Plant Physiol. (1977) 59, 1111-1115

# Transient Changes During Soybean Imbibition[1]

Received for publication October 12, 1976 and in revised form February 8, 1977

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## ABSTRACT

**Air dry cotyledons of soybean (*Glycine max* Merr. var. Wayne) imbibe water rapidly for about 10 minutes followed by a slower, linear rate of uptake. Leakage of solutes out of the coytledon likewise shows an initial rapid period, followed by a slower, nearly linear rate after 5 to 10 minutes; both the rapid and the steady rate leakage are greater for initially drier seeds. Respiratory activity of cotyledons as measured by manometric techniques becomes apparent after about 10 minutes of imbibition while polarographic studies of ground particles suggest that $O_2$ comsumption begins almost immediately upon wetting. Initial wetting of the seed causes the release of adsorbed gases, and a series of changes in volume of the seed-water mixture are charted. The data are interpreted as indicating that extensive physical changes occur in the first few minutes of water entry, including a rearrangement of membranes changing them from a relatively porous to a less permeable condition, and a release of adsorbed gases which cause an inflation or swelling of the seed.**

The imbibition of water converts the seed from a quiescent body with very low respiratory rate into a dynamic organism, active in respiration, in biosynthesis, and capable of growth (11). Shull (24) found that imbibition by peas occurs at a fairly steady rate for 5 to 8 hr, at which time water uptake is complete. Simon (25) has pointed out that the dry seed should be expected to have porous membranes due to the absence of sufficient water to maintain a hydrophilic/hydrophobic orientation of the lipids in membranes, and he presented evidence of a rapid leakage of solutes out of pea seeds during the first 10 min of imbibition, presumably because of the poor structural arrangement of membranes.

The present experiments will attempt to describe some kinetic activities during imbibition as they may relate to membrane rearrangement and other physical changes during the initial wetting of the seed.

## MATERIALS AND METHODS

Certified 1975 seed of "Wayne" soybean (*Glycine max* Merr.) were used. Moisture content, determined by drying at 100 C for 24 hr, was 7 to 8%. Most experiments were done using individual cotyledons isolated from dry seed. To determine the time course of imbibition of water, air dry cotyledons were weighed, placed in distilled $H_2O$ for a time, removed, blotted, weighed, and returned to the distilled $H_2O$.

Leakage of ionic material from imbibing seeds was determined by conductivity studies. Six cotyledons (0.5 g) were placed in 10 ml of distilled $H_2O$ in a test tube which could accommodate the probe of an Electro Mark Analyzer (Markson Science, Inc., Del Mar, Calif.). The imbibing solution was stirred frequently. Leakage of material absorbing at 280 nm was determined by placing single cotyledons in 3-ml cuvettes in a Beckman DU spectrophotometer; the imbibing solution was stirred by frequent use of a medicine dropper to withdraw and return the water.

$O_2$ consumption by cotyledons or seed particles was measured at 25 C with a differential respirometer (Gilson Medical Electronics, Inc., Middleton, Wis.). Particles of various sizes were prepared by grinding dry seed in a Wiley mill and separating the ground material through a sieve series. The seed material (0.5 g of a single size range) was placed in the main chamber of 15-ml reaction flasks with 1 ml distilled $H_2O$ in the side arm. The manometer system was allowed to reach thermal equilibrium and either closed before or 1 to 2 min after tipping in the water from the side arm.

A polarographic technique was also used to determine the timing of the onset of respiration of ground particles during wetting. One tenth g of seed particles (0.5-1 mm in diameter) was added to distilled $H_2O$ in a test tube which just accommodated the probe of a Fieldlab oxygen analyzer (Beckman Instruments). The test tube which was partially immersed in a 25 C water bath contained a small magnetic stirring bar. Effective volume for the respiring tissue was about 1.1 ml. Background $O_2$ consumption (with no tissue in the system) was about 10 $\mu$l/hr.

A "dilatometer" employing Archimedes' Principle was made from a 1,000-ml round bottom flask fitted with a stopper through which was inserted the stem of a 10-ml burette. The volume of the flask when filled to the stopper was determined. Dry, whole seed or ground particles were weighed and placed in the flask, and the flask was rapidly filled with distilled $H_2O$ and stoppered. The volume of water added was determined for calculations of seed volume and density. The stoppering action raised the water into the calibrated portion of the burette so that changes in total volume of seed and water could be observed as fluctuations in the burette readings. The flask was shaken periodically to improve aeration within $O_2$ depleted layers.

## RESULTS

**Rapid Transient of Water Uptake.** To examine the kinetics of water uptake during the first stages of imbibition, cotyledons of soybean were placed in water, and then removed briefly for weighing at intervals (Fig. 1). Although absolute amounts of uptake varied from trial to trial, in each of more than 100 trials there was an initial, rapid rate of uptake which declined after about 10 min to a slower, linear rate. The constant rate of weight gain lasted at least 1 hr before slowing as the tissue approached saturation. The rapid inrush of water may be considered to be "uncontrolled" and the extent of such uptake can be estimated by extrapolating the linear part of the curve to the ordinate. The amount of uncontrolled uptake varies directly with the initial moisture content of the tissue (unpublished results).

**Loss of Intracellular Material During Imbibition.** Measurements of 280 nm absorbing material in the imbibing medium (Fig. 2) show that a decelerating phase of leakage occurred

[1] Published as Paper No. 5284, Journal Series, Nebraska Agricultural Experimental Station.

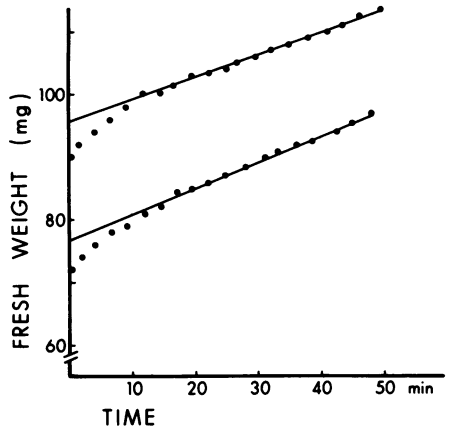


FIG. 1. Fresh weight gain of imbibing cotyledons. Each set of points represents a single cotyledon placed in distilled $H_2O$ (20 ± 1 C) at zero time.

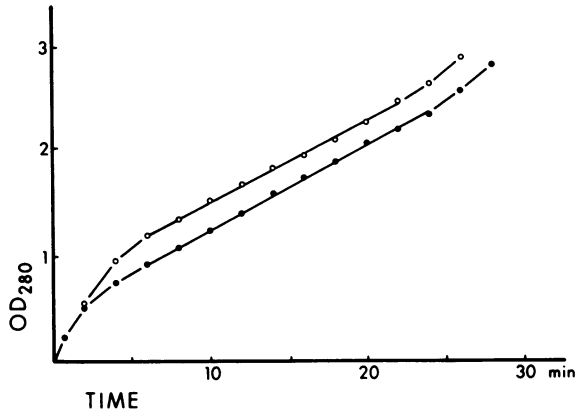


FIG. 2. Leakage of 280 nm-absorbing materials from imbibing cotyledons. Each set of points is data from a single cotyledon placed in the cuvette at zero time. Temperature of imbibition was regulated at 25 C.

during the first 4 to 5 min of imbibition, after which there was a linear rate of loss. Another means of measuring the kinetics of leakage from the seed is through the increases in electrical conductivity of the ambient solution (Fig. 3). There was a period of rapid efflux of solutes for the first 4 to 5 min of imbibition, after which an essentially linear efflux occurred. The upward inflections in leakage curves (Figs. 2 and 3) at about 20 min coincided with a fracturing of the cotyledons which resulted in increased surface area and release of inter- and intracellular materials.

If the interpretation is correct that there is a rearrangement of the membranes into effective barriers to water and solute passage, then the drier the seeds, the greater the extent of rearrangement that might be anticipated. To examine this possibility, seeds were placed over concentrated $H_2SO_4$ to lower the moisture content, or placed in a humid atmosphere to obtain a higher moisture content, and the kinetics of electrolyte leakage was examined (Fig. 4). Certain deductions can be made from these data. First, the slope of the steady rate of leakage varies with the initial amount of moisture in the seed, dry seeds showing a much greater rate of leakage than moist seeds. (This interpretation is perhaps confounded by the greater and more rapid fracturing of drier seed.) Second, if one estimates the amount of "uncontrolled" electrolyte loss by extrapolating the slope of the linear portion of the curve to the ordinate, one can deduce that the amount of leakage in the initial, rapid phase varies inversely with the amount of initial moisture in the seed. Similar experiments using $A$ at 280 nm to measure the leakage of solutes give identical results: the seeds with low initial moisture showed a steeper leakage curve, and a greater loss of solutes in the initial, rapid phase of leakage (data not shown).

**Respiration and Desorption Activities During Imbibition.** Assuming respiratory activity to be primarily a function of hydrated organized mitochondrial unit membranes, one might expect to find that during initial imbibition, normal respiratory activity was hindered. In order to examine this possibility, cotyledons were placed in a respirometer and the onset of respiration was measured following tipping in water. When cotyledons were measured in this manner (Fig. 5) there was a period of about 15 min in which respiration was minimal. Since we were interested in the initial response to wetting, it seemed reasonable to compare the respiratory activities of whole cotyledons and particles ground to various sizes where the initial wetting of the tissues might be more rapid. Results with particles of two sizes (Fig. 5) show that the smaller particles had a slightly more rapid initia-

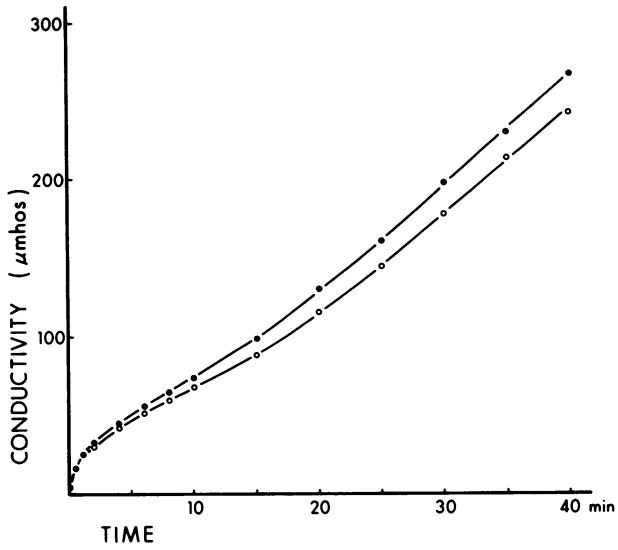


FIG. 3. Leakage of electrolytes from imbibing cotyledons. Six cotyledons (0.5 g) were placed in 10 ml distilled $H_2O$ (22 ± 1 C) at zero time.

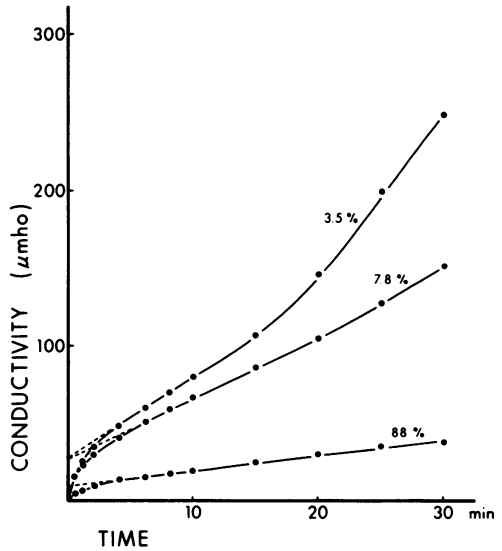


FIG. 4. Effect of seed moisture content on leakage of electrolytes. Sets of six cotyledons of various moisture levels were placed in 10 ml distilled $H_2O$ (22 ± 1 C) at zero time. Moisture content indicated for each set was determined by drying identically pretreated seed.

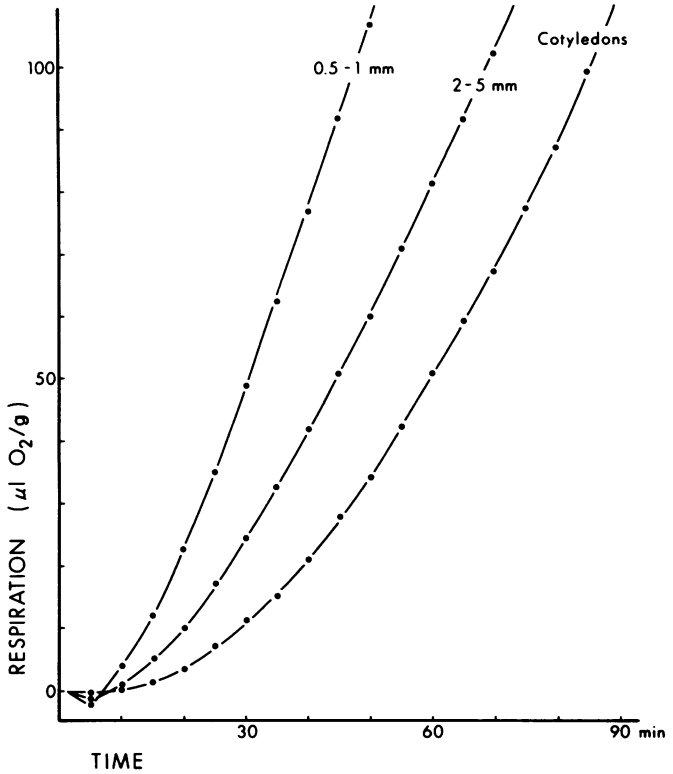


FIG. 5. Respiratory time course of imbibing cotyledons and two sizes of ground seed particles. Water was tipped onto the cotyledons or particles at −2 min. The respirometer system was closed at zero time and readings were taken every 5 min. Six cotyledons (0.5 g) or 0.5 g of particles (0.5–1 mm or 2–5 mm in diameter) were used in each flask. Each data point is the mean of three replicates.

tion of respiratory activity, starting at between 5 and 10 min after the onset of imbibition and accelerating for about 30 min until linearity was reached.

The data for Figure 5 were obtained by closing the respirometer system 2 min after the water had been tipped in, since it was found that adding water to dry seeds or particles after the manometer system had been closed resulted in increases in the volume of the flask contents (*e.g.* Fig. 6). Such a sudden volume increase was reported by Haber and Brassington (5), who interpreted it as being a release of adsorbed gases from the seed as it was wetted. Experiments done with 1 mM KCN as the imbibing solution (Fig. 6) showed a lesser rate of $O_2$ consumption, which suggests that the gas exchange occurring after 10 or 20 min was at least partially due to metal-containing oxidases. In order to get a more precise look at the onset of respiration, $O_2$ consumption by particles in water or 1 mM KCN was measured in an $O_2$ electrode (Fig. 7). The results indicate that $O_2$ utilization during the first minutes of wetting was much higher than had been indicated by respirometry, even in later stages of imbibition. The data also indicate that the initial $O_2$ uptake is insensitive to KCN.

**Volume and Density Changes During Imbibition of Whole Seed.** Our experience with the abrupt changes in respirometer volumes during initial imbibition led us to examine over-all volume changes during imbibition. This was done by completely filling a flask with water and seeds, immediately stoppering the flask, and following the subsequent changes in total volume of seeds plus water (Fig. 8). The volume increased for about 5 min followed by a reduction in the total volume; after about 20 min, the total volume of the seeds plus water began to climb steadily for several hours. During the course of the experiment, a small amount (about 2 $cm^3$/100 g of seed) of gas collected in the dilatometer and was drawn off with a syringe. It is not known whether this represents air exuded from the seeds or bubbles trapped on their surfaces; so it is not included in the volume changes. Even so, it is clear that the seed is increasing in volume more than can be accounted for by the entry of water; measurements of the pre- and postimbibed seeds show that the density decreases from 1.23 g $cm^{-3}$ in the dry state to 1.06 g $cm^{-3}$ after 5 hr imbibition, during which time fresh weight has increased by 103%. Assuming a density of 1 g $cm^{-3}$ for water, the postimbibition density should be about 1.1 g $cm^{-3}$.

## DISCUSSION

While most nondormant seed studies deal with processes occurring relatively late in germination, the experiments reported here bear on some of the very earliest germination events, those associated with imbibition. The data suggest that several very significant physical and biochemical processes are taking place in the first few minutes of water uptake. There is an inrush of water accompanied by a rapid loss of solutes from the cells and a

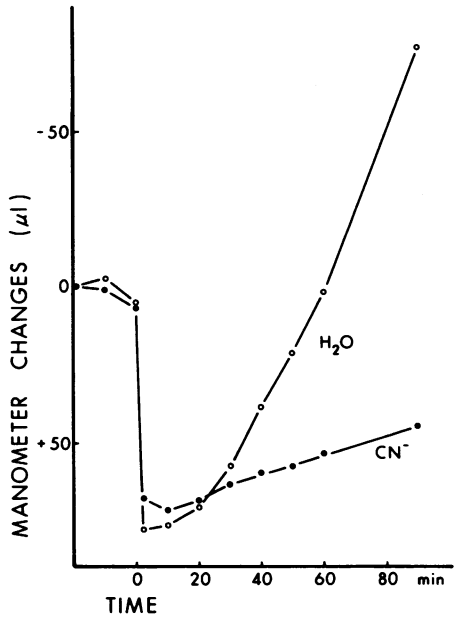


FIG. 6. Effect of desorbed gases and cyanide on respirometer measurements. Six cotyledons (0.5 g) were placed in each flask and the respirometer system was closed 20 min before tipping in distilled $H_2O$ or 1 mM KCN (at zero time).

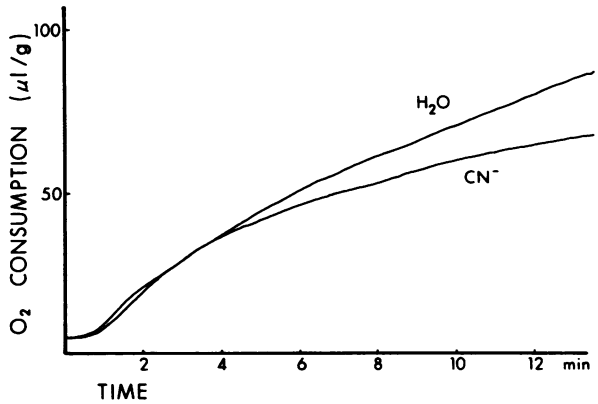


FIG. 7. Polarographic determination of respiratory onset in distilled $H_2O$ and 1 mM KCN. Seed particles (0.1 g, 0.5–1 mm in diameter) were added and the electrode probe inserted at zero time. Thermal equilibration of the probe requires about 0.5 to 1 min.

release of adsorbed gases. The cell membranes reorganize from a presumably porous condition (25) into effective semipermeable barriers and functional units. Respiration begins with a very short lag time and declines from an initially high rate as hydration proceeds. There is also associated with imbibitional wetting a trapping of gases within the seed which may alter subsequent water uptake. Each of these events will be discussed briefly and some of their implications for ultimate seed vigor examined.

Previous authors have reported on various components of this set of experiments. Hallam *et al.* (7) observed a rapid uptake of water in rye embryos for the first 10 min; Simon (25) noted the rapid leakage of solutes from pea seeds during an initial 10-min period, and Hendricks and Taylorson (9) have noted the same phenomenon in seeds of several species. Eyster (3) reported that raising the moisture content of seeds could protect them from the damage associated with rapid water imbibition, and the role of initial moisture content has since been noted also by other authors (16, 18, 20, 23, 26). Pollock (21) showed that seeds which started imbibition at higher moisture levels leaked less and produced more vigorous seedlings.

If hydration of seed tissue were simply a physical wetting and osmotic uptake phenomenon, one might predict that the rate of fresh weight gain would be initially high and would then show a continuous exponential decline until the seed becomes saturated. Experience has shown, however, that uptake of water is marked by an initial, rapid inrush which decelerates to an essentially linear rate by about 10 min and continues at the steady rate until equilibrium is approached. The seemingly steady-state nature of the process raises the question of whether hydration might be under some mode of biological control. However, Uhivits (27) and Hallam (6) have presented evidence that water permeates live and dead seed identically during the initial stages of wetting. We suggest the following entirely physical explanation for the observed linear nature of imbibitional fresh weight gain.

Dry seed tissue (5) as well as dry nonbiological materials (12) contain adsorbed atmospheric gases. The adsorbed molecules have a reduced volume as a consequence of their association with the solid phase. When displaced by water, the gases return to their original volume or a volume dictated by the pressure of the system into which they are released. When a dry seed or cotyledon is submerged in water, the free atmospheric gases and adsorbed gases will be trapped within the tissue. As the wetting front moves inward, gases are desorbed and expand, causing the gas phase to become essentially a pressurized bubble which might resist the inward movement of water. As in a capillary, where the rise of water is offset by gravity, the cohesiveness of water at the water-gas interface would counteract the adhesive wetting. As the pressure increases within the trapped bubble, the solubilization of the gases will increase and they will eventually escape; but this process will presumably take place slowly and become in essence the rate-limiting step for a linear rate of imbibition.

Direct evidence of a significant and measurable desorption process during wetting comes from the literature (5, 12) and Figure 6. Indirect evidence for the desorption-backpressure hypothesis comes from the following observations. Cotyledons which have been humidified in a moist atmosphere take up about one-third more water in the first 10 min of submersion than do nonhumidified cotyledons, but their subsequent linear uptake rates are essentially equal. From manometric studies, we know that dry seed placed in a humid atmosphere cause the pressure/volume of gases within the system to increase (*e.g.* Fig. 6). These two observations taken together suggest that the quantity of adsorbed gases is reduced in humidified seed and a backpressure resistance may develop only after greater hydration.

The increase in total volume of the imbibing system (seed plus water) during imbibition is surprising, since the wetting of polyelectyrolytes should result in a combined volume decrease (8). The volume increases occur only with whole seed, not with ground seed particles, and seed with a higher initial moisture content exhibit a smaller effect. We suggest that the swelling itself and the increased swelling of drier seed are manifestations of the desorption backpressure process: the internal bubble causing the seed to "balloon."

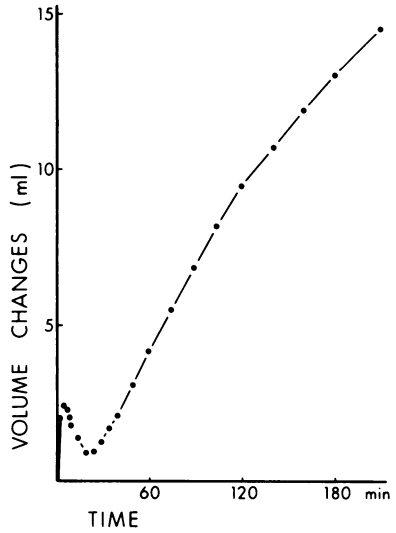


FIG. 8. Total volume changes in an imbibing system. Dry seed (300 g, 245 ml) plus 885 ml distilled $H_2O$ were combined in the dilatometer (see text) and total volume changes recorded at intervals.

The density of many seeds actually drops below unity during imbibition and they float. It is possible that this ballooning effect seen during imbibition could contribute to the damage commonly experienced by rapidly imbibed seed such as the cracking of cotyledons and embryonic axes (4, 13, 22). (Mazurak [12] has shown that wetting of dry soil particles causes an explosive desorption of gases leading to disintegration of the soil aggregates.) It seems particularly significant that humidifying seed increases the amount of initial water inrush but decreases the amount of cotyledonary cracking as well as vigor losses normally associated with rapid imbibition (3, 16, 21, unpublished observations).

The assumption made in the leakage studies is that we are measuring responses of a relatively synchronous and uniform population of cells. Since wetting of the innermost cotyledonary tissue occurs well after the outer layer (28), rapid leakage of intracellular materials should be occurring continuously until all cells are wet. However, as wetting proceeds inward from the surface of the cotyledons, the diffusion pathway becomes longer (25), swelling of protoplasts may be presumed to increase diffusive resistance, and net inward movement of water will be against the leakage diffusion gradient. This combination of factors suggests that diffusion of materials out of cotyledons into the imbibing solution will be most rapid for the outermost cells but drastically reduced in underlying tissue. We feel that inflections in the conductivity/optical density curves reflect changes in the leakage of intracellular contents mainly from the outer cells. The intracellular origin of electrolytes and of 280 nm absorbing material is suggested by the fact that cotyledons which have been washed and redried produce identical leakage patterns (unpublished data and 25), and that cotyledons with high moisture content but previously unexposed to free water leak smaller amounts (Fig. 4).

While respirometer studies indicated that respiration begins after a 10- to 15-min lag period and then accelerates (Fig. 5 and refs. 1, 2, 19, 29), polarography (Fig. 7) shows that $O_2$ consumption by ground particles in water begins almost immediately

and declines with time. Nygaard (17) used an $O_2$ electrode to study pine pollen and found that respiration (and phosphorylation) began within 1 min of wetting, and that the rate was linear after about 4 min. There are at least two possible explanations for the discrepancies in results obtained from the two techniques. In light of our findings and those of Haber and Brassington (5), the desorption of gases during initial imbibition obscures $O_2$ consumption. Unpublished studies with 1 M $CN^-$ indicated that gas desorption may go on for some time after the initial burst of desorption; so the manometer readings will be lower than actual $O_2$ consumption until desorption is complete. Another complicating factor in both respirometry and polarography is the variable speed at which the tissue is wetted. The smaller the particle the more rapidly it will become fully hydrated and begin respiring at its full potential. Figure 5 shows the relationship graphically. A semilog plot of particle volume against time required to attain linearity of apparent respiratory rates (Fig. 9) extrapolates to zero time at a particle volume of about $10^{-3}$ $mm^3$. These values suggest that wetting of cell-sized particles may lead to nearly instantaneous $O_2$ consumption.

Nawa and Asahi (14, 15) have produced data with pea cotyledons and isolated mitochondria which suggest that dry mitochondria are functionally "immature" and gain respiratory competency only after several hours. We cannot explain the differences between their $Q_{O_2}$ values of isolated mitochondria and ours for seed particles unless peas are radically different from soybeans, or their isolation procedure altered the normal course of respiratory onset, or the $O_2$ consumption we measured was not of mitochondrial origin.

In light of their partial $CN^-$ insensitivity (Figure 6 and 7 and ref. 30), it is probable that some oxidases other than Cyt oxidase are consuming $O_2$ during early stages of wetting. Levitt (10) suggests that some protective role may be assigned to $O_2$ scavenger enzymes such as peroxidases and superoxide dismutase. Yentur and Leopold (30) have shown that a cyanide-insensitive "alternate" respiration occurred during the initial wetting of soybean, but faded out at the time imbibition was completed.

Collectively, these experiments indicate that dynamic changes in the first few minutes of water imbibition may be related to the relatively poor functioning of membranes. The existence of faulty membranes combined with a release of adsorbed gases from seed components may well be factors limiting the physiological effectiveness of the seed in terms of viability and subsequent vigor.

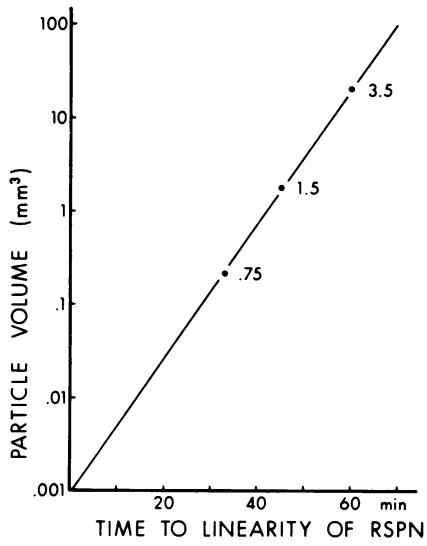


FIG. 9. Relationship of particle size to time of linearity for apparent respiration. Seed particles with an average diameter of 0.75, 1.5, or 3.5 mm were placed in a respirometer and wetted at zero time. The approximate times to attain a linear rate of respiration for each particle size were 33, 45, and 60 min, respectively (average of three repetitions). Numbers at data points are mean diameters (assumed to be average between largest and smallest particles).

**Note Added in Proof.** Since sending this manuscript to the printers, we have discovered that much of the manometric volume change seen when $H_2O$ is tipped onto seeds is due to an increase in $H_2O$ vapor. We believe that the dry seed material holds the $H_2O$ vapor pressure in its vicinity below saturation. When $H_2O$ is tipped into the main chamber, $H_2O$ vapor as well as desorbed gases increase the volume of gases.

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