because the embryo cannot generate enough growth potential to overcome the mechanical restraining force of the external seed layers, principally the endosperm. Red light, acting on the phytochrome system, induces a potential for growth which enables embryos to penetrate these layers. The data of this thesis have developed this concept of how photodormancy is broken, in quantitative terms. The water and osmotic potentials involved in light- and dark-treated embryo germination, both with and without external restraining forces, have been derived in terms of the basic equation for water relations in plant tissue.

Scheibe found that use of an osmotically active incubation solution "restores" photodormancy to excised embryos. In terms of physical forces, the osmoticum acts as an "artificial endosperm," with the added feature that its force can be varied simply by changing the concentration of osmoticum. By thus changing the strength of an imposed barrier to germination, one can measure the maximum force

(in terms of a lowered water potential) which light- and dark-treated embryos can develop (Figure 4 and Table 6). Use of different osmotica leads to different values for these forces, indicating the introduction of errors by penetration of the osmotica into the cells. Therefore the experiments described in this thesis were terminated at different stages of germination. The forces preventing germination were plotted against the course of germination in order to obtain the extrapolated value for the force needed to prevent germination in the very first stages of radicle elongation (Figures 8-13). This value is equivalent for different osmotica; the error introduced by penetration of osmotica into the osmotic space of the tissue is eliminated. As germination begins, light-treated embryos have the ability to develop up to a 0.30 molal lower water potential than dark-treated embryos (Figure 15). This potential difference is developed within an hour after radicle elongation begins.

In osmoticum as germination begins (16 hours after light treatment) the water potential of light-treated embryos is -0.35 molal. Since the force needed to penetrate the external seed layers is 0.16 to 0.38 molal, whole seeds should be prevented from germinating by 0.00 to 0.19 molal osmoticum (-0.35 + 0.16 = -0.19; -0.35 + 0.38 = +0.03, but light-treated whole seeds germinate in water so the value of +0.03 must be 0.00 or less). Kaufmann (1969), using a different

variety of lettuce, germinated whole, evidently non-dormant, seeds in soil which was separated from osmoticum (PEG 6000) by a cellulose acetate dialysis membrane which presumably allowed for equilibration of water potentials without leakage of osmoticum into the soil. He found that osmotic potentials of -0.13 to -0.19 molal prevented germination. These values compare favorably with those computed for the seeds used in this thesis.

For embryos germinating in water, the water potentials are the same for both light- and dark-treated tissues, but the light-treated embryos elongate much more rapidly (Figures 14 and 6). The conclusion from these results is that in water, extra growth potential induced by red light treatment is not allowed to accumulate but is rapidly translated into growth: a decrease in water potential below 0.00 to -0.10 molal is quickly adjusted upwards through growth.

Dark-treated embryos develop the same water potential in water as in an osmoticum. Penetration of osmoticum must occur before these embryos will grow in an osmotic potential below 0.0 to -0.1 molal. These facts may explain some interesting characteristics of the curves in Figures 8, 9, 10 and 11, in which water uptake is plotted against external osmotic concentration for embryos germinating in osmoticum.

The "dark, PEG 4000 graph" (Figure 10) has a strictly linear plot when the osmotic concentration preventing

growth is around 0.1 molal and linear plots which become hyperbolic when the osmotic concentration preventing growth is much above 0.1 molal. The dark, mannitol graph (Figure 8) has linear plots which tail off hyperbolically in all cases but which have a consistent leveling in slope around 0.1 to 0.2 molal (especially when the embryos are just beginning to germinate). These variations may perhaps have the following explanation. The strictly linear part of the PEG 4000 curves may represent cases in which the external osmotic concentration is low enough that no entry of osmoticum need occur for growth (see the 16:10 and 17:05 data). The linear-hyperbolic parts of the PEG 4000 curves may represent instances in which entry has occurred, causing the hyperbolic section of the curves. In the case of mannitol, which enters the cells (embryos) much more rapidly than PEG 4000, all curves tend to become hyperbolic; but they have a characteristic change in slope around 0.1 to 0.2 molal, the point at which penetration must occur for growth to take place. The tailing off, when it occurs, is therefore the representation of the time delay introduced when osmoticum penetration must occur before growth begins. The graphs for light-treated embryos in each osmoticum show a characteristic leveling in slope between 0.1 and 0.2 molal which may be explained as above. In the case of light-treated embryos, however, both penetration of external osmoticum and build-up of internal

growth potential can contribute to overcoming the growth restraint created by an external osmoticum.

In the case of germination in water, the water relations equation has been completely solved. The osmotic potential was evaluated in two different manners, each of which eliminates error due to tissue manipulation.

Rates of penetration of deuterated water after various osmotic stresses show a sharp increase as the external osmotic potential decreases below that at which incipient plasmolysis occurs. When the tissue is fully plasmolyzed, the rate of D_2O entry slowly increases and appears to be linearly dependent on external osmotic concentration (Figure 23). This latter relation holds when pressure potential is zero and, thus, the internal osmotic volume is directly proportional to the external osmotic strength. The mathematics of water entry at this stage are complicated, depending at the same time on the ratio of vacuolar to free space and on the surface area and shape of the embryo. Assuming a spherical vacuole at plasmolysis and a cubical cell wall structure, the volume of free space does not increase linearly as does external osmotic concentration (Figure 26); however, the relationship would appear to be linear for relatively small osmotic changes, and this may explain the apparent linearity of graphs in Figure 23 when pressure potential is no longer a factor involved. Another worker has found a similar relationship in leaves (Hammel, 1968).

The rather large osmotic range over which the rate of D_2O penetration continues to increase before pressure potential becomes zero can be interpreted in several ways: (1) All cells of the tissue might have the same point of incipient plasmolysis; this point would be the external osmotic potential at the maximum rate of fall in D_2O as pressure potential becomes zero ("#2" values in Figure 23 and Table 7). This interpretation seems unlikely since reduction of a still-positive pressure potential would probably not change the rate of D_2O penetration appreciably. (2) Some cells might have one point of incipient plasmolysis and some another. (3) Finally, all cells may have the same osmotic potential ("#1"), but near the point of incipient plasmolysis externally located cells might come to osmotic equilibrium in the 10 minute osmotic stress period whereas interior cells might not. The 10 minute period is amply sufficient for complete plasmolysis to occur throughout the tissue (no decrease in seconds required to fall in 100% D_2O occurs if incubation time in hypertonic KNO_3 is extended beyond 2-5 minutes), but might not suffice when the rate of water exchange is less rapid. Longer osmotic incubation periods were not used, however, since uptake of the osmoticum itself would have become a problem.

The second method for evaluating the osmotic potential is an interesting adaptation of traditional methods, made possible by radioisotopes: the osmoticum used to plasmolyze

the tissue is itself the measure of the extent of plasmolysis. After a one hour incubation in one of several concentrations of cold osmoticum, each containing a standard unit of labeled osmoticum, uptake into the tissue might be expected to follow an isotope dilution curve based purely on the increasing concentration of unlabeled osmoticum. As Figure 24 shows, this is generally the case, although to some extent uptake appears to increase with concentration. At plasmolysis, however, it becomes clear that what is being measured is uptake of osmoticum into the free space, which drastically increases in volume on plasmolysis. After plasmolysis the increase in the volume of free space even overrides the effect of isotope dilution, thus causing a net increase in uptake as concentration rises. Possibly, then, the apparent increase in uptake with concentration at osmotic potentials above the point of incipient plasmolysis (left side of graph) is related to the slight increase in free space which might result as pressure potential decreases due to increased external osmotic stress. Alternatively, a concentration-dependent mechanism for uptake of osmoticum might exist in these concentration ranges.

With measurement of the osmotic and water potentials of water-germinated, light- and dark-treated embryos, it becomes apparent that, as in the other plant systems discussed earlier (p. 18), the growth of lettuce embryos is dependent on a pressure potential which is greater than a

certain minimal positive value. This dependency thus appears to be a phenomenon of general occurrence in plants. The water potential of growing plant tissue is usually around -0.1 molal, the osmotic potential around -0.4 molal.

There are instances in which growth is reported to occur at more negative water potentials (at which the pressure potential would be near zero). In these cases, however, water potentials were determined with the gravimetric method and are probably (Brouwer, 1963; Wadleigh and Gauch, 1948) or definitely (Thimann and Schneider, 1938) abnormally low due to penetration during the determination period or even before (in cases in which osmoticum was introduced into nutrient solution bathing the roots, and the water potential of leaves was determined later).

Quantitative determinations of seed coat and endosperm resistance verify what is already obvious: light-treated embryos have the growth potential to penetrate these layers, dark-treated embryos do not. Light-treated embryos develop the full extent of their increased growth potential by 15 hours after light treatment and probably before this time--that is, as soon as elongation begins. By 16 hours after light treatment, light-treated whole seeds are 80-90% germinated (in terms of radicle protrusion); and at this time there is a 2 hour lag in the water content of light-treated embryos compared to that of the whole seeds. The lag continues to increase, however, and by 34 hours after red light

it is over 6 hours. After radicle protrusion, it is difficult to envision continuing decreases in water uptake as being caused by physical restraint by the external layers.

The possibility that the outer layers of the seed may contain inhibitors affecting germination must be considered. Dry lettuce seeds have on extraction yielded substances which inhibit elongation of Avena coleoptiles (Poljakoff-Mayber et al., 1957) and germination of lettuce seeds (Wareing and Foda, 1956). These compounds disappear as germination of the seeds occurs. Unfortunately, the location of such inhibitors within the seed is not known.

Scheibe (1965) showed that red light-treated half-embryos in 0.46 molar mannitol showed 92% germination after 33.5 hours whereas similarly treated half-seeds showed 77% germination. Far-red-treated embryos and seeds gave 1% and 4% germination respectively. Thus, although photodormancy is not directly related to presence of the external layers, Scheibe's results and the data in Figure 25 indicate that presence of these layers somehow slows germination or growth rate of the embryo.

What Has Not Been Accomplished and Why

A complete understanding of the growth physics of lettuce embryos requires a knowledge of the method by which light-treated embryos are able, under stress, to develop a much lower water potential than dark-treated ones.

As discussed earlier, a demonstration that decreased water potential can be explained by decreased osmotic potential does not necessarily give osmotic potential the role of primary effector of growth. In the case of Avena coleoptiles, neither decrease of the osmotic potential nor cell wall loosening (decrease of the pressure potential) can at present be said to have primary responsibility for causing growth: both are necessary if net water uptake is to occur. Nevertheless, it would be satisfying to show that in lettuce embryos, light treatment results in a decreased water potential which is linked to a lowered osmotic potential.

Unfortunately, light-treated embryos build up an increased growth potential under two conditions which make direct determinations of osmotic potentials rather difficult: (1) The potential is built up when embryos germinate in an osmoticum. Presence of the osmoticum in the free space and inner space of the tissues makes determinations of the osmotic potential, by standard means or by those used in this thesis, impossible on an accurate, practical basis. (2) Embryos in whole seeds, just prior to protruding through the external layers, build up the required water potential. But the phenomenon is extremely transient in the individual seed while extending in a germinating seed population over about 2 hours, i.e., it is not synchronized. This situation rules out the use of several convenient methods for osmotic

potential determination. The external layers cannot be removed for the determination. The relevant osmotic volume is so small at this pre-germination stage that plasmolysis would have no significant effect on the rate of D_2O exchange. In the absence of usable direct methods, the most reasonable manner of arriving at the value for osmotic potential is an indirect one such as examination of the osmotic contents of the cells.

Osmotically Active Constituents of Germinating Lettuce Embryos

Many studies which have been made in an attempt to relate dormancy and its breakage to metabolic changes in the seeds (embryos) suffer from one or both of two faults. First, many of the studies were made on seeds in advanced stages of germination (in lettuce embryos, after 24 hours or so of imbibition). Such studies are valuable in terms of providing information on germination in general, but they say very little about dormancy or its breakage: the mechanism involved in these latter phenomena are active before germination is observed and possibly in the very first stages of radicle protrusion. Second, studies on dormancy must be able to eliminate results attributable to germination in general from those relating to dormancy in particular. In lettuce seeds, this apparently insurmountable distinction can be dealt with by removing the seed layers external to the embryo and allowing both light- and dark-treated seeds to

germinate. For metabolic studies, if embryos in the same stage of germination are selected for comparison, the effects of germination per se are eliminated. In lettuce seeds, as has been seen, germination must be carried out in an osmoticum if one hopes to find metabolic differences due to phytochrome action. Differences may be found in water-germinated embryos, but one would reasonably expect them to be smaller than those found in osmotically germinated embryos, and their relation to the quantitative values for water and osmotic potentials would be less obvious.

In osmoticum, light- and dark-treated embryos have water potential differences of -0.30 molal in the early stages of germination. If the osmotic potential is at least secondarily related to this one should find a 0.30 molal difference in osmotically active embryo constituents at this stage. Since lettuce embryos are germinated in distilled water and, of course, do not photosynthesize, the only possible source of a lower osmotic potential is the breakdown of large molecules into smaller ones. To meet the requirements of a fuel system for an osmoticum generator: a compound (1) must normally be broken down into smaller units; and (2) must occur in large enough amounts to provide the required osmotic potential on breakdown. From a table of the chemical composition of lettuce seeds, it appears that four groups of compounds meet these requirements (Mayer and Poljakoff-Mayber, 1963).

	Air-Dry Seeds (mg/g)
Protein Nitrogen	37
Fat	370
Sucrose	30
Phytic Acid	20
(Total Dry Weight	960)

In addition, some classes of compounds apparently not studied--for instance organic acids--might be osmotically active to a significant degree.

Since the imbibed seed is 41% water by weight, the concentration of sucrose is 0.15 molal; and sucrose would only double its osmotic contribution upon complete breakdown to glucose and fructose. However, since most of the seed water is not osmotically active in the early stages of germination, and the bulk of the sucrose could conceivably be located near osmotically active regions of the embryo, it is possible that sucrose contributes significantly to the light-dark osmotic potential difference. Unfortunately, the data on which the above values are based are not detailed with respect to intra-embryo location of the various substances. Since even half-seeds (which actually are closer to the lower one-third of seed and embryo weight) in osmoticum show a light-induced growth promotion, the major part of the constituents of the cotyledons are not necessary for dormancy breakage. Also, of course, the external layers themselves are not involved.

If, in germination, new osmotic space is created in a distinct manner--new vacuoles being formed, for instance--

then the water taken up during radicle elongation can be taken reasonably to represent the main fraction of cell solution active in expressing osmotic potential. Whether or not the relevant compounds are in this fraction is still unknown. In the case of vacuoles, some success might result from use of methods of extracting whole vacuoles--especially smaller ones (Matile, 1968).

The Osmotically Silent Period

Between the time when red promotion is only 50% reversible by far-red (8.5 hours after red light) and the time when 50% of the radicles have protruded (14.5 hours) is the osmotically silent period. In lettuce seeds, virtually nothing is known about these 6 hours, although synthesis of gibberellins has been reported to occur as early as one hour after red light (Köhler, 1966). (Considering the temperature used in the gibberellin experiments--26°--and the fact that most seed lots have considerably shorter periods of far-red reversibility than did the one used in this thesis, it is likely that this gibberellin synthesis may occur in seeds which have lost far-red reversibility.) Undoubtedly, much can be learned about breakage of photodormancy through chemical and microscopic studies of the osmotically silent period.

Reflection Coefficient and Growth

The fact that various osmotica penetrate into cellular osmotic space at different rates is expressed in terms of the reflection coefficient. This coefficient is given the value of 1.0 if absolutely no penetration of osmoticum occurs, and 0.0 if penetration is rapid, as for water. (It is interesting that external D_2O can itself act as an effective osmoticum for a brief period before exchange with internal H_2O is complete [Evans, unpublished].) For various osmotic substances values of the reflection coefficient range between 0.0 and 1.0. Thus, various osmotica have different effects on the rate of water flux and therefore on the rates of plasmolysis and deplasmolysis due to penetration of osmoticum:

$$J = L_w (\Delta\psi_p + \sigma\Delta\psi_o)$$

J = water flow/cross section

$L_w = 1/r$

r = membrane resistance

σ = reflection coefficient

These considerations suggest a manner for solving a currently unresearched and to some extent unrecognized problem in water relations: If the external osmotic potential is lowered to the point at which the cellular water potential becomes zero and growth stops, what happens to the systems which produce growth under conditions of normal

turgor--i.e., the osmotic systems and/or those which loosen cell walls? One can imagine (1) that potential generators are dependent upon a pressure potential of a certain magnitude and are shut off when pressure potential decreases below this value. On the other hand, (2) they might continue to generate growth potential which, upon release from osmotic stress, would give rise to a rapid but transient increase in growth rate. Also, continuous generation of growth potential would result in a gradual overcoming of the external osmotic stress.

Each phenomenon which would result from the second possibility has been reported. Thus Ray and Ruesink (1963) found that Avena coleoptile sections rapidly resumed growth after an osmotic stress which initially prevented water uptake. Green (1969) showed that Nitella behaved in a similar manner; in addition, on removal of the osmotic stress, a transient increase in growth rate (above the growth rate of unstressed cells) was observed.

It should however be pointed out that these effects are not necessarily caused by a continued production of growth potential: penetration of osmoticum into the tissue would also account for them. If penetration of osmoticum is occurring, however, the use of several different osmotica with different reflection coefficients will show that the time required for the tissue to resume growth after osmotic stress is positively correlated with the rate of

entry of osmoticum into the tissue. Also, if the overshoot in growth rate which follows removal of osmoticum is caused by the decrease in internal osmotic potential due to osmotic penetration, the amount of overshoot should also be positively correlated with the rate of osmoticum penetration (negatively correlated with the value of the reflection coefficient). There is a wide range in molecular weights of PEG; and if it is remembered that osmotic potential is not strictly dependent on molality, PEG could provide an excellent range of osmotica for such experiments.

X. TABLES

Table 1

Factors other than red light which can induce germination of dormant lettuce seeds.

Factor Inducing Germination	Reference
low temperature	70, 73
gibberellins	24, 9, 54, 6, 7
kinetin	64, 42, 73
thiourea	24, 45
chloramphenicol	8, 31
actinomycin D	8
CO_2	83
ethylene	1, 77

Table 2

Factors which can induce dormancy in lettuce seeds.

Factor Inducing Dormancy	Reference
gamma radiation (low)	34
gamma radiation (high)	35
IAA	52
coumarin and related compounds	50, 51
high temperature	83, 72, 5
abscisin	49

Table 3

The effect of FUDR and thymidine on germination of lettuce embryos incubated in water or 0.46 molal mannitol on filter paper. Final percent germination.

	H_2O control	10^{-5}M FUDR	10^{-5}M FUDR + 10^{-4}M thymidine
light-treated; water	100	100	100
light-treated; mannitol	100	20	79
dark-treated; water	100	100	100
dark-treated; mannitol	100	24	68

Table 4

Relation between initial density and rate of fall for plant tissue in a D_2O gradient.

Assume a tissue of density 1.04 and 80% water by volume takes up water in a growth process until its density is 1.02.

1. The tissue of density 1.02 now has 90% water:

density 1.04; 80% water		density 1.02, 90% water
8.00	8 volumes of water	9.00
2.40	2 volumes non-water	1.20
10.40/10 = 1.04		10.20/10 = 1.02

2. For the two tissues to fall in a 50% D_2O column they must achieve the density of 50% D_2O: 1.05.

density 1.04; 80% water	density 1.02, 90% water
8.10 (6.00 + 2 x 1.05)	9.30 (3.00 + 6 x 1.05)
2.40	1.20
10.50/10 = 1.05	10.50/10 = 1.05

   The density 1.04 tissue has now exchanged 2 volumes of water for 50% D_2O whereas the density 1.02 tissue has exchanged 6 volumes of water for 50% D_2O.

3. For the two tissues to fall in a 60% D_2O column after achieving the density to fall in a 50% D_2O column they must bring their density up to 1.06.

density 1.04; 80% water	density 1.02, 90% water
8.20 (4.667 + 3.333 x 1.06)	9.40 (2.333 + 6.667 x 1.06)
2.40	1.20
10.60/10 = 1.06	10.60/10 = 1.06

   The density 1.04 tissue has now exchanged 3.333 volumes of water for 60% D_2O whereas the density 1.02 tissue has exchanged 6.667 volumes of water for 60% D_2O.

4. The effect of tissue density on exchange rate is probably twofold. First, for tissue sections of the same size, the lower the density of the section the larger volume of water which must be exchanged for the section to fall through an increment of the gradient. Second, as the tissue exchanges more water, exchange must occur further into the section.

Table 5

Relation between molecular weight and osmotic pressure for polyethylene glycol. Determinations were made with a vapor pressure osmometer.

Osmoticum	Molal Concentrations with Equal Osmotic Potentials
Mannitol	0.483
PEG 200	0.440
PEG 600	0.327
PEG 1000	0.253
PEG 4000 (MW = 3350)	0.100

Table 6

Effect of two different osmotica on germination of red light- and dark-treated lettuce seed embryos.

Osmoticum	Molal concentration permitting 50% final germination (20° liquid culture)		Light minus dark in terms of mannitol
	red light	dark	
Mannitol	0.65	0.47	0.18
Polyethylene glycol 4000	0.085 (= 0.39 mannitol)	0.062 (= 0.21 mannitol)	0.18

Table 7

Penetration of 0.075 molal PEG 4000 into germinating embryos.

1. Dark- and light-treated embryos were placed in osmoticum at 18:50 hours after treatment. 10λ of incubation medium contained 3413 cpm of ^{14}C-PEG-4000. 50 dark-treated embryos contained 28λ water; 50 light-treated embryos contained 37λ water.

Treatment	cpm/50 embryos	Percent penetration
Dk.-treated; 30 min incubate	894	9.4
Lt.-treated; 30 min incubate	1153	9.1
Dk.-treated; 60 min incubate	1227	12.8
Lt.-treated; 60 min incubate	1412	11.2

2. In a similar experiment dark- and light-treated embryos were placed in osmoticum at 18:00 hours and removed at 24:00 hours. 50λ of incubate contained 7250 and 6970 cpm for dk. and lt. respectively. 50 dark-treated embryos contained 27λ water; 50 light-treated embryos contained 35λ water.

Treatment	cpm/50 embryos	Percent penetration
Dk.-treated; 360 min incubate	1458	37
Lt.-treated; 360 min incubate	1420	29

3. Similar experiments using ^{14}C-mannitol showed that by 48 hours of osmotic treatment, penetration as measured by this method is 75-85% complete.

Table 8

Points of incipient plasmolysis as determined by the rate of D_2O penetration into osmotically stressed tissue (see Figure 23).

Hours after red light or dark treatment	Point of incipient plasmolysis Method #1	Method #2
	MOLAL MANNITOL EQUIVALENT	
14.5 light	---	less than .47
	---	less than .47
15.5 light	.32	.40
	.34	.40
	---	.45
	---	.39
16.5 light	.36	.39
	---	.45
	---	.45
	---	.44
	?	.34
	?	?
17.5 light	.34	.44
	.32	.45
	---	.47
	---	?
18.5 light	?	.47
	---	.40
	---	.40
	---	.40
16.5 dark	---	.47
17.5 dark	.36	?
	---	.45
	.22	.40
	---	.40
	---	.40
	.42?	?
	.36	.50?
18.5 dark	.32	.47
	.36	.44
	---	.45
	---	.43
	.34	.39
	---	.45
	---	?
19.5 dark	---	.45
	---	?
	---	.45
	---	.44
	?	.60

continued

Table 8 - continued

Hours after red light or dark treatment	Point of incipient plasmolysis Method #1	Method #2
	MOLAL MANNITOL EQUIVALENT	
20.5 dark	.34	.40
	.36	?
	---	.47
	---	?
	.36	?
21.5 dark	.39	.45
	---	?
	---	.45
	---	.47
	?	.40

Table 9

Force necessary to penetrate seed layers external to embryo.

Rod Diameter (mm)	Force to Penetrate per Rod Surface Area (gm/mm^2)	Molal Force
0.25	8/0.098	0.38
	9	
	10	
	7	
	9	
	9	
	7	
	8	
	10	
	8	
0.40	18/0.25	0.31
	18	
	19	
	14	
	18	
	20	
	19	

Area of spherical segment = $2\pi rh$

1 molal = 22.4 atmospheres (A.)

1 A. = 0.0447 molal = 10.33 gm/mm^2

XI. FIGURES

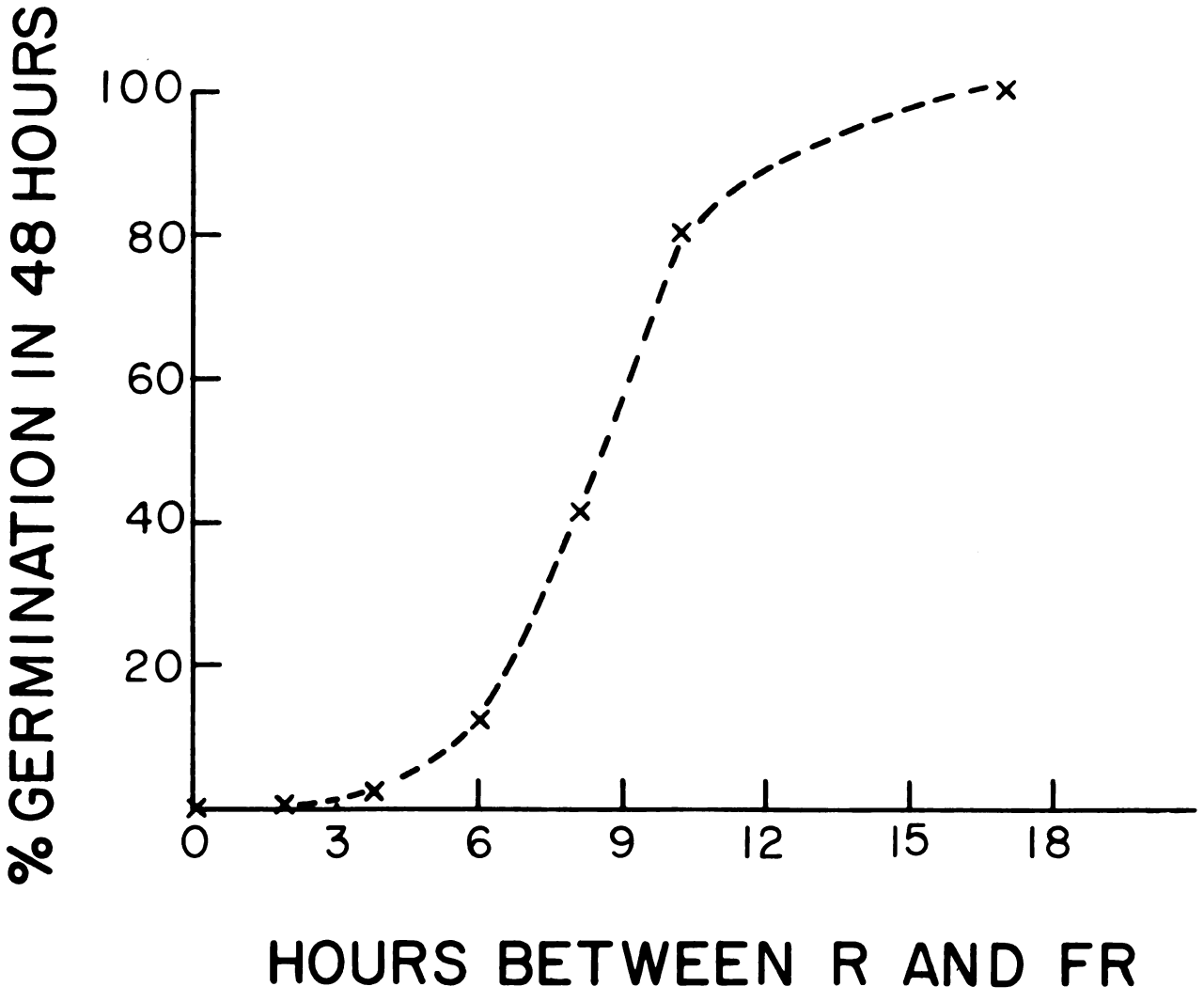

Figure 1. A far-red reversal curve for whole seeds incubated on filter paper. Sample size: 300-600 seeds.

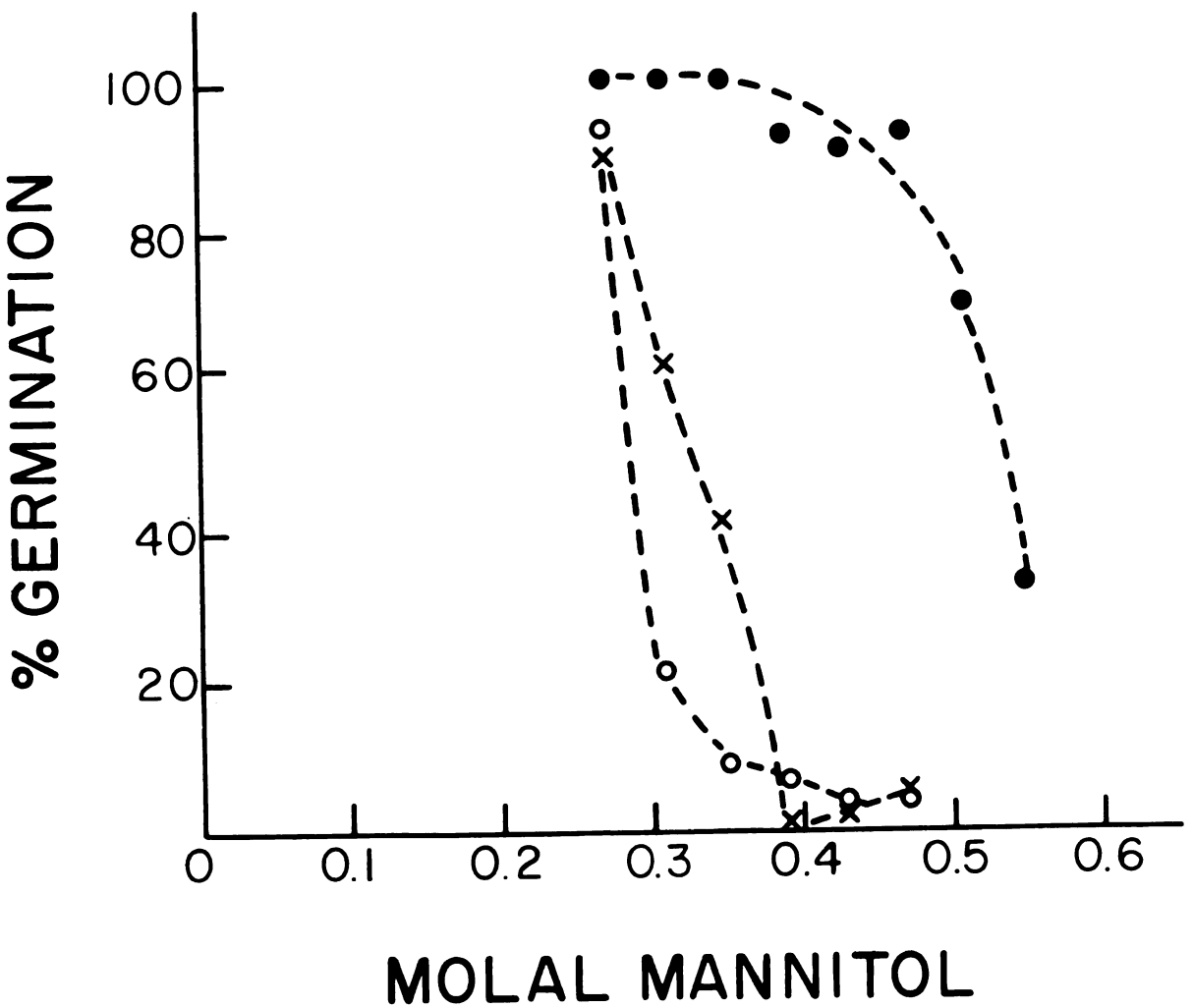

Figure 2. The effect of osmoticum on the germination of lettuce embryos incubated on filter paper for 72 hours. o FR, x dark, ● R. Sample size: 80 embryos.

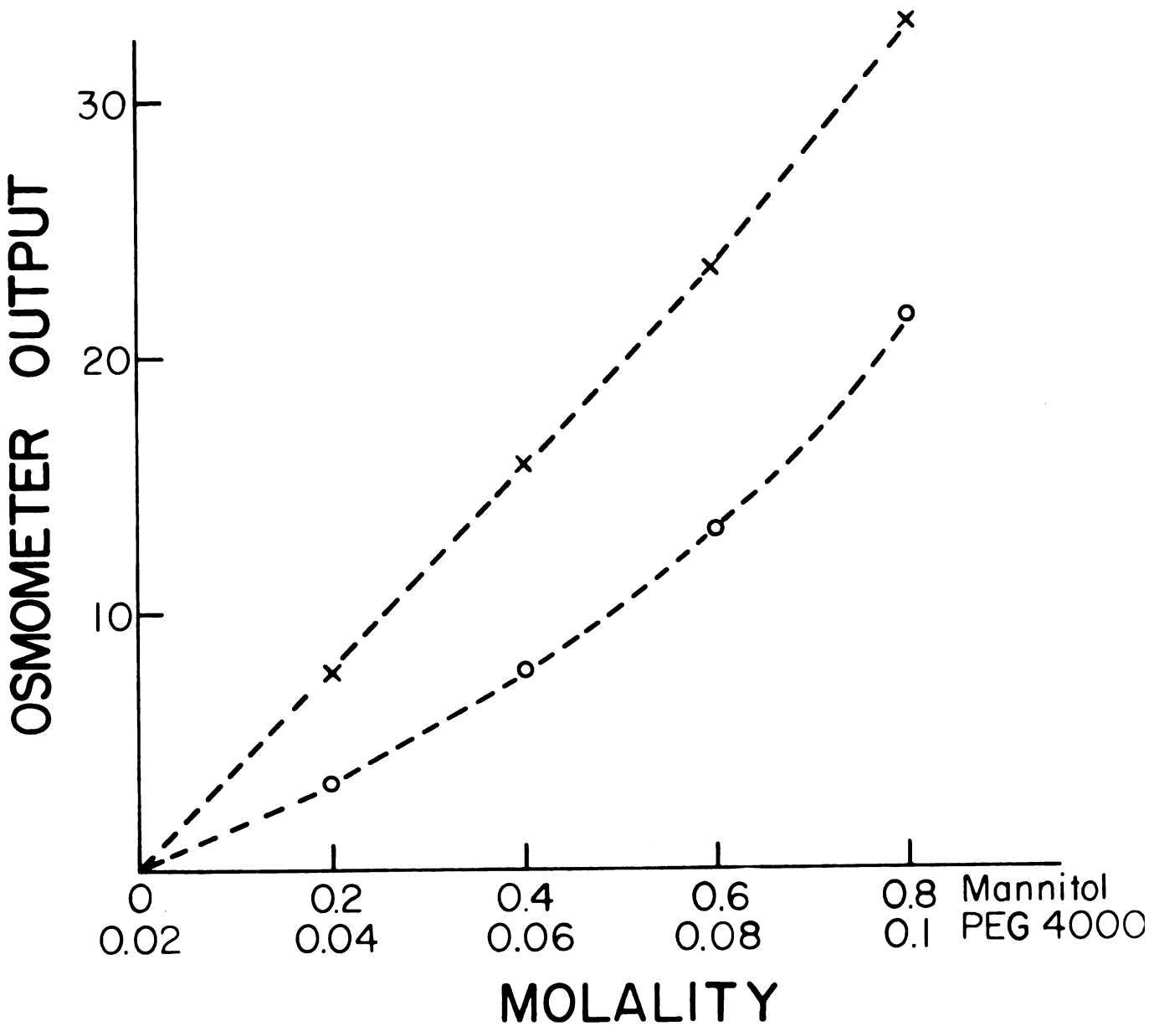

Figure 3. Relative osmotic pressures of mannitol and of PEG 4000. Averages of several determinations. x mannitol, o PEG 4000.

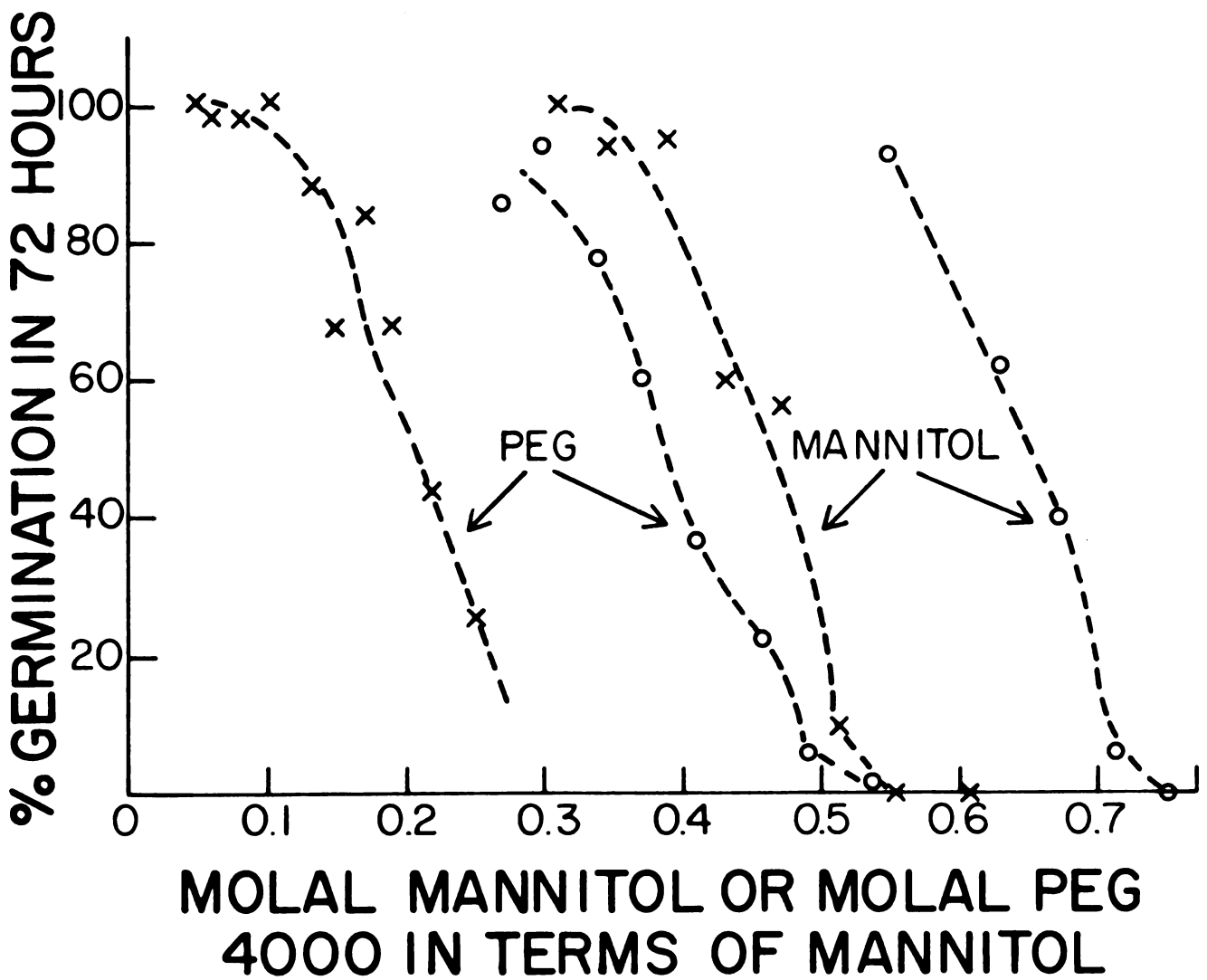

Figure 4. The effect of osmoticum on the germination of lettuce embryos incubated in solution for 72 hours. x dark, o R. Sample size: 80 embryos.

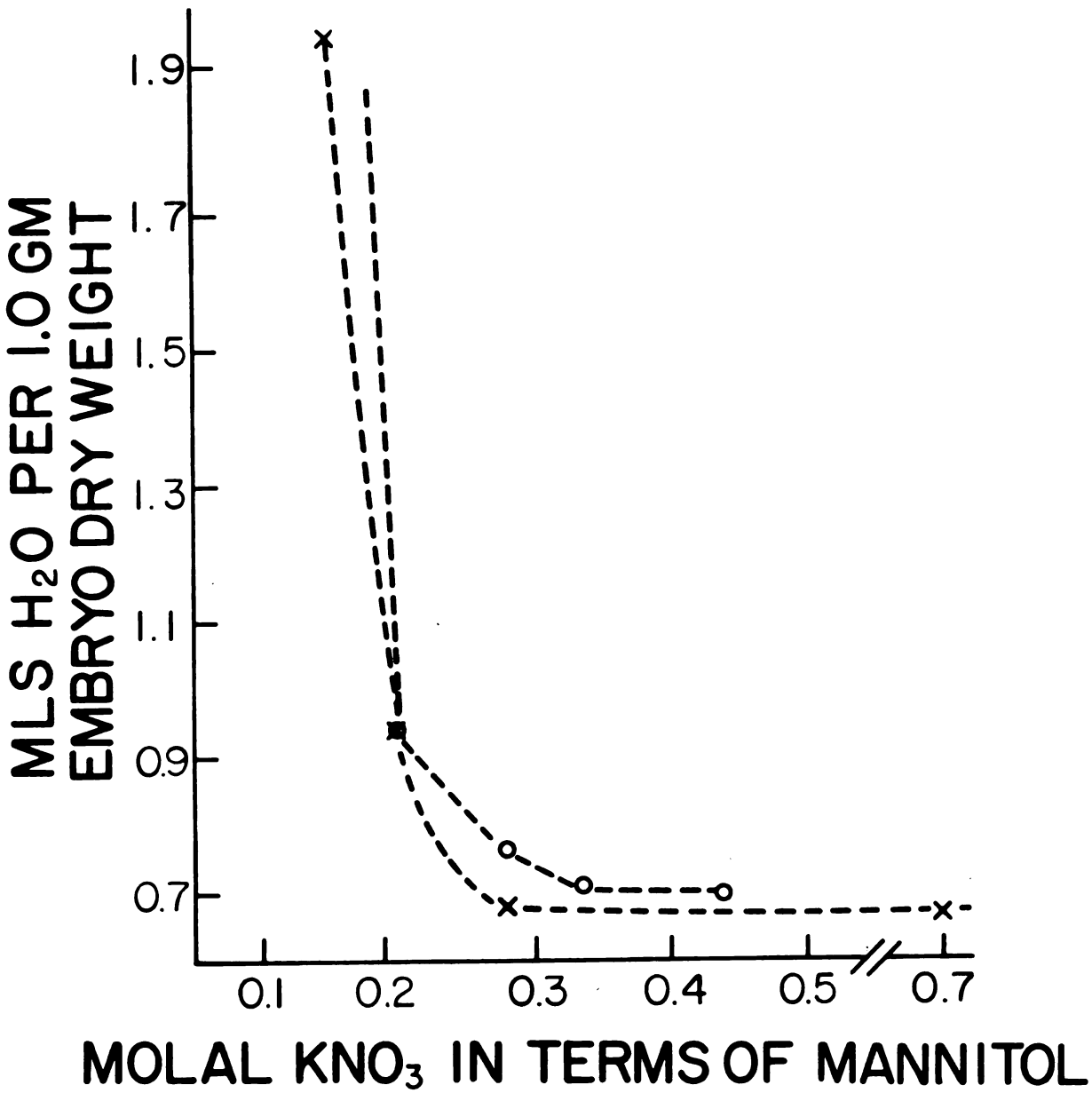

Figure 5. The effect of KNO_3 on the germination of lettuce embryos incubated for 68 hours in solution. x dark, o R. Sample size: 80 embryos.

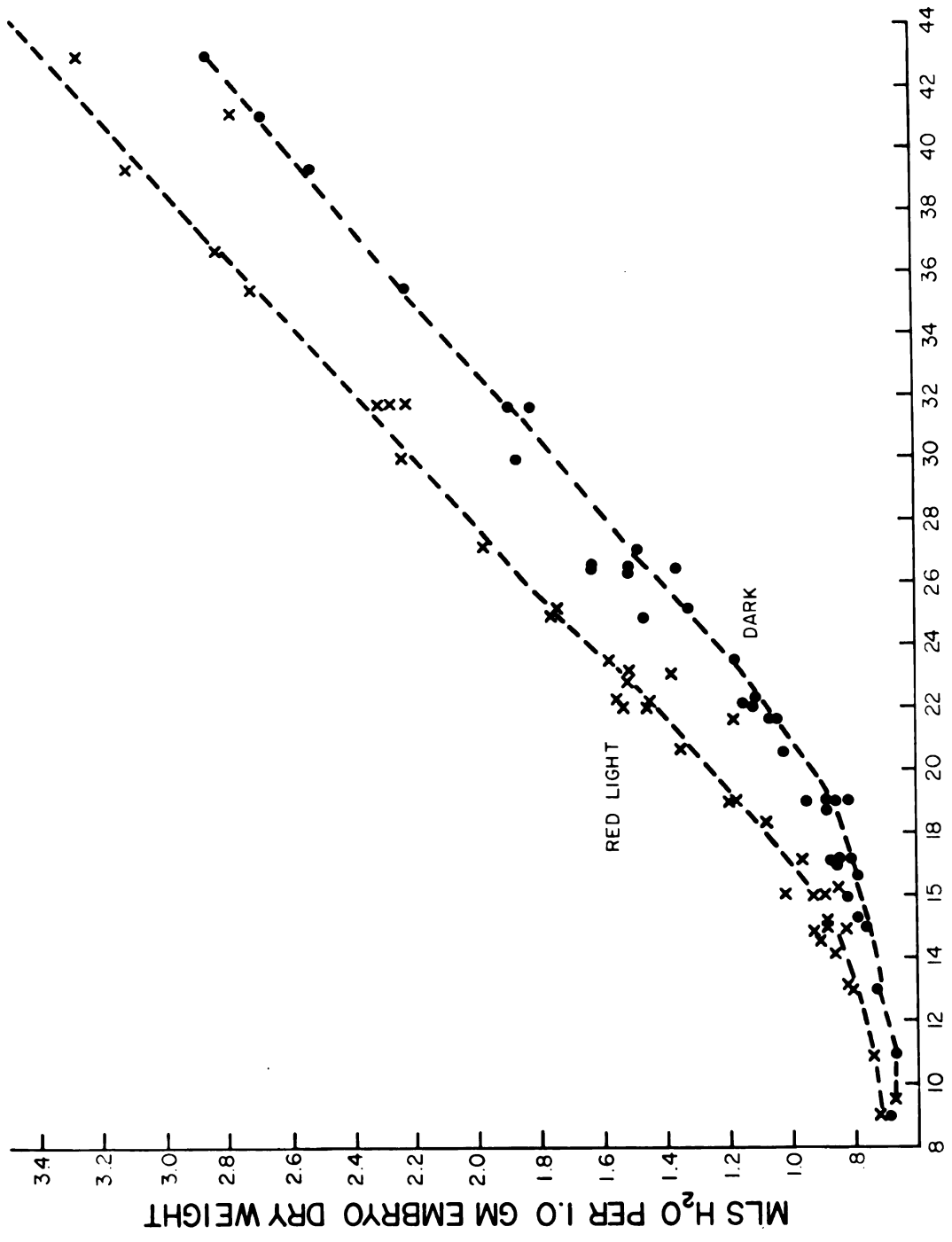

Figure 6. Water content of embryos germinating in water. Sample size: 80 embryos.

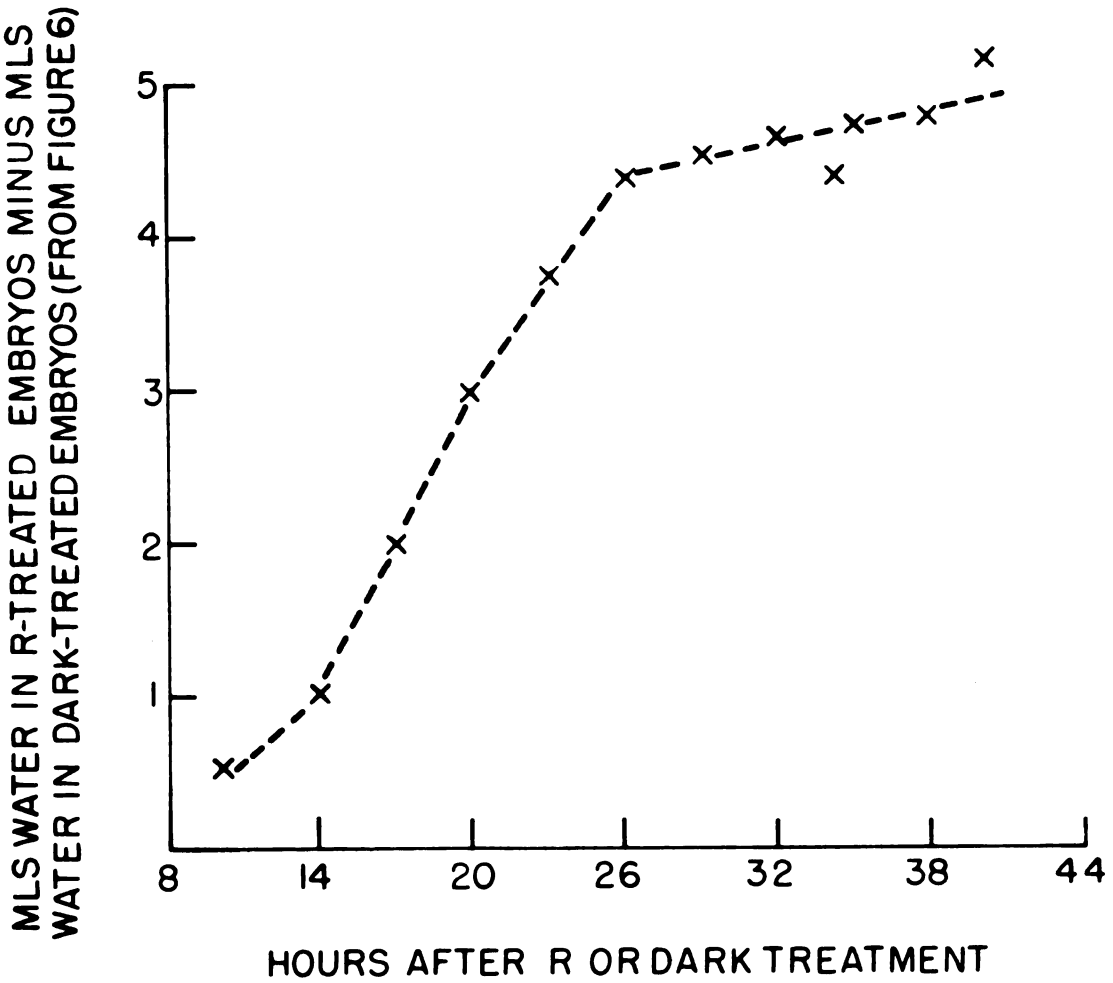

Figure 7. The increased rate of water uptake in R-treated embryos.

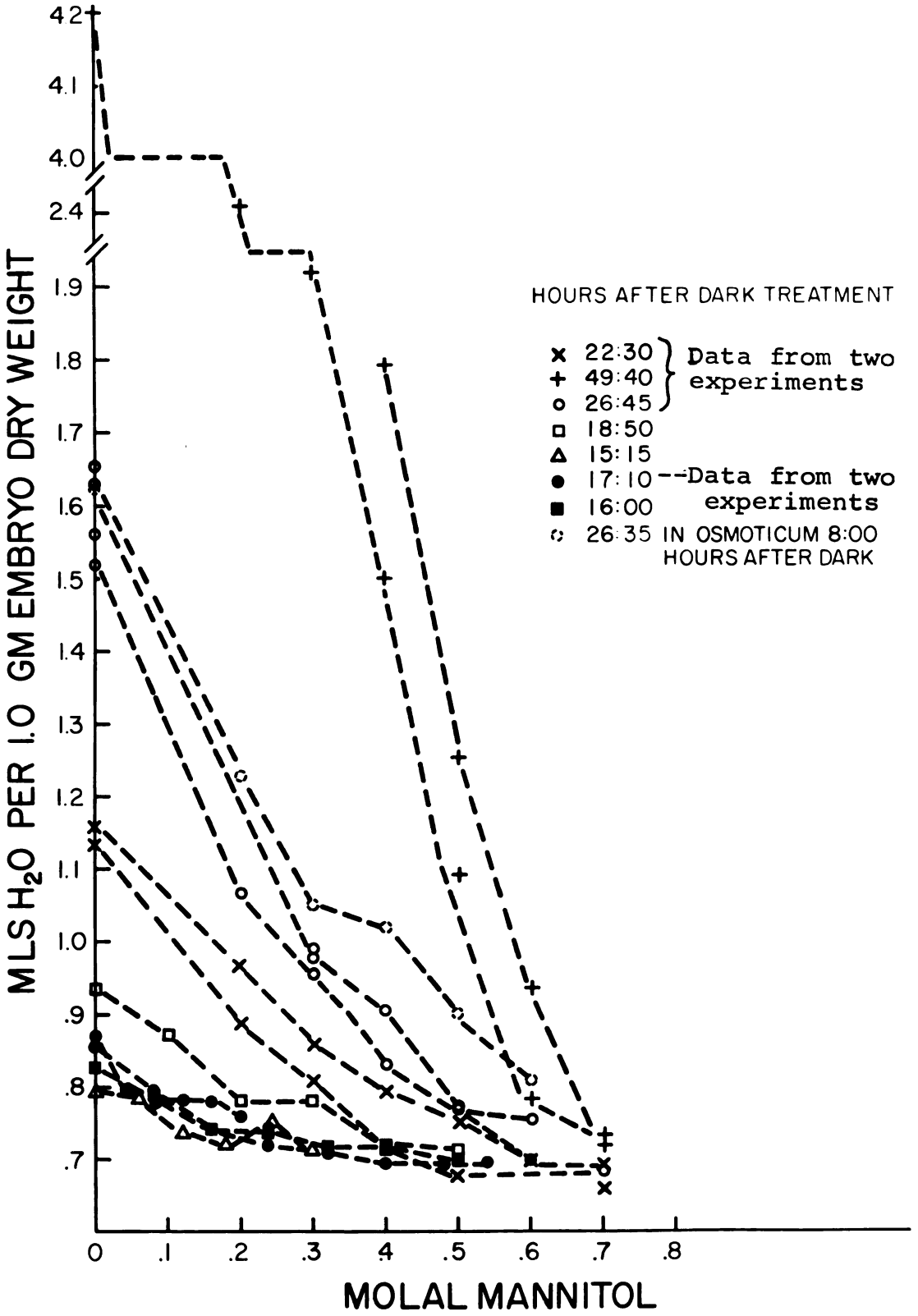

Figure 8. Water uptake by embryos incubated in osmoticum from 2 to 3 hours after dark treatment. Sample size: 80 embryos.

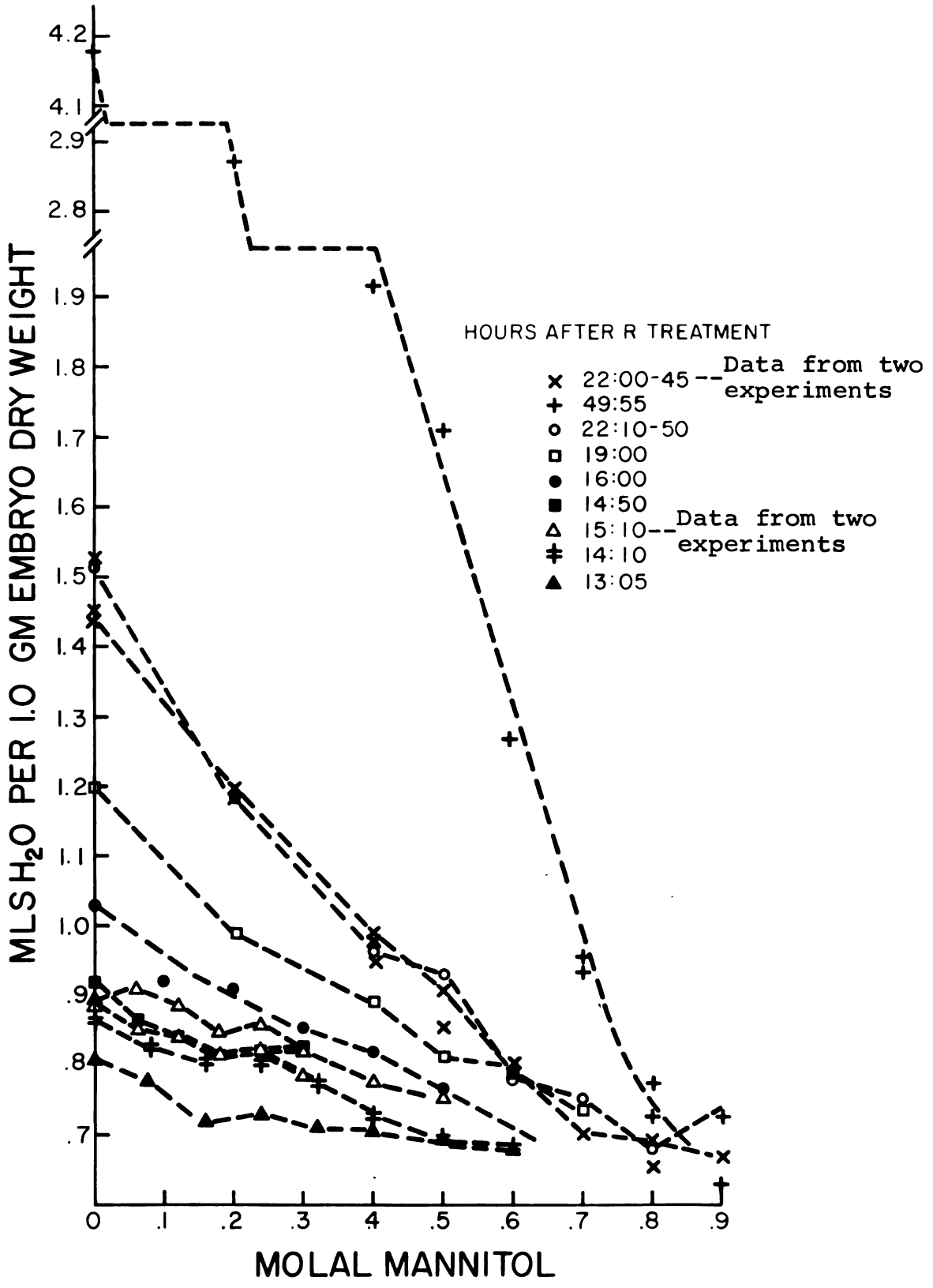

Figure 9. Water uptake by embryos incubated in osmoticum from 2 to 3 hours after R treatment. Sample size: 80 embryos.

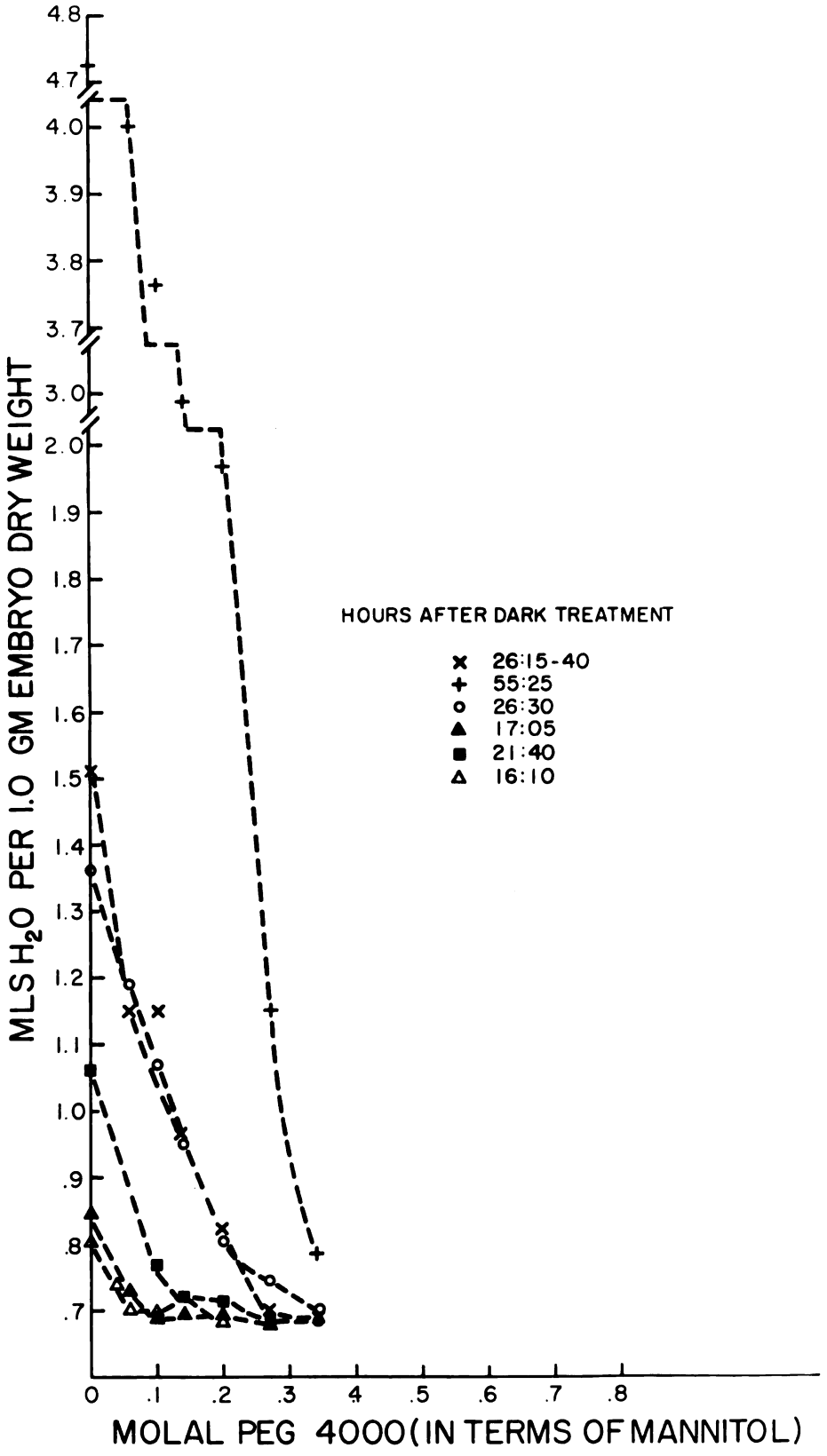

Figure 10. Water uptake by embryos incubated in osmoticum from 2 to 3 hours after dark treatment. Sample size: 80 embryos.

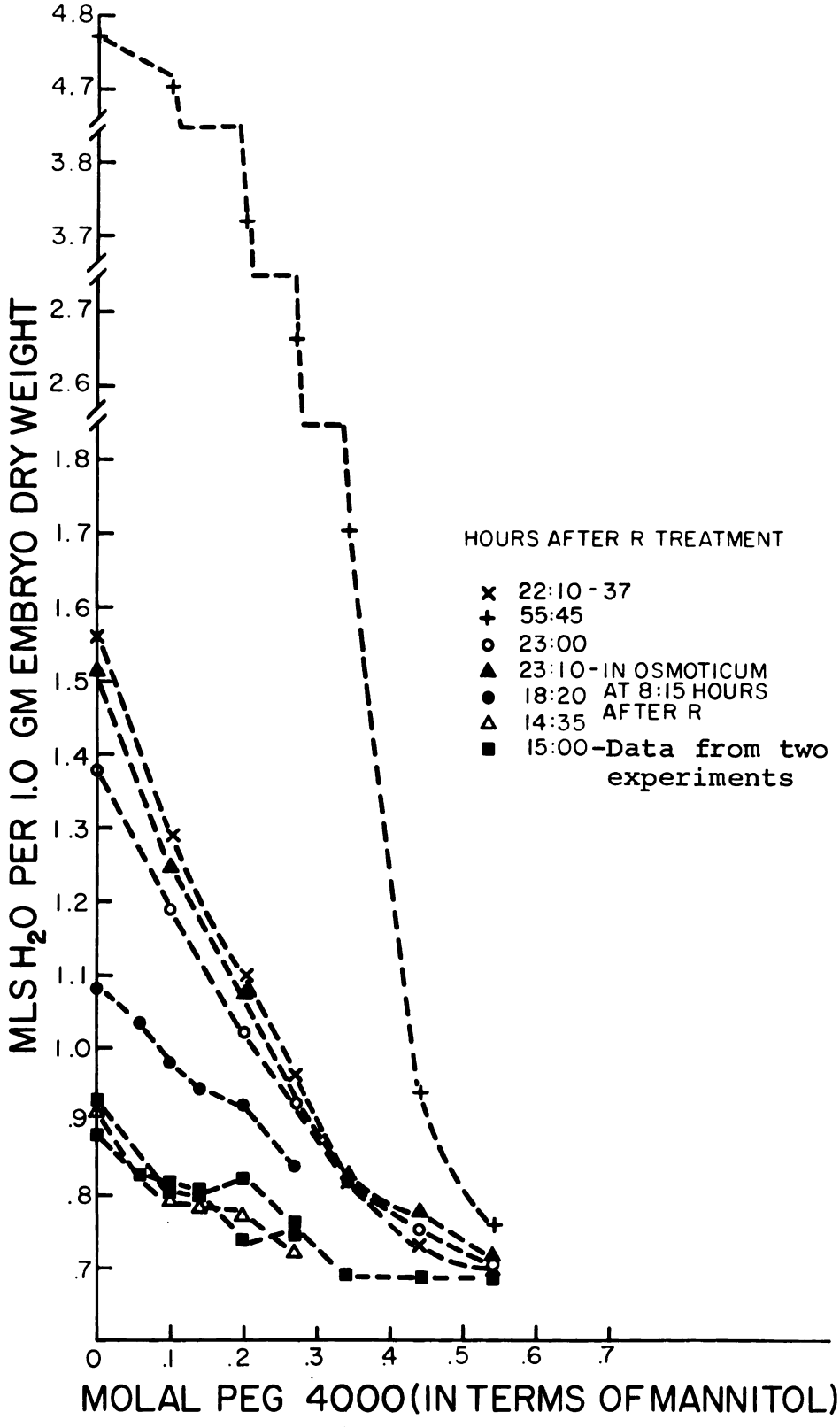

Figure 11. Water uptake by embryos incubated in osmoticum from 2 to 3 hours after R treatment. Sample size: 80 embryos.

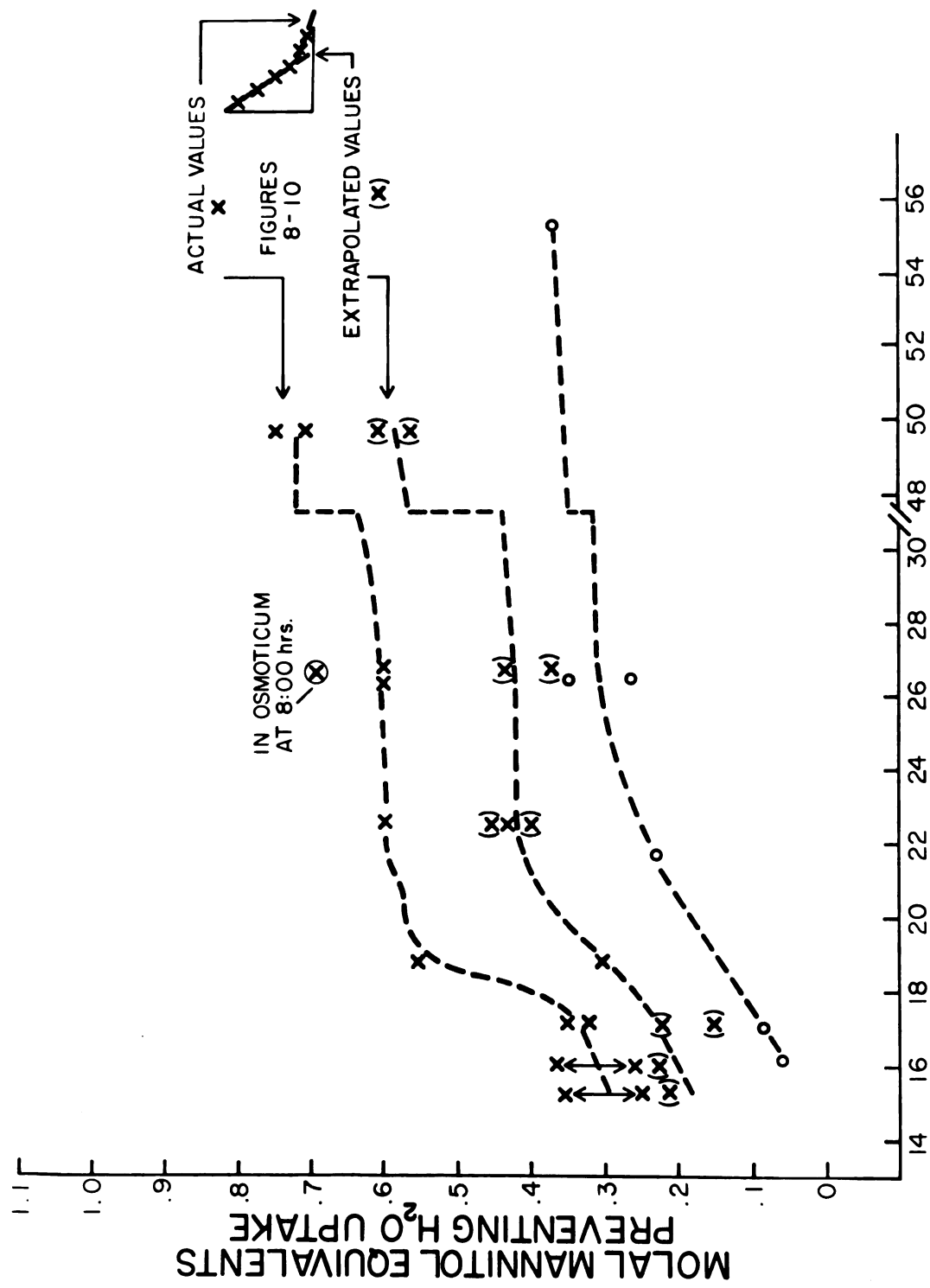

Figure 12. Osmotic concentrations preventing growth of dark-treated embryos versus hours since dark treatment. Embryos incubated in osmoticum since 2 to 3 hours after dark treatment. From figures 8 and 10. x mannitol, o PEG 4000.

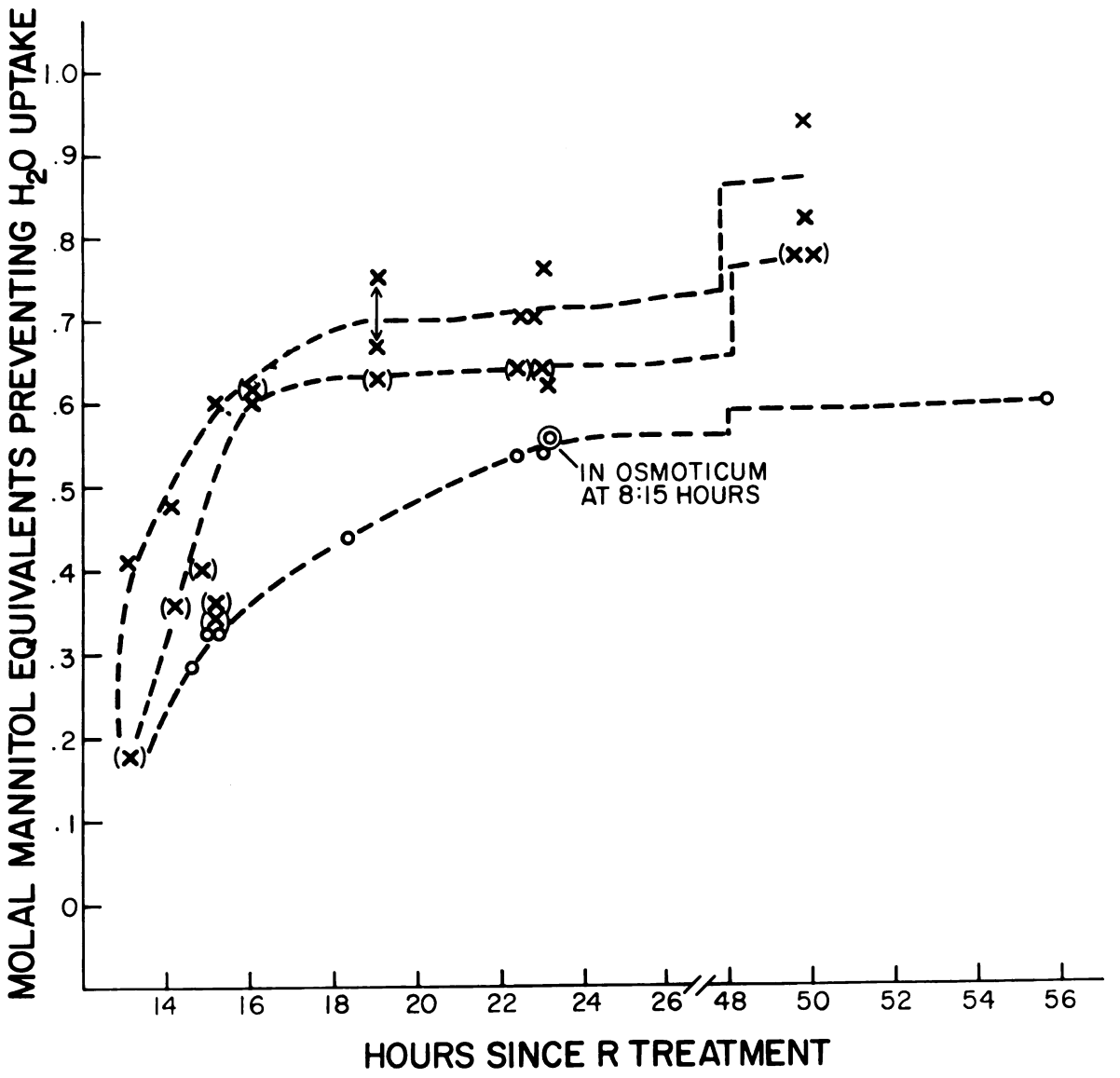

Figure 13. Osmotic concentrations preventing growth of R-treated embryos versus hours since R treatment. Embryos incubated in osmoticum since 2 to 3 hours after R treatment. From figures 9 and 11. x mannitol, o PEG 4000.

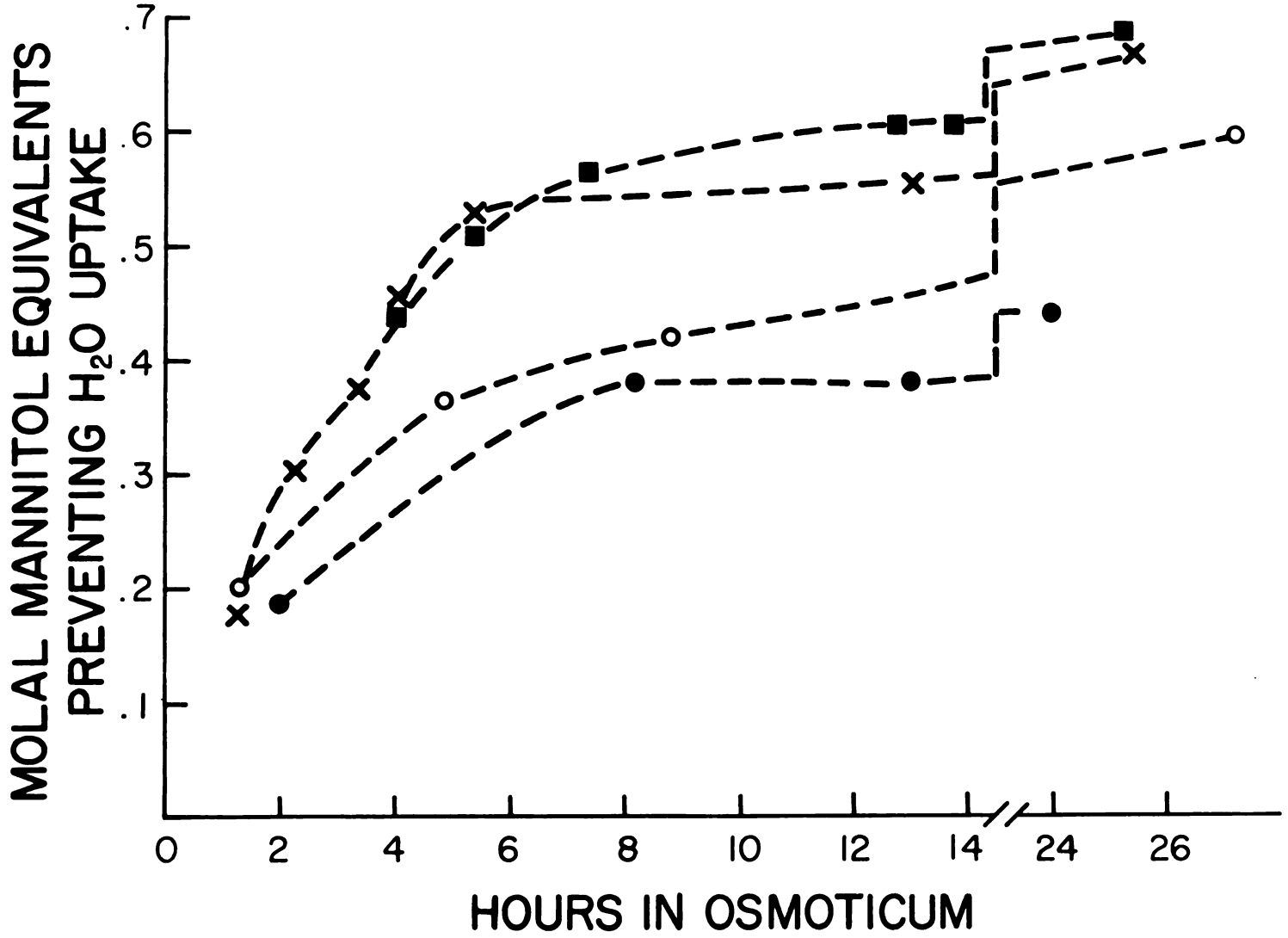

Figure 14. Osmotic concentrations preventing growth of embryos versus hours in osmoticum. Embryos incubated in water until 15.5 hours (for light-treated) and 18.5 hours (for dark-treated). From figures 18, 19, 20, and 21. x mannitol, R-treated; ■ mannitol, dark-treated; O PEG 4000, R-treated; ● PEG 4000, dark-treated.

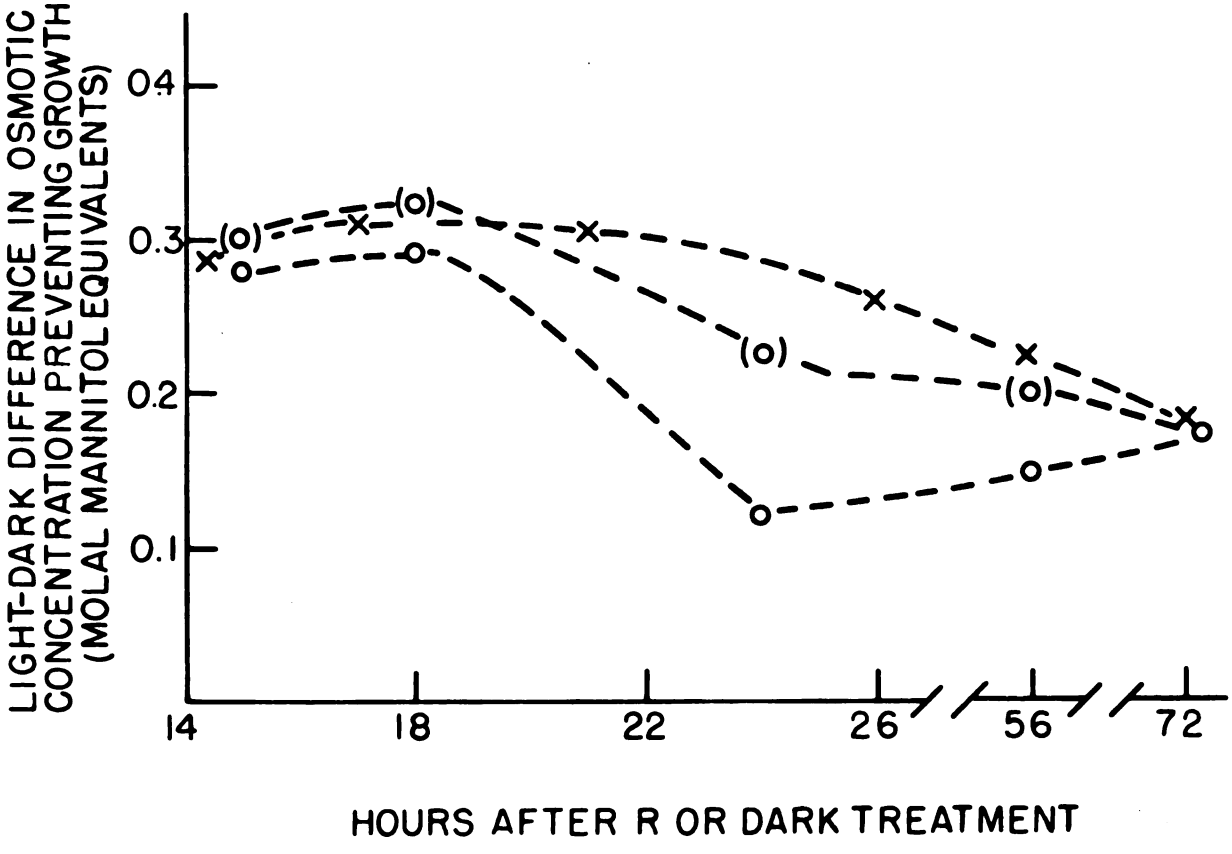

Figure 15. Differences between osmotic concentrations preventing growth of R- and dark-treated embryos germinating in osmoticum. From figures 12 and 13. O mannitol, x PEG 4000.

Figure 16. An experiment designed to show a build-up of water potential in osmotically-stressed, dark-treated embryos.

Δ dark-treated embryos germinated in H_2O.
x as Δ but transferred to a new flask 19-20 hours after dark treatment.
O dark-treated embryos incubated in 0.07 molal PEG 4000 until 19 hours after dark treatment, then in H_2O.
Sample size: 80 embryos.

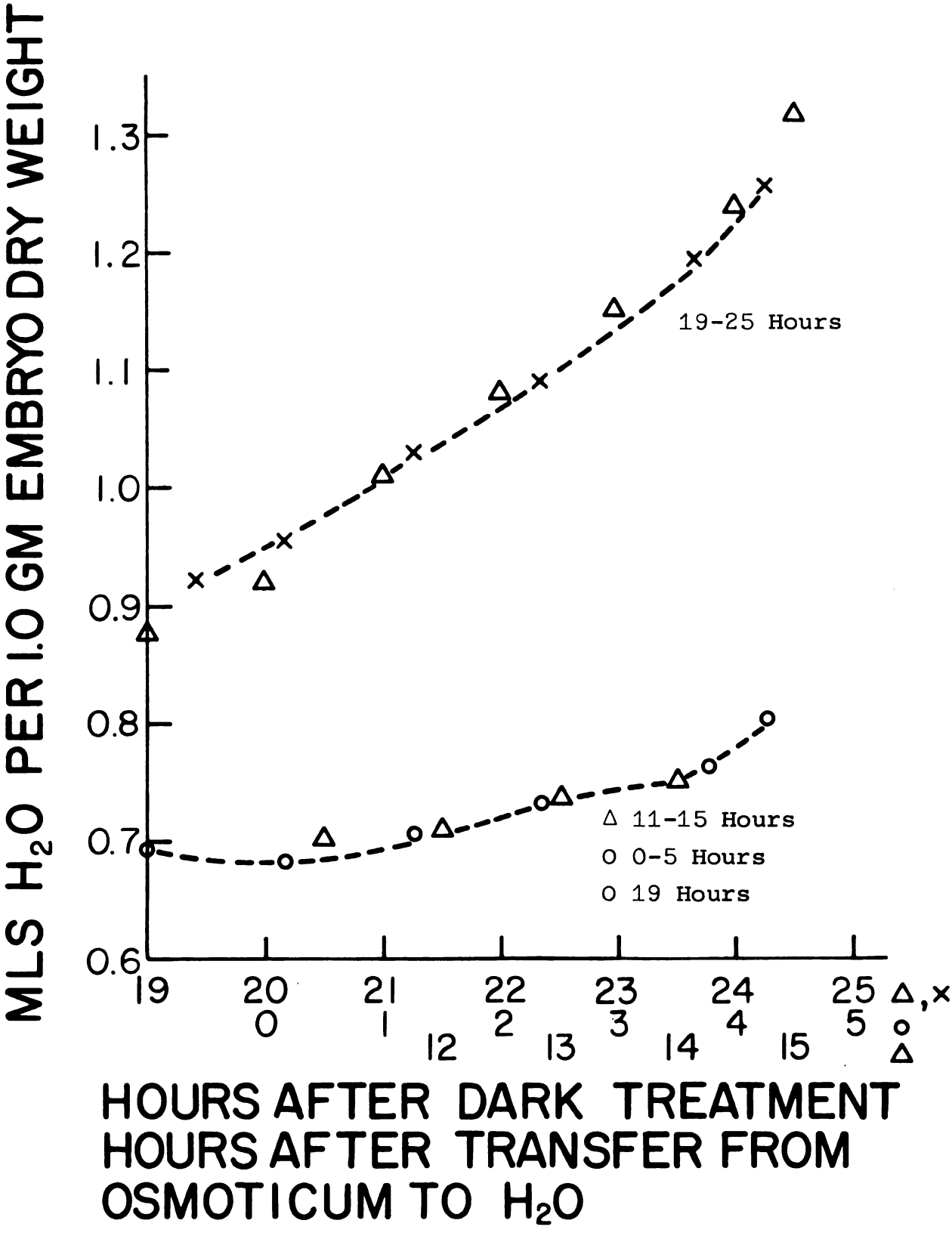
MLS H2O PER 1.0 GM EMBRYO DRY WEIGHT
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
19-25 Hours
Δ 11-15 Hours
o 0-5 Hours
o 19 Hours
19
20
0
21
1
12
22
2
13
23
3
14
24
4
15
25
5
Δ,x
o
Δ
HOURS AFTER DARK TREATMENT
HOURS AFTER TRANSFER FROM
OSMOTICUM TO H2O

Figure 16

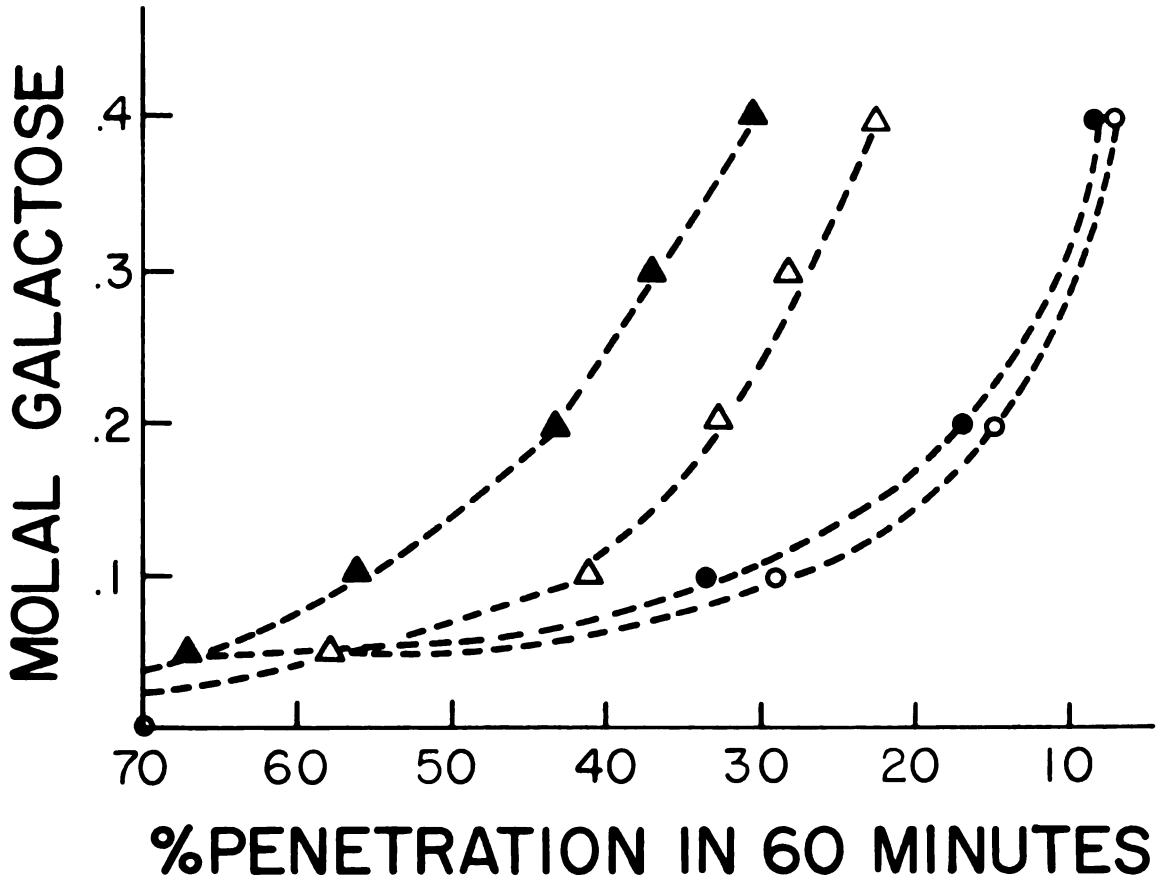

Figure 17. The rate at which the water in embryos (fresh weight minus dry weight) equilibrates with external osmoticum as a function of external osmotic concentration. From figure 24. △ light-treated embryos, ▲ dark-treated embryos, O and ● theoretical effect of concentration on penetration for light- and dark-treated embryos respectively (based on 0.05 molal data) if penetration rate is independent of concentration.

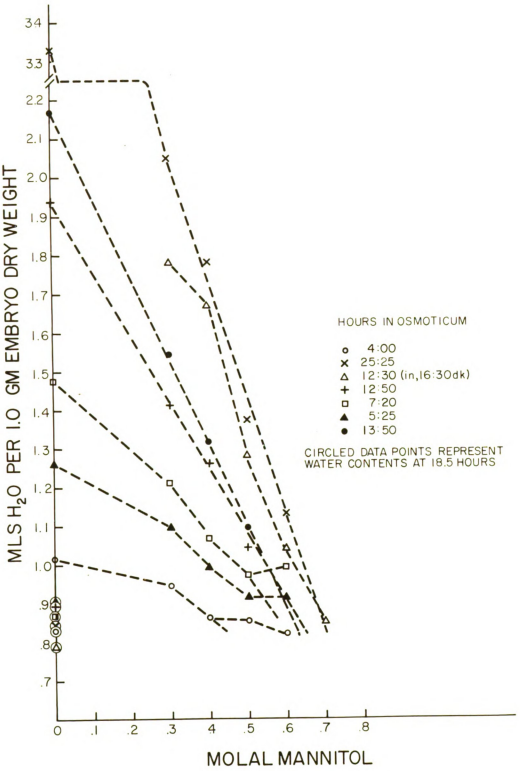

Figure 18. Water uptake by embryos incubated in osmoticum from 18.5 hours after dark treatment. Sample size: 80 embryos.

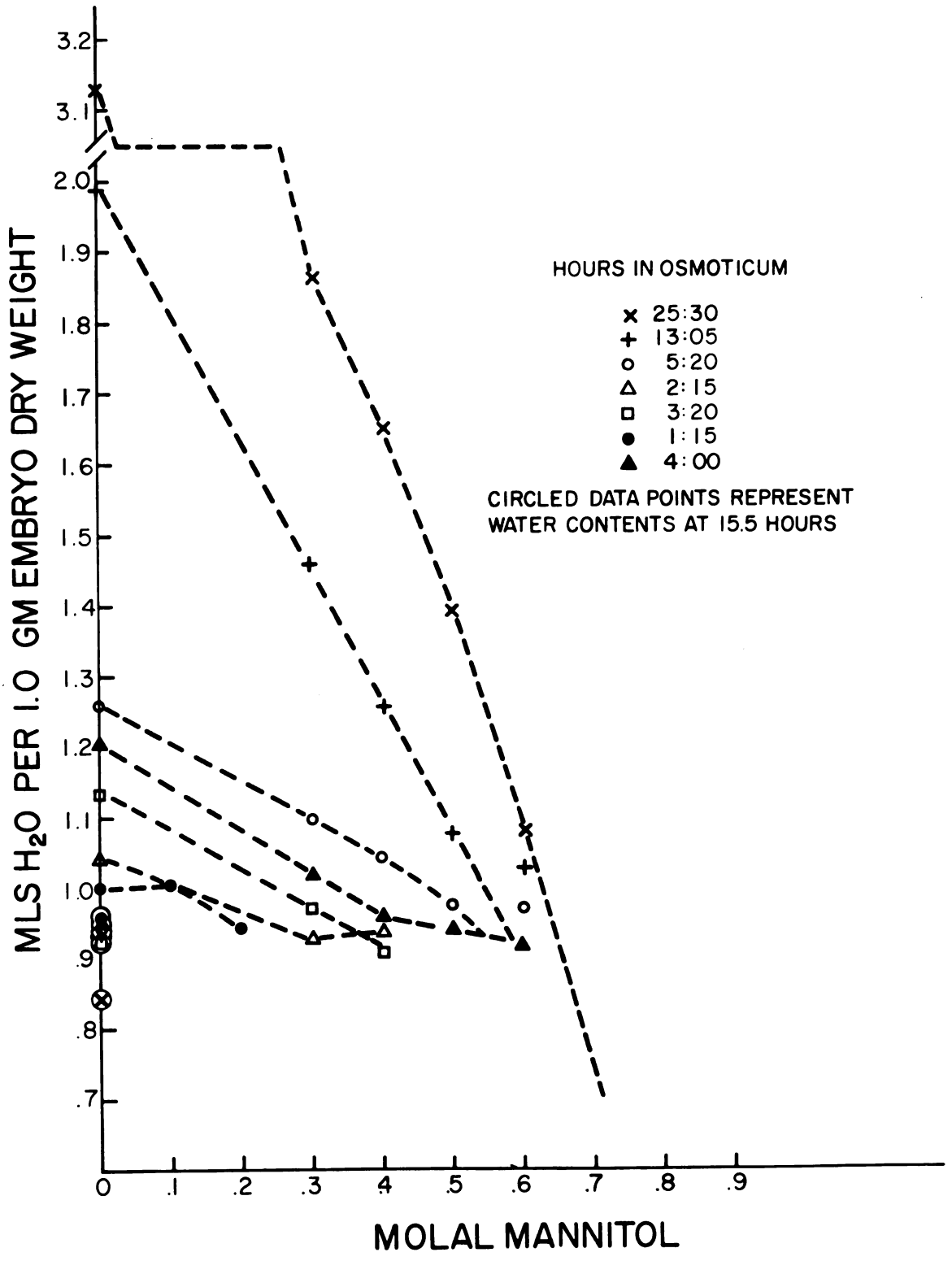

Figure 19. Water uptake by embryos incubated in osmoticum from 15.5 hours after R treatment. Sample size: 80 embryos.

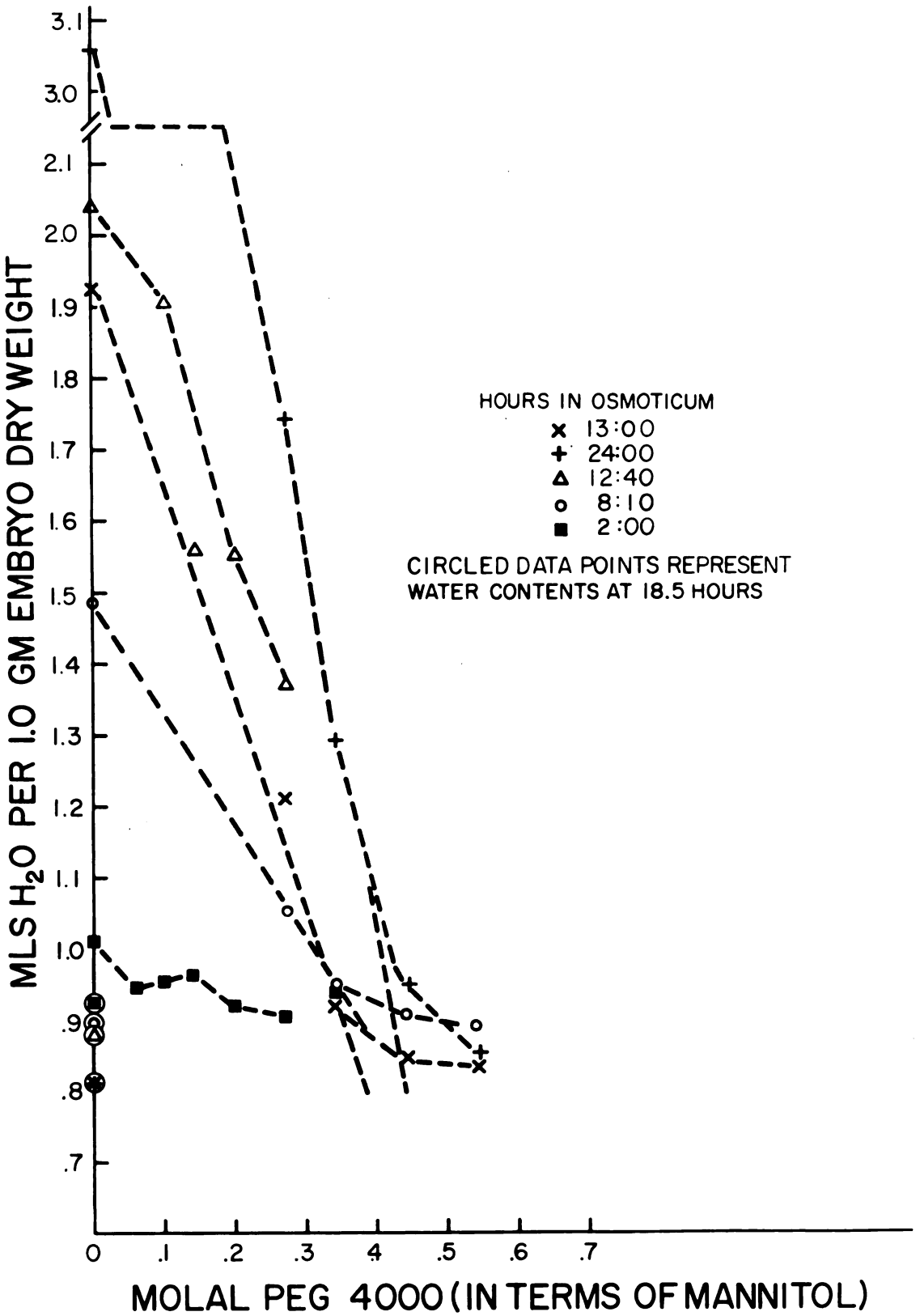

Figure 20. Water uptake by embryos incubated in osmoticum from 18.5 hours after dark treatment. Sample size: 80 embryos.

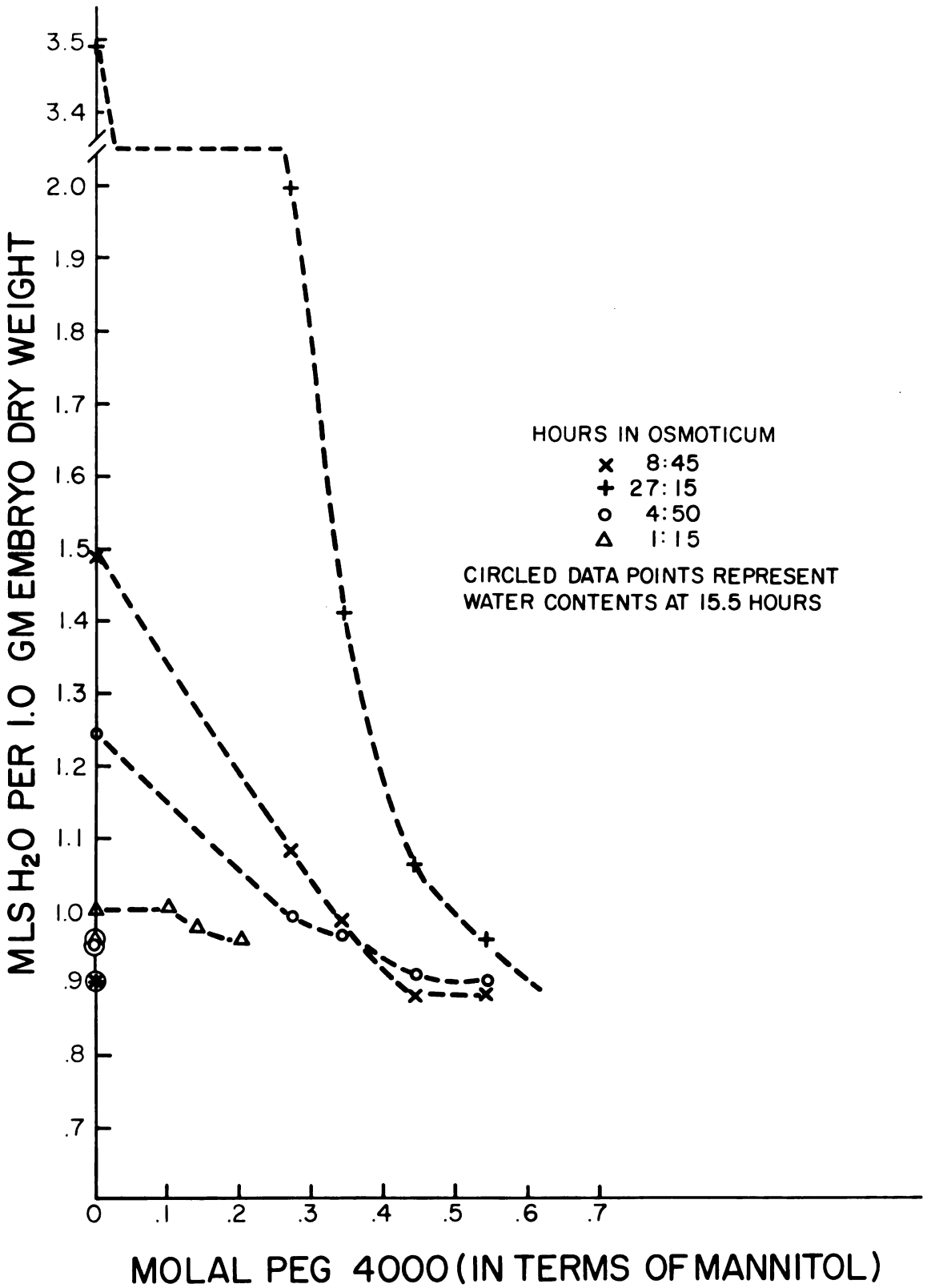

Figure 21. Water uptake by embryos incubated in osmoticum from 15.5 hours after R treatment. Sample size: 80 embryos.

Figure 22. The behavior of germinating embryo sections in a D_2O-osmoticum gradient. O embryo section 22 hours after dark treatment, x embryo section 22 hours after R treatment. The two examples illustrate the fact that density (which is itself determined by the germination stage) affects rate of fall in the gradient, and in the case of more dense embryo sections obscures the point of i.p. The dark-treated example has, if it is an average embryo, only 83% of the water content of the light-treated example.

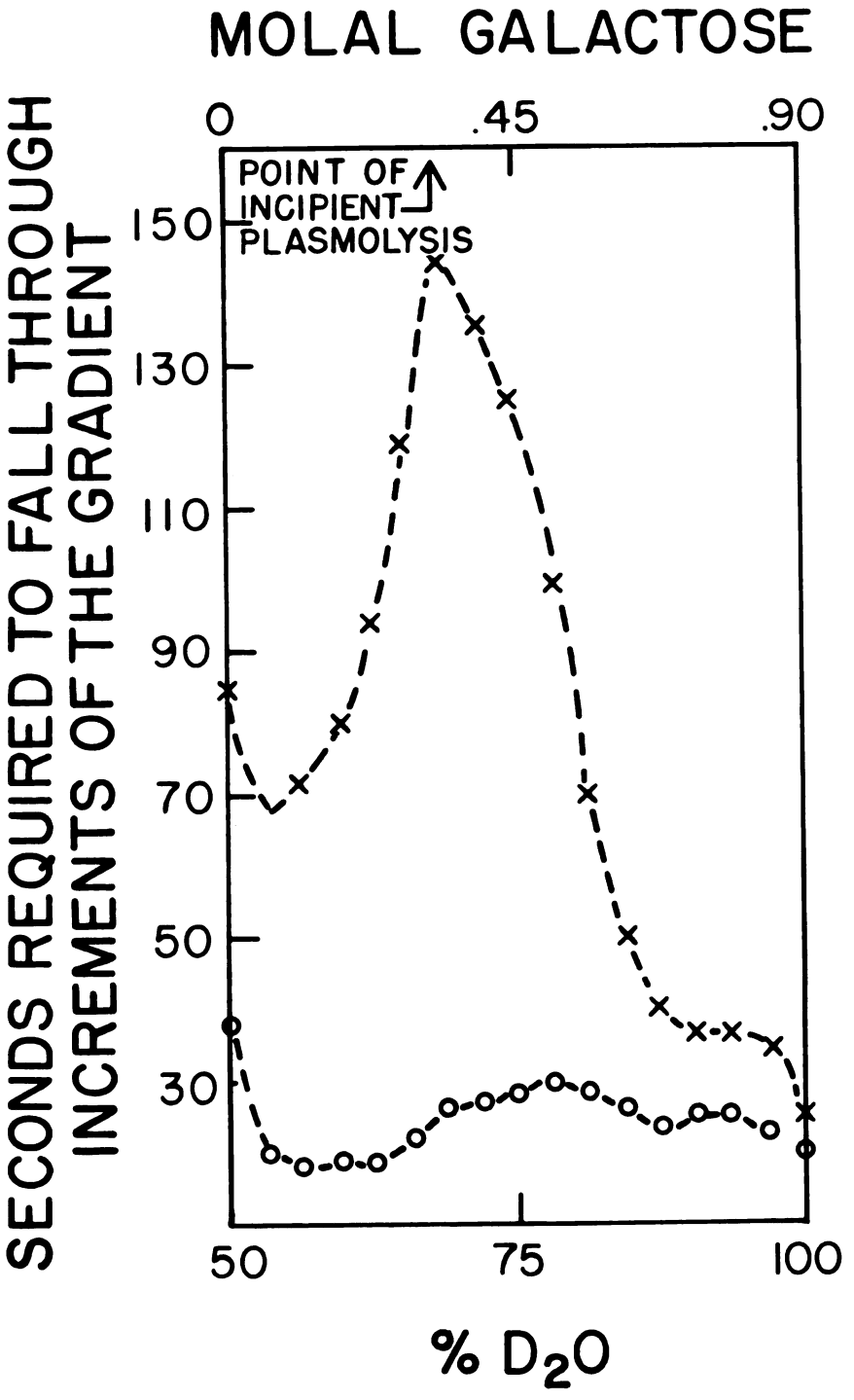
MOLAL GALACTOSE
0
.45
.90
POINT OF INCIPIENT PLASMOLYSIS
SECONDS REQUIRED TO FALL THROUGH INCREMENTS OF THE GRADIENT
150
130
110
90
70
50
30
50
75
100
% D2O

Figure 22

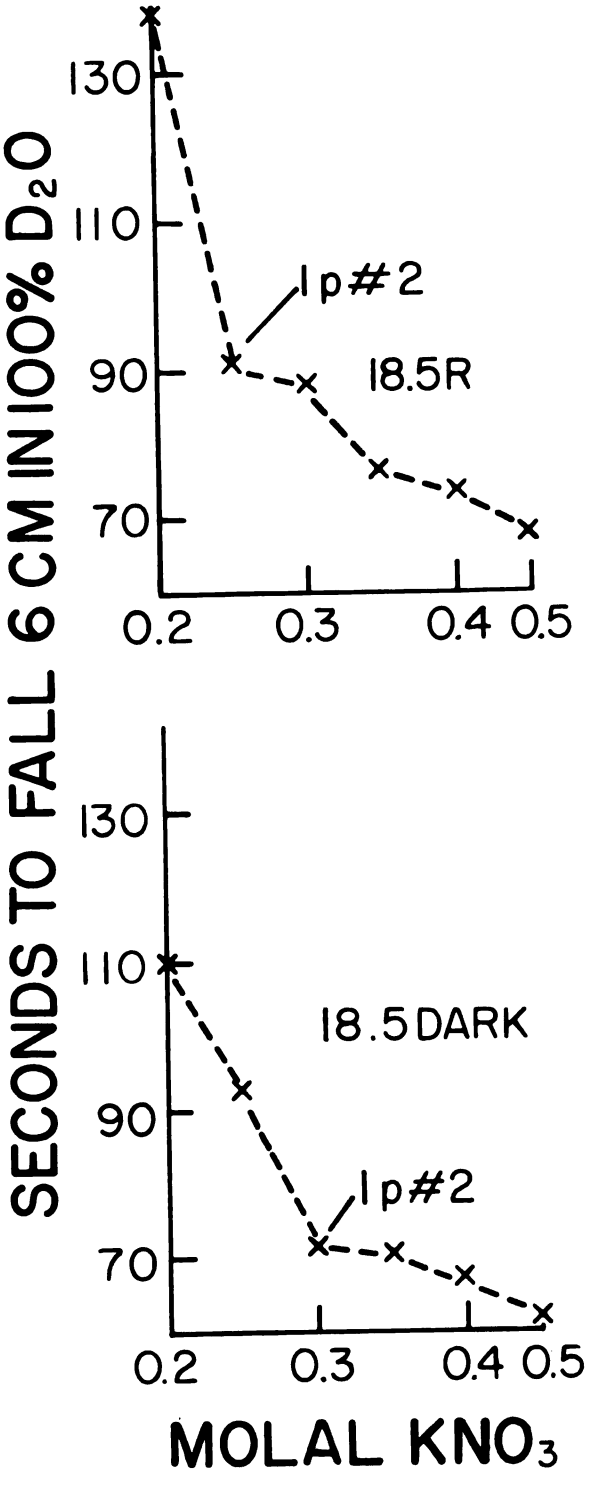

Figure 23. Examples of points of incipient plasmolysis determined by pre-stressing tissue in KNO_3, then measuring the length of time for the tissue to fall 6 cm in 100% D_2O. Sample size: 25 embryos, a 1mm section from the elongating zone of each.

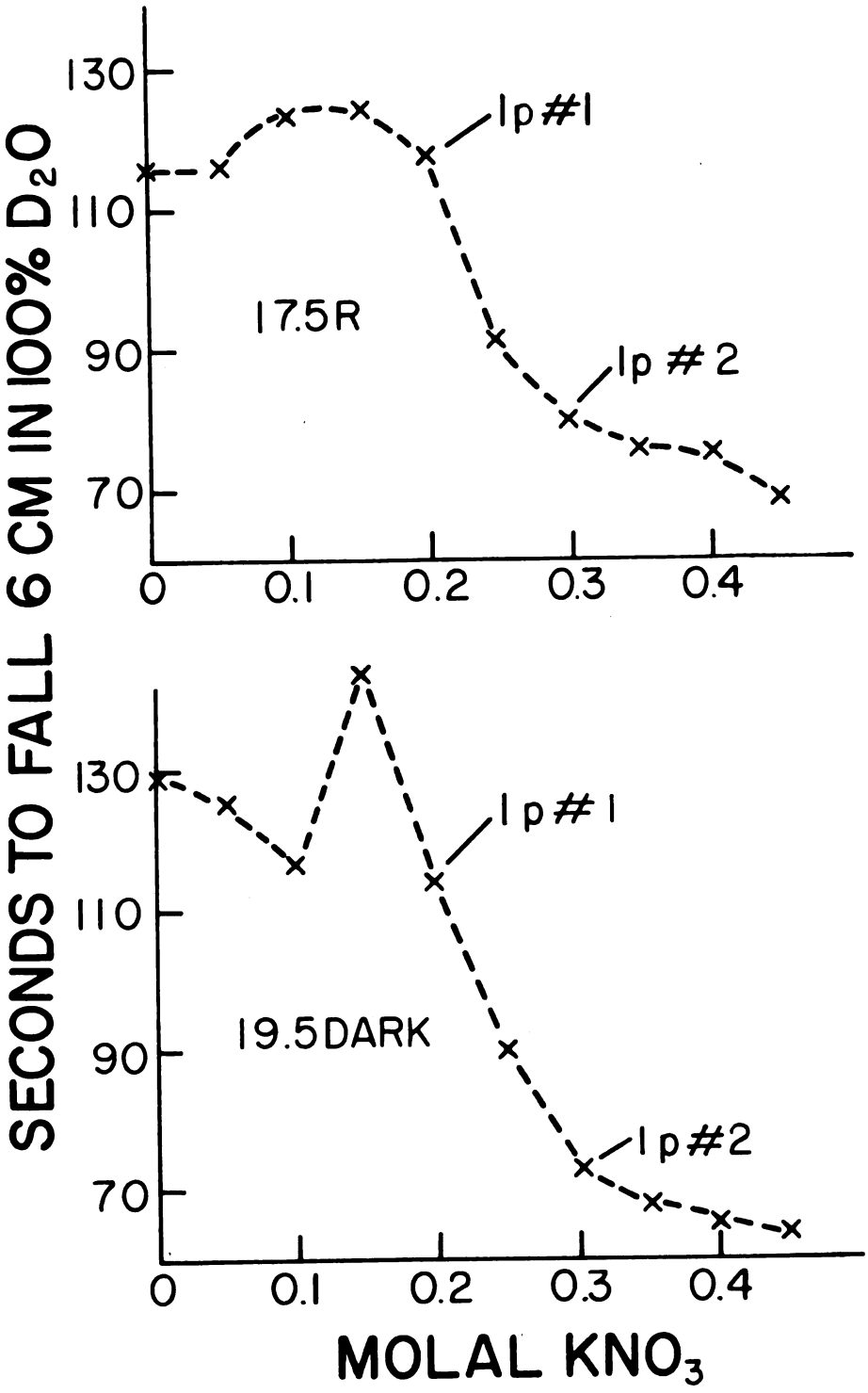

Figure 23 continued

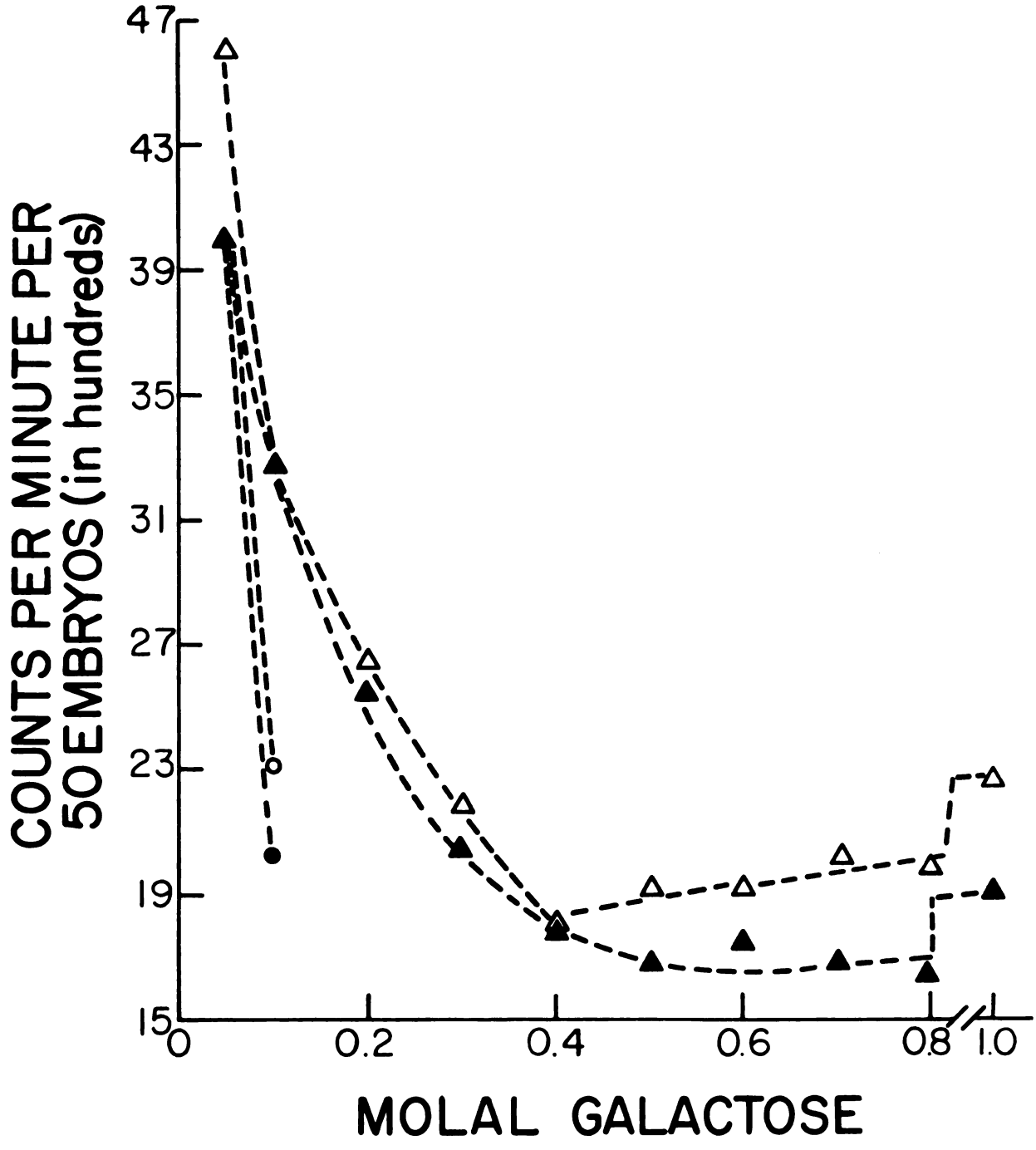

Figure 24. Uptake of carbon-labeled galactose by embryos 19.5 to 20.5 hours after R or dark treatment. △ R, ▲ dark, O and ● theoretical R and dark values based on isotope dilution of the 0.05 molal data and assuming that uptake is not related to concentration.

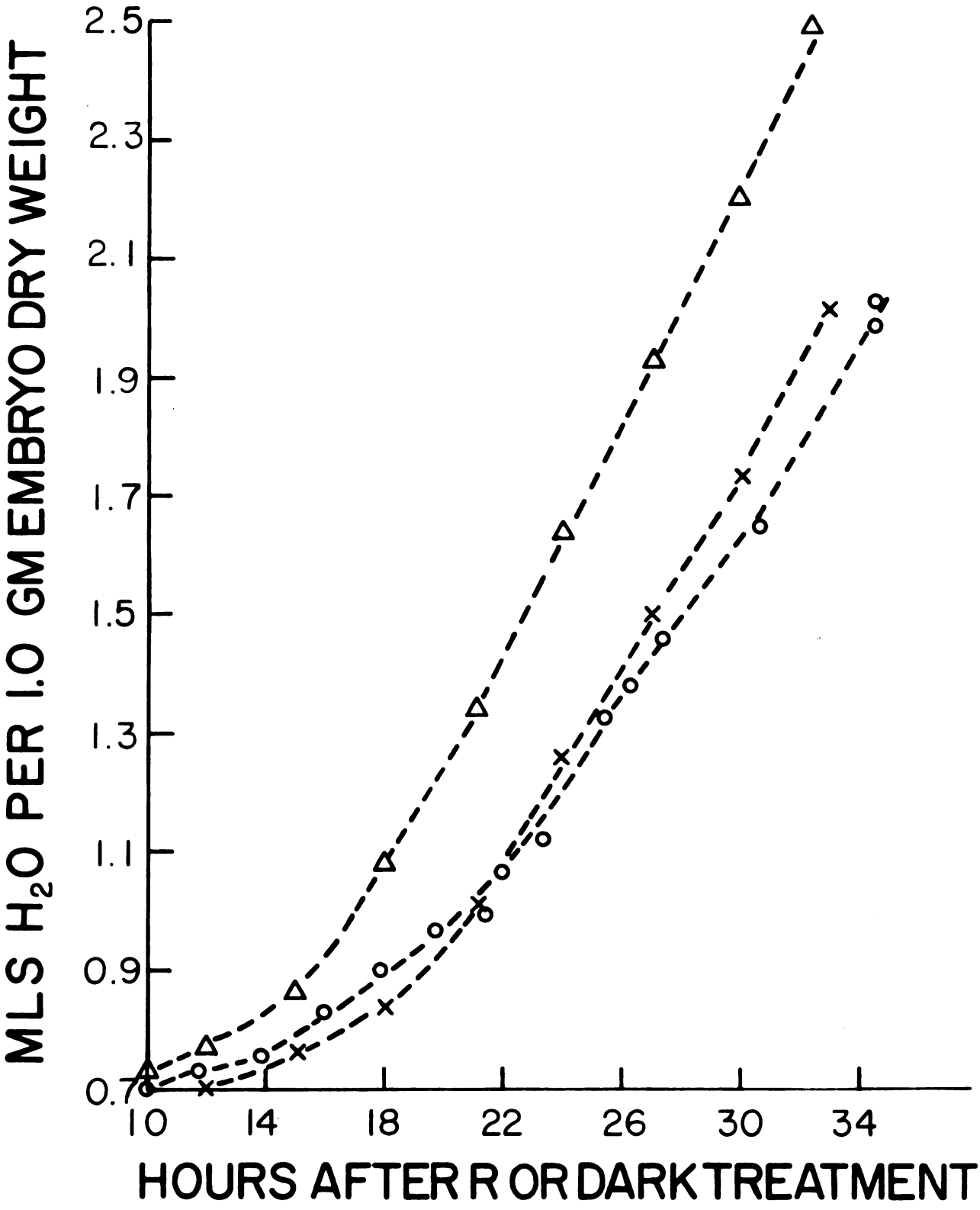

Figure 25. The water content of embryos and seeds germinating in water. x dark embryos, Δ R embryos, O R seeds. Sample size: 80 embryos or seeds.

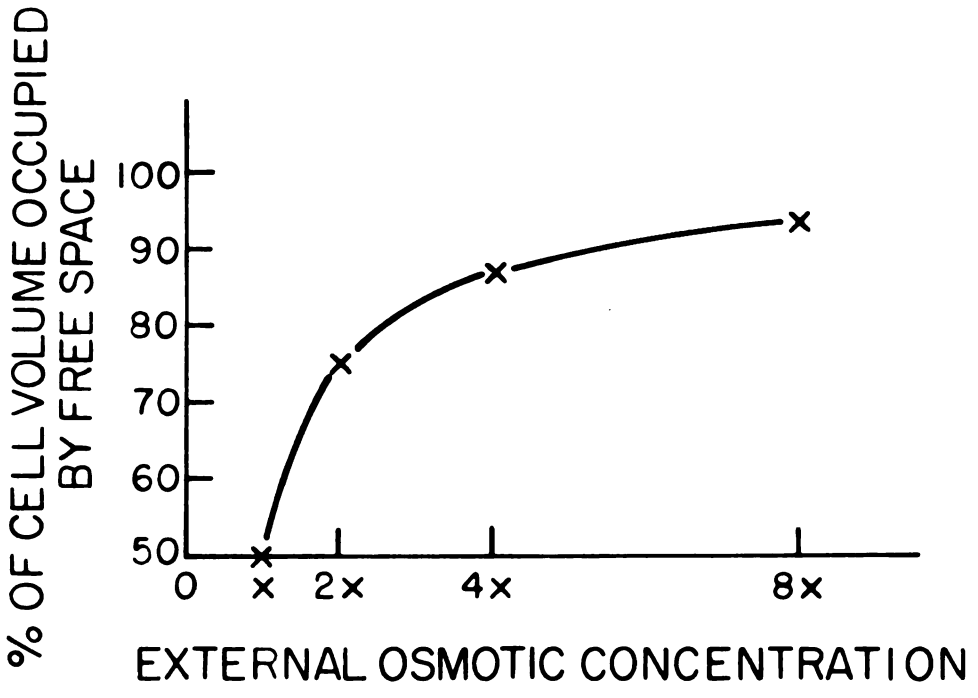

Figure 26. The variation in free space with external osmotic concentration after plasmolysis has occurred.

Assume a cubical cell. At incipient plasmolysis the cell contents become spherical. The ratio of free space to cell contents will be $1.33\pi R^3$ to $8R^3$ or about 0.5. Thereafter, as the external osmotic concentration doubles, volume of cell contents will be halved and the volume of free space will rise accordingly.

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