SUMMARY

The distribution of two quinones with coenzyme Q activity which are broadly distributed in higher plants has been determined. Particulate fractions from spinach leaves, the white and green portions of Pandanus leaves, and the roots and shoots of corn seedlings have been examined with respect to the amount of coenzyme Q and Q_{254} present. Coenzyme Q is found in both white and green tissue and appears to be concentrated in mitochondria, whereas Q_{254} is concentrated in chloroplasts and chloroplast-containing tissue. It is proposed that coenzyme Q is involved in mitochondrial electron transport and that Q_{254} may be involved in photosynthetic electron transport.

I wish to thank Dr. D. E. Green for his encouragement of this investigation, Dr. Robert L. Lester for paper chromatography of the quinones, and Mrs. Wanda Fechner for her excellent technical assistance. *Pandanus vechii* was kindly supplied by Dr. R. H. Roberts of the Department of Horticulture, University of Wisconsin.

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STUDIES OF THE REST PERIOD. I. GROWTH, TRANSLOCATION, AND RESPIRATORY CHANGES IN THE EMBRYONIC ORGANS OF THE AFTER-RIPENING CHERRY SEED [1,2]

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Rest or dormant periods are common in the plant kingdom, and have been widely studied in such organs as buds, seeds, tubers, and corms. The seed provides particularly favorable material for investigating the rest period since it may, to a considerable degree, be considered a closed system, complete with its store of organic materials, minerals, and growth factors, and depending on the external environment only for water, oxygen, and favorable temperature and light conditions. The dry seed contains an embryonic plant in an arrested condition; germination may be considered to be the resumption of growth. Toole et al (18) and Evenari (4) have pointed out that germination requires the coupling of respiratory energy to growth, but that little is known concerning the actual mechanisms of germination.

While the seed may be considered to be a closed

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system, it cannot be treated as a homogeneous system since it is structurally complex with physiological specialization closely associated with this structural complexity. Generally speaking, the potentiality for active synthesis and growth is restricted to the embryonic axis, while the cotyledons and/or endosperm are primarily concerned with the storage and subsequent mobilization of reserve materials. Since a seed is heterogeneous and complex, investigations of the germination process must treat the seed in its complexity and study the physiology of the individual organs and their interrelations. Such an approach was suggested by Sherman (15) but has received relatively little experimental treatment.

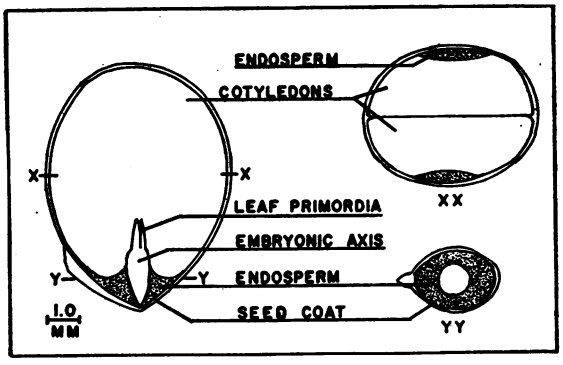
Most dry seeds contain all essential elements for germination except water. However, in many seeds the simple uptake of water will not permit germination because the growth of the embryo is blocked by the presence of a resting or dormant condition (see (2) and (13) for discussion of terminology). Crocker (3) described 6 classes of blocked growth which are obviously associated with blocks at different stages of germination and in different parts of the seed. It is clear in Crocker's classification that mechanical restriction of transport or expansion is relatively simple compared with "embryo dormancy" and "secondary dormancy." These latter, while classified by Crocker as distinct types, probably are identical (1, 6, 17, 20) and are closely similar to, if not identical with, the rest period of buds of trees (1, 6). We will refer to the result of any block to growth which resides within the embryo as the rest period.

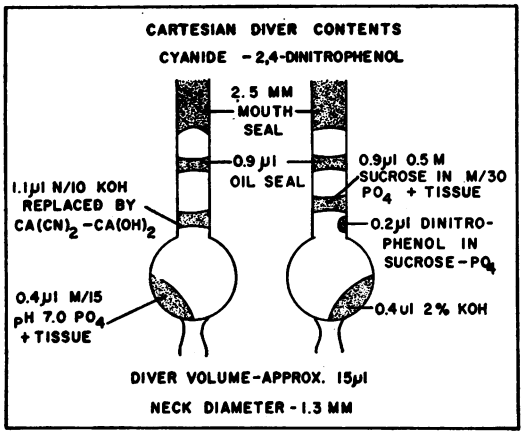
The rest period is frequently studied because of its importance in agriculture and forestry. However, rest is also of great theoretical importance because it results from a block to growth within the growing cells; understanding the nature of this block could contribute directly to the understanding of the factors which control cellular growth and development. However, it is much easier to define the rest period in writing than it is to measure it in the laboratory. For example, germination is generally measured as the emergence of the primary root from the seed coat. However the question arises as to whether growth by cell division or enlargement prior to rupture of the seed coat is part of germination. To avoid this difficulty Toole et al (18) defined germination as "the resumption of growth" and Evenari (4) considers germination to be those processes preceding the resumption of growth. The rest period may be defined as a blocking of germination, but we must recognize that this is not an adequate working definition for at least 2 reasons: 1) The block to growth may not be complete, thus some growth may continue during the rest period as in the embryos described in this paper; and 2) the rest period can only be defined in terms of failure to grow. In the laboratory, it is normal practice to remove the block to growth by after-ripening at low temperatures and then to test for growth by placing the material at higher temperatures. There is, however, ample evidence (1) that these higher temperatures themselves can reverse the effects of low temperature and tend to re-establish the rest period; according to Crocker's terminology (3), secondary dormancy is established. Thus it is essential to have a measure of the actual growth potential of the system which at least minimizes this reversing effect. It is for this reason that recent workers such as Abbott (1) and Visser (20) use a more comprehensive measure of "germination capacity" rather than germination, and a similar procedure is followed in the work presented in this paper.

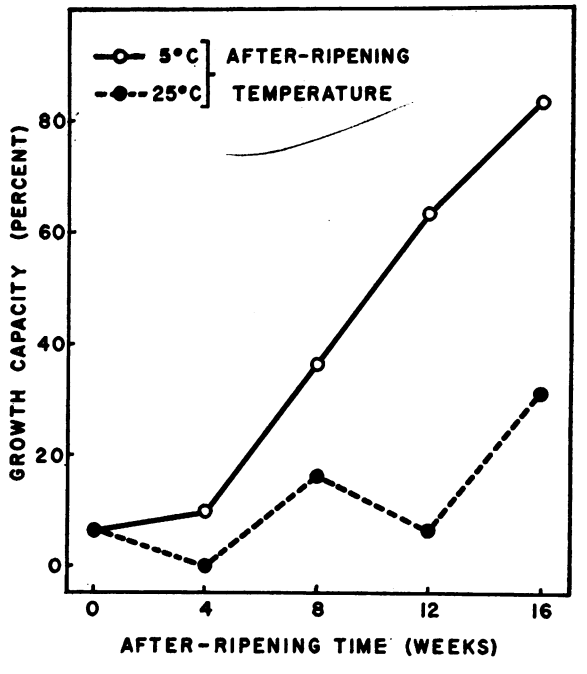
In the experiments reported in this paper the cherry seed was chosen as experimental material since it contains an embryonic axis with a well developed root and shoot, has a definite rest period (8, 15, 19), and is readily available. In early experiments, we found that low-temperature after-ripening increases the respiration rate of cherry seeds. We then investigated the relative effect of low temperature on the various seed organs and found that the respiration of the parts of the embryonic axis increases much more than the whole seed. Immediately following this respiratory stimulation is growth by cell division and probably also cell enlargement, together with changes in dry weight due to translocation and utilization of nutrients. Because these changes tend to complicate the interpretation of the respiratory data by changing the basis for expression (respiration per unit dry weight, cell, or organ) and because few data of this type are available in the literature (18), we have included a rather detailed study of this type of change. We have also analyzed the respiratory increases, making use of 2,4-dinitrophenol (DNP) to permit determination both of respiratory rate and respiratory capacity as measured by the maximum respiration rate obtained in the presence of DNP. The results of these experiments suggest that the initial effect of low-temperature after-ripening is to increase the efficiency with which respiratory enzyme systems operate, thus providing a greater supply of available energy to the embryo and triggering a complex of reactions leading eventually to active growth.

Materials and Methods

Plant Material: Seeds of the sour cherry, *Prunus cerasus* L. var. Montmorency, from the 1957 crop of the University of Delaware farm were used for the bulk of the work reported here, while seeds from the 1954, 1955, and 1956 crops were used for preliminary experiments. No difference was noted between these several crops. The fruits were pitted immediately after harvest and the pits were thoroughly washed and allowed to air-dry in a single layer at room temperature. They were stored at room temperature until needed, at which time they were cracked. The undamaged seeds were sterilized by immersing for 10 minutes in a filtered suspension of 5% Pittchlor (Columbia-Southern Chemical Company) in water. After-ripening was carried out on







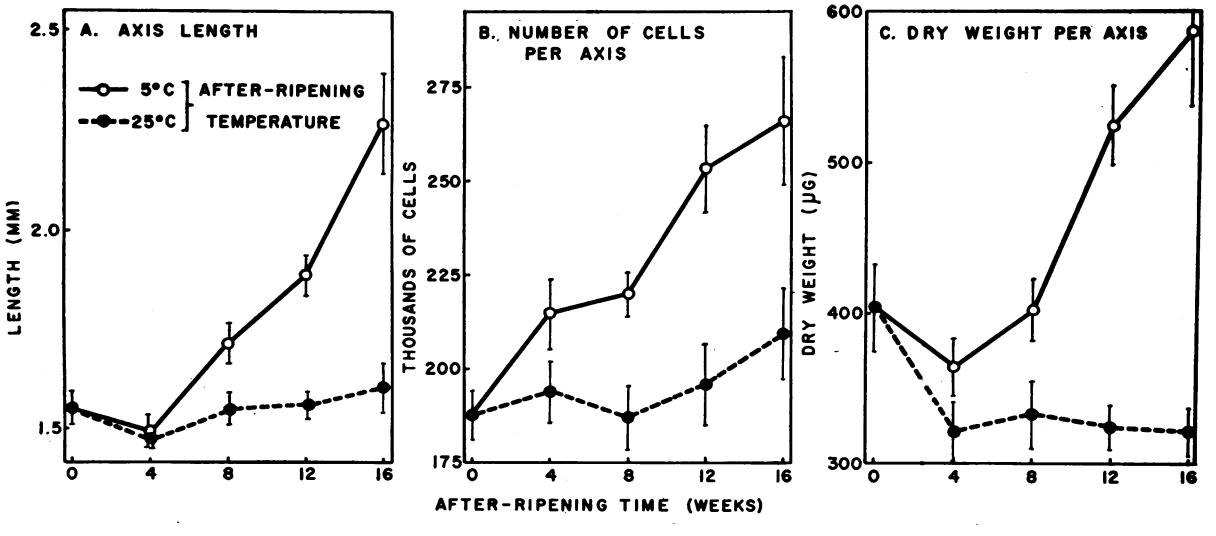


FIG. 1 (*upper left*). A diagram of the structure of the cherry seed.

FIG. 2. (*center left*). Cartesian diver contents in experiments with 2,4-dinitrophenol and cyanide.

FIG. 3 (*upper right*). Growth capacity of cherry embryos as a function of time and after-ripening temperature. Growth capacity is based on relative growth rates and stage of development reached by excised embryos during a 2-week period at 25° C.

FIG. 4 (*bottom*). The effect of after-ripening time and temperature on growth and dry weight in the embryonic axis of cherry seeds. The vertical lines represent ± the standard error of the mean.

sterile moist sand, with water added as needed to keep the seeds moist but not submerged. For most purposes, approximately 40 seeds were after-ripened in a 125 ml Erlenmeyer flask. However, there occasionally seems to be an interaction between seeds in such a flask when 1 seed germinates and stimulates germination of several surrounding seeds. To avoid this complication, most of the data used in this paper were obtained from seeds after-ripened individually on sand in 13 × 120 mm test tubes. After-ripening was carried out in the dark at 5° C and at 25° C; any contaminated or structurally abnormal seeds were discarded.

Figure 1 shows the structure of the cherry seed. Germination in this seed is epigeal; the embryonic leaf primordia become the 1st true leaves of the seedling. Dissection, carried out with the seed submerged in water and observed with a 10 × dissecting microscope, involved separating the cotyledons from the embryonic axis by diagonal cuts at the cotyledonary node. The leaf primorida were removed by cutting at the point of petiole attachment. Thus the portion of the embryo referred to as "embryonic axis" includes the root primordium, hypocotyl, and shoot apex.

Germination and growth capacity were measured as part of a study on growth rates according to the following procedure: 500 ml tall form beakers were lined with germination blotting paper, partially filled with washed sand moistened with water, covered with a Petri dish half, and sterilized. Seeds, 15 per beaker, were inserted between the blotting paper and glass in such a position that they could be observed and measured with a horizontal microscope. All tests were performed at 25° C in the dark except for brief periods of observation under room light. Germination was measured by placing whole seeds in the beakers; a seed was considered to have germinated if the root emerged from the seed coat within 2 weeks. However, in closely related seeds, Abbott (1) and Visser (20) have shown that growth in embryos excised from the seed coats is a better measure of germination ability than that described above for whole seeds. Since we were also interested in obtaining visual observations on changes in the leaf primordia during germination, seed coats were excised, 1 cotyledon was removed, and the embryonic axis with 1 cotyledon was placed in the beaker with the axis toward the glass for observation and measurement. As a measure of growth capacity, the seeds were divided into 4 classes according to the changes occurring within a 2 week period:

CLASS 1: Embryos not growing: Leaf primordia and axis with little or no elongation. Color of embryo mostly white with occasionally yellow areas.

CLASS 2: Slight growth: Leaf primordia enlarging and turning yellow. Some thickening and yellowing of the axis. Cotyledons white with occasional yellow spots.

CLASS 3: Weak growth: Leaf primordia enlarging and turning yellow. Considerable elongation in the axis with the yellowing of the root tip. Partial yellowing of the cotyledons.

CLASS 4: Active growth: Rapid elongation and yellowing of the leaf primordia and axis, together with yellowing of the cotyledons.

These classes were assigned the values 0, 1/3, 2/3, and 1 respectively. Then the number of embryos in each class multiplied by the class value, divided by the number of embryos tested (20 to 30), and expressed as percent, was taken as the growth capacity of the material. The results of this experiment are shown in figure 3.

ANALYTICAL METHODS: If we examine a cherry seed, we find that the amount of experimental material available in the embryonic axis and leaf primordia is only a very small part of that in the whole seed. For example, an average non-after-ripened seed has a dry weight of 56.5 mg and contains an embryonic axis with a dry weight of 405 μg and 2 leaf primordia each with a dry weight of about 12 μg. Thus in comparing changes in leaf primordia with changes in whole seeds, we are dealing with materials differing in size over a 1000-fold range. Since only a limited number of seeds can be dissected within a practical period of time, it has been necessary to use a range of methods, from the "conventional" to the "ultramicro."

Several measurements were made to compare the size of the seed parts and to serve as a basis for expressing other data. Linear measurements on the leaf primordia and embryonic axis were made with an ocular micrometer in a 10 × dissecting microscope. Dry weights were obtained on these same organs using "fishpole" type quartz fiber balances (12). Several balances were used; the size of the balances was chosen to give dry weights of the organs accurate to at least 1 %. Dry weights of whole seeds were obtained with a Roller-Smith balance sensitive to 0.02 mg. Cell counts were obtained using the maceration technique described by Sunderland and Brown (16). We have also studied changes in total nitrogen and phosphorus; these data will be presented in another paper.

During the course of these investigations we attempted to obtain the volume of the embryonic leaf primordia and axis by the method of Lϕvtrup (11). Briefly, this method involves measuring the reduced weight of an organism with the Cartesian diver balance (10) and the density from the level at which the organism comes to rest in a physiologically-inert density gradient. From these 2 measurements, the volume of the organism can be calculated. While the reduced weight measurement offered no problem, density measurement proved difficult. The axis and leaf primordia are relatively dense, 1.06 to 1.08, in comparison with the amoeba, density 1.02, for which the method was originally developed. The solutions available for these densities were so viscous that it was difficult to find a definite rest point in the gradient. We used a high molecular weight dextran preparation (kindly supplied by the Commercial Solvents

Corporation through Dr. F. Kavanagh), but even this preparation was quite viscous at these densities. Nevertheless, this method may in the future provide a convenient and accurate way of measuring the volume of meristematic plant tissues which have complex shapes.

Respiration, measured as oxygen uptake, was determined by 3 methods. In each case, the gas atmosphere was air and a temperature of 25° C was used. The rates were essentially constant for the period of 4 to 6 hours during which measurements were made. For whole seeds, standard Warburg techniques were used. Groups of 10 to 20 seeds were placed on moist filter paper in the bottom of a 20 ml vessel, and measurements were made without shaking. For the embryonic axis, the differential volumeter described by Grunbaum et al (7) was employed. The particular instruments used were made with capillaries of 0.8 to 1.0 μl/cm volume and approximately 1 ml flasks. The axes were placed, 5 in a flask, on moistened filter paper disks.

Respiration of leaf primordia was measured using standard Cartesian diver techniques ((9) and other papers from the Carlsberg Laboratory). Figure 2 illustrates the filling schedule for the various experiments. All solutions were prepared using water redistilled from an all-glass (Pyrex) still. The oxygen consumption per diver was kept at a minimum of about 10×10^{-3} μl/hr by using pairs of leaf primordia from non-after-ripened seeds and from seeds after-ripened at 25° C. With seeds after-ripened at 5° C a single primordium was placed in a diver. In most cases, this resulted in an oxygen uptake below 80×10^{-3} μl/hr/diver although a few ran as high as 140×10^{-3} μl/hr/diver. However, empirical measurements showed that even with these high rates, diffusion of oxygen to the primordium and absorption of carbon dioxide were not limiting.

In experiments with 2,4-dinitrophenol (DNP), the normal respiration rate was measured for a period of 2 to 3 hours before the seal containing the leaf primordium was mixed with a side drop containing DNP. The rate of oxygen uptake in the presence of DNP was allowed to stabilize for about 1 hour and the linear rate was then measured during the period 1 to 3 hours after mixing. Early experiments with DNP indicated that respiratory rates during these rather long experiments were stabilized somewhat if the primordia were suspended in 0.5 M sucrose in M/30 phosphate buffer pH 5.3. Other than this slight stabilizing effect, the sucrose used in this series of experiments seemed to have no influence on respiratory rates.

For cyanide inhibition measurements, the $Ca(CN)_2$–$Ca(OH)_2$ method of Robbie (14) was used, with the HCN concentrations calculated as for the Warburg apparatus. Two measurements were made for each primordium. First a normal rate was determined during a 2 to 3 hour period by absorbing the CO_2 in a seal of 0.1 *N* KOH. This seal was then replaced with the appropriate $Ca(CN)_2$–$Ca(OH)_2$ mixture and the linear rate of oxygen uptake was determined for a period of 2 to 3 hours after the initial exposure to HCN. Although in the Cartesian diver the $Ca(CN)_2$–$Ca(OH)_2$ mixture is not as effective in CO_2 absorption as KOH, probably because of the formation of a surface film of $Ca(CO_3)_2$, empirical measurements showed that the rate of CO_2 absorption did not become limiting.

The sampling procedure followed in these experiments involved analyses of non-after-ripened seeds and of seeds after-ripened for 4, 8, 12, and 16 weeks. The non-after-ripened seeds were sterilized and allowed to imbibe water for 2 to 5 days before analysis. At each of the subsequent time periods the measurements with living material were completed within a 3 to 5 day period. At each sampling time 1 group of 15 seeds from each treatment was removed from after-ripening conditions and was immediately excised and the respiration rate of 1 or both leaf primordia measured. One primordium was then used for cell counting and the other was dried and used for dry weight measurement and total nitrogen analysis. The embryonic axis was macerated for cell counting. Another group of 15 seeds was excised and the leaf primordia dried for total phosphate analysis while the embryonic axes, in groups of 5, were used for respiration measurements. These were then dried and used for dry weights and total nitrogen and phosphate analysis. Three groups of 10 seeds each were used for respiration measurements on whole seeds and were then excised for growth measurements. Finally, 20 to 30 seeds were used to measure the germination of intact seeds. It should be noted in the sampling procedure for leaf primordia that the primordia from any one seed are so closely matched that a "matched leaf" technique could have been employed.

Results

The data obtained show that after-ripening at 5° C leads to 5 % germination after 12 to 16 weeks; a high germination percentage is not achieved under these conditions until after 18 weeks. After-ripening at 25° C does not lead to normal germination. As shown in figure 3, excision of the embryo leads to much earlier growth, according to the classification used almost full capacity after 16 weeks at 5° C. Excision of embryos after-ripened at 25° C leads to a slight increase in growth capacity by 16 weeks.

One of the qualitative characters taken as a measure of growth is a change in the growing portions of the embryo from a white opaque to a yellow translucent appearance. This change is apparently due to 2 factors: 1) the synthesis of pigments and 2) the emptying of the reserve materials from the cells. The latter can be seen clearly in microscope sections of the material. This means that growth in these cells is accompanied by utilization of storage materials or their conversion into protoplasm and cell wall material in situ. For this reason, dry weight or total nitrogen may be poor bases for expressing other data. We have therefore investigated such other measures of growth as organ size and number of cells.

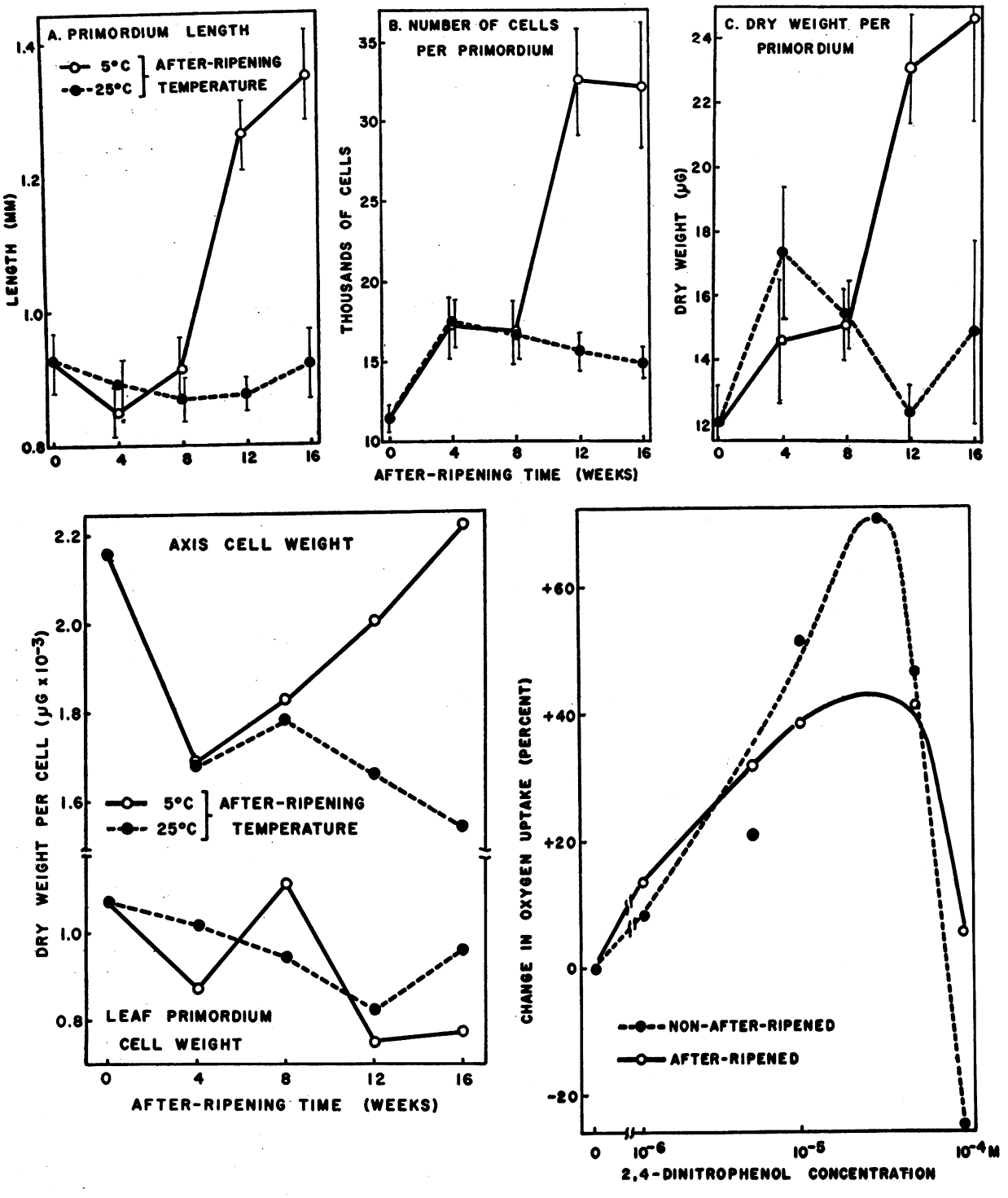


FIG. 5 (*top*). The effect of after-ripening time and temperature on growth and dry weight in the embryonic leaf primordia of cherry seeds. The vertical lines represent ± the standard error of the mean.

FIG. 6 (*lower left*). Changes in dry weight of the cells of the embryonic axis and leaf primordium as a function of after-ripening time and temperature.

FIG. 7 (*lower right*). The effect of 2,4-dinitrophenol concentration on the oxygen uptake of cherry embryonic leaf primordia.

In figures 4 and 5 are presented data on changes in the embryonic axis and leaf primordia during after-ripening at 5° C and at 25° C. A number of changes are obvious and fairly constant, although the exact times of these changes are not necessarily synchronized in the axis and leaf primordia. Specifically, at 5° C both the axis and leaf primordia show an increase in length roughly correlated with the increase in the growth capacity. This increase in length is due at least in part to the production of new cells. The increase in length and number of cells is also accompanied by an increase in the total dry weights of axis and leaf primordia (figures 4 C and 5 C). In the axis, the dry weight per cell tends to drop and then increase again (figure 6), while in the leaf primordia it tends to drop, although somewhat erratically because the rate of cell division is not constant. The difference in amount of variability between leaf primordia and axis in this respect is magnified by the fact that during the 1st 4 weeks of after-ripening the increase in cell number in the axis could be accounted for by a single division in 15 % of the cells, while in the leaf primordia it was the equivalent of a single division in 51 % of the cells. Thus cell division in the leaf primordia is proportionately much more active. It should be noted that the dry weight per cell in the leaf primordium is less than half that in the axis. This is at least partially the result of a difference in cell size as can be seen in microscope sections. Whether this also represents a difference in amount of stored material cannot now be determined.

In comparing embryos after-ripened at 5° C, and thus being prepared for active growth, with embryos after-ripened at 25° C and increasing little in growth capacity, a number of differences are obvious. According to most of the measurements made, increases with after-ripening time at 5° C are not paralleled by increases in embryos after-ripened at 25° C. However, if we examine the changes during the 1st 4 weeks of after-ripening, we find that these are actually quite similar at both 5° C and 25° C, the divergence appears in most cases only after 4 weeks.

It should be recognized that the growth shown in figures 4 and 5 can be seen only by means of the precise measurements used to obtain these data. On the other hand, the data used to estimate the growth

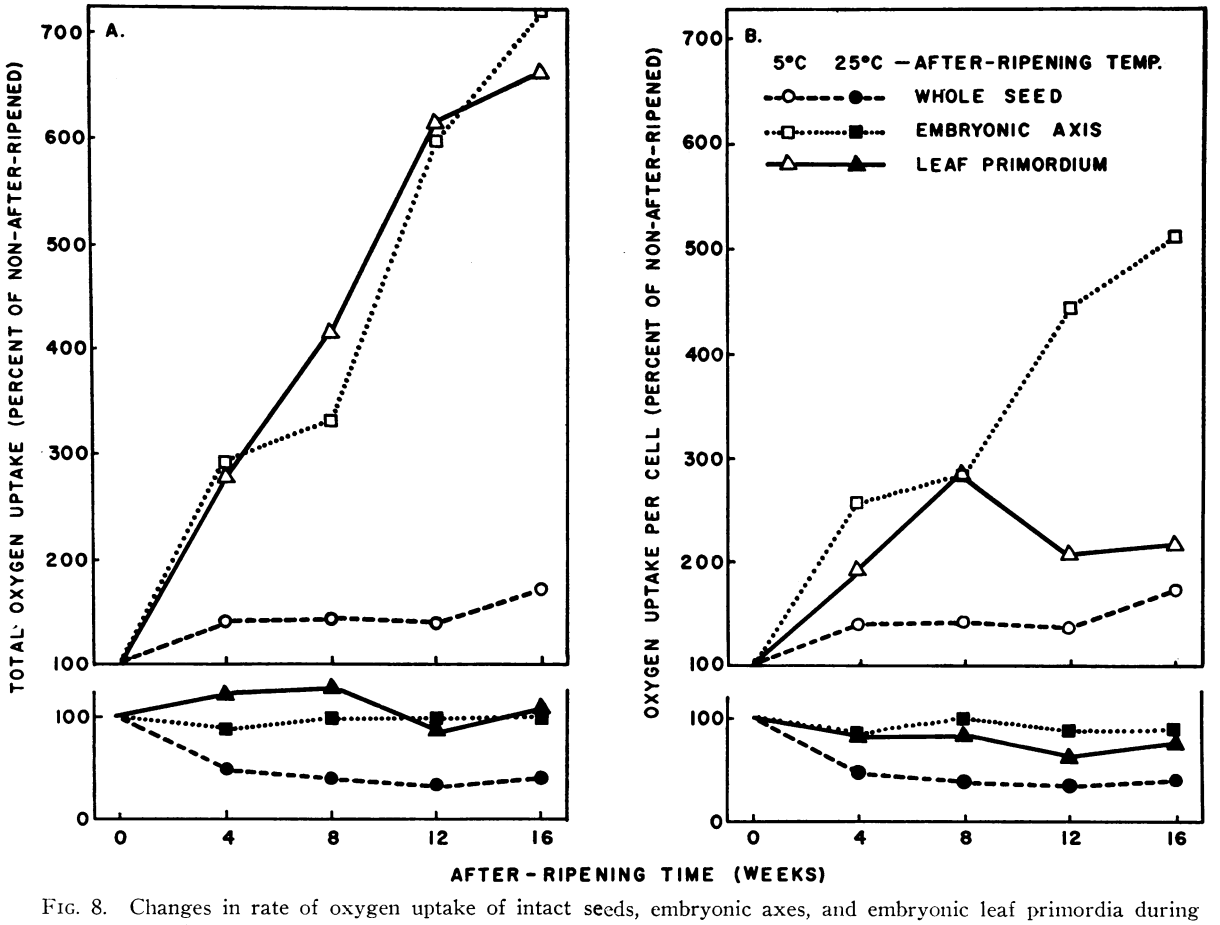


FIG. 8. Changes in rate of oxygen uptake of intact seeds, embryonic axes, and embryonic leaf primordia during after-ripening at 5° C and at 25° C. In A the data are expressed on the basis of the individual, using the non-after-ripened individual as 100 %. In B these data have been calculated on a cell basis by using the data presented in Figures 4 and 5.

capacity as presented in figure 3 represent changes of a much higher order of magnitude, actually growth visible to the naked eye.

In figure 8 the changes in respiration rate in the various parts of the seed are presented as a function of after-ripening time and temperature. Because of the great difference in absolute amounts, the data are expressed on a percentage basis, using the rates for non-after-ripened seeds as 100 %. For the whole seed, embryonic axis, and individual leaf primordium this basic value is 5.29, 0.246 and 11.1 $\times$ 10^{-3} μl/hr/ individual or organ, respectively. Figure 8 A shows that the respiration of whole seeds after-ripened at 5° C increases during after-ripening. However, this increase for whole seeds, a maximum of about 70 %, is small compared to the increase for the embryonic axis and leaf primordia of almost 600 % in 16 weeks. It should be noted that for both the leaf primordia and axis this increase is almost linear with time and does not show a 4 week lag as does the growth capacity and increase in dry weight of the embryonic axis.

In seeds after-ripened at 25° C the respiration rate of whole seeds actually declines to less than half that originally present. The respiration rate of the embryonic axis and leaf primordium does not change significantly with time.

Figure 8 B shows the respiration rate calculated on a cell basis and plotted as a function of after-ripening time and temperature. To do this with whole seeds we have assumed that there are few or no cell divisions in the cotyledons and endosperm, an assumption which is probably valid. Since the axis and leaf primordia are small compared to the rest of the seed, their cell divisions contribute little to the total cell number, and the change per cell on a percentage basis is therefore identical with the value for the whole seed. At 5° C the respiration rate per cell rises for the 1st 8 weeks in both axis and leaf primordium, and thereafter drops in the leaf primordium when most cell divisions occur.

At 25° C after-ripening has little effect on the total oxygen uptake of the axis and leaf primordium cells, with a decrease apparent in the seed as a whole.

Recognizing the great increase in respiration rate which precedes and accompanies an increase in growth capacity, the question arises as to whether this increase is due to the activation of enzymes previously present, to the synthesis of new enzymes, to the synthesis of other possible rate-limiting factors, or to the transport of such rate-limiting factors from the cotyledons to the growing cells of the embryo. In an attempt to answer these questions, 2,4-dinitrophenol (DNP) was used as an uncoupling agent to obtain an approximate measure of the rate-limiting step in the respiratory chain.

Preliminary experiments with leaf primordia showed that DNP can increase respiration at a concentration of 3 $\times$ 10^{-5} M (fig 7). These experiments also showed that the degree of increase is greater in non-after-ripened than in after-ripened primordia. Therefore, as part of the experiment previously described, DNP at 3 $\times$ 10^{-5} M was added to leaf primordia after the normal respiratory rate had been measured. The results are shown in figure 9. Figure 9 A shows that the amount of increase declines with after-ripening time at 5° C. However, at 25° C during the 1st 4 weeks there is a very sharp increase in DNP action; this is followed by a decline with time. The percent increase of primordia after-ripened at 25° C remained throughout the experiment at least twice as high as that of primordia after-ripened at 5° C.

When the DNP is applied to the leaf primordia, the resulting rate of oxygen uptake might be referred to as "respiratory capacity" and should be a measure of the limiting step in the respiratory chain if substrate and cofactor quantities are not limiting. This respiratory capacity is plotted in comparison with normal respiration rates in figure 9 B. With after-ripening at 5° C both the respiratory rate and capacity rise continuously from the beginning of after-ripening. At 25° C however, the respiratory rate remains approximately constant, but the respiratory capacity increases during the 1st 4 weeks to almost the same level as in material after-ripened at 5° C; thereafter it falls off. Indeed, had a measurement been made at about 3 weeks, it might have shown an identical value at 5° C and at 25° C. Much the same trend is shown in figure 10 with the results calculated on a per cell basis. If we then calculate the actual respiration rate of the leaf primordia as a percentage of their respiratory capacity, it is clear (fig 9 C) that the percent of utilization climbs more or less continuously during after-ripening at 5° C, but at 25° C it declines very sharply during the 1st 4 weeks, thereafter rising somewhat with time but never getting to much over half the degree of utilization shown by the leaf primordia from seeds after-ripening at 5° C. While this experiment was terminated prior to much actual growth, other experiments with more advanced material, such as that shown in figure 7, indicate that this trend for more complete utilization of the respiratory potentialities of the system continues at least into the early stages of active growth.

The fact that the stimulation by DNP declines as the system approaches a growing condition suggests that the rate-limiting step in respiration shifts with the degree of after-ripening and changing growth capacity. This shift seems to be from the phosphorylation system toward a rate-limiting step in the respiratory chain itself, although these data alone might equally indicate a rate limited by respiratory substrate. However, evidence for the respiratory chain as the rate-limiting step is shown in figure 11, where cyanide sensitivity curves for non-after-ripened and after-ripened (15 to 18 weeks at 5° C) leaf primordia are shown. In this case, the after-ripening was carried somewhat closer to actual growth than in the previous case, and the DNP increase was comparable to that shown for after-ripened primordia in figure 7. These data show that after-ripened primordia are more sensitive, both in regard to HCN concentration and total

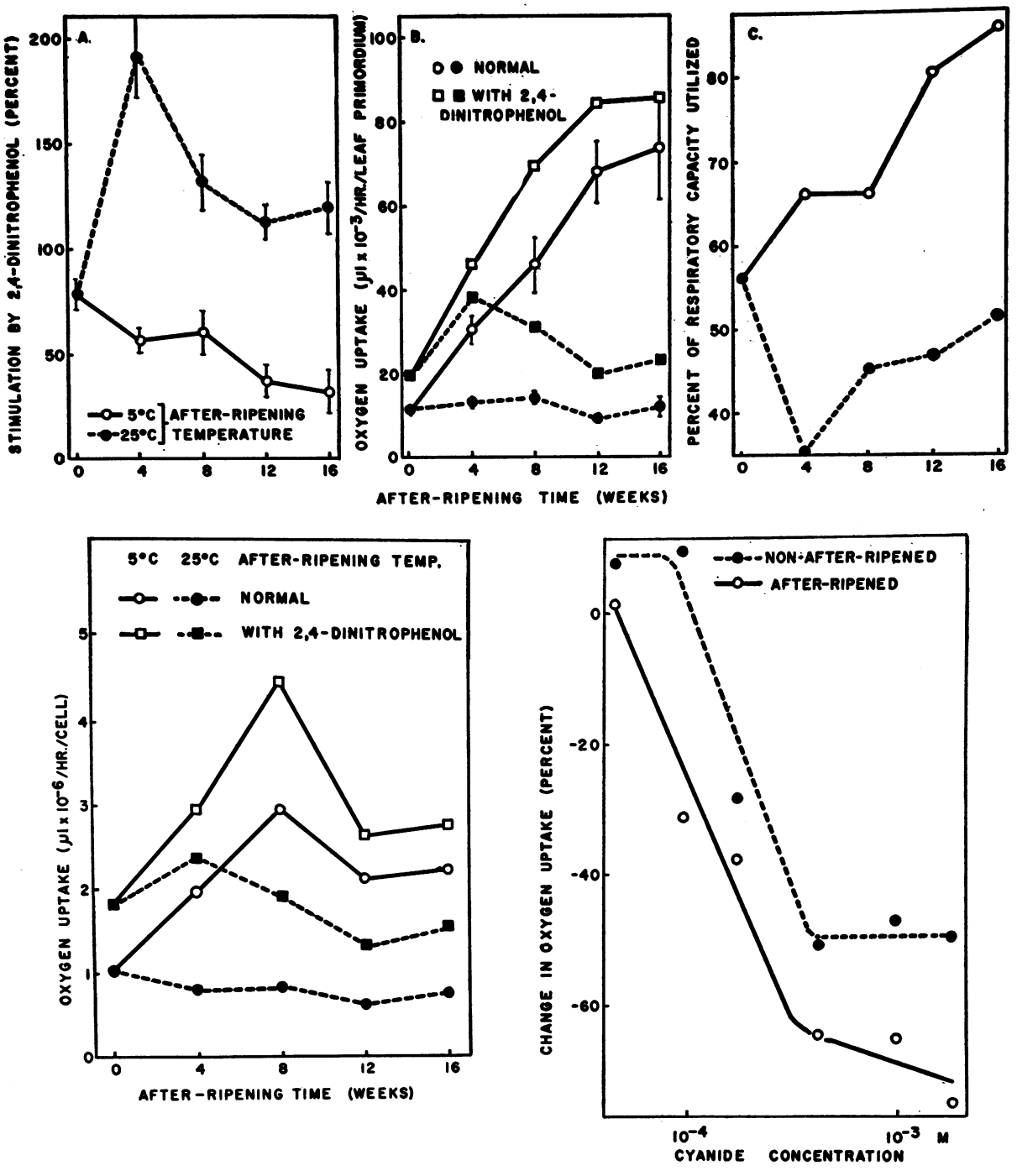


FIG. 9 (*top*). The effect of after-ripening time and temperature on respiration rate and respiratory capacity of embryonic leaf primordia. "Respiratory capacity" is here defined as the maximum respiration rate obtained in the presence of 3×10^{-5} M 2,4-dinitrophenol. The vertical lines represent ± the standard error of the mean.

FIG. 10 (*lower left*). Changes in respiratory rate and capacity per cell in embryonic leaf primordia as a function of after-ripening time and temperature. These data are calculated from the data presented in figures 5 and 9.

FIG. 11 (*lower right*). The effect of HCN on the oxygen uptake of cherry embryonic leaf primordia from non-after-ripened and from after-ripened seeds.

inhibition, than are non-after-ripened primordia. This is what would be expected if the capacity of the respiratory chain itself became more the limiting factor in respiration.

Discussion

In their recent review article, Toole et al (18) noted that one of the first evidences of the onset of germination is the increase in respiration rate, but that the sequence of enzymatic events has not been established. Thus the data presented in this paper need to be interpreted relative to the normal germination process as well as to the rest period. Indeed, it is possible to consider that the changes which occur slowly during a period of low temperature after-ripening are similar to those which must occur very quickly during the 1st hours of germination in a seed germinating without a rest period.

One of the interesting conclusions from these data is that, for the cherry seed at least, early growth of the embryo is not restricted to cell elongation, but includes cell division. It is particularly interesting to note that in the leaf primordia a significant number of cell divisions occur during the 1st 4 weeks, and occur equally at 5° C and 25° C. At this time the embryo is not capable of any further growth. This might be interpreted to mean that these early cell divisions are independent of the rest period block, possibly a continuation of development which was arrested by seed maturity.

One of the difficulties in interpreting changes relative to the ending of the rest period is the problem of distinguishing cause from result. When the rest period ends and active growth begins, obviously a large number of profound physiological and biochemical changes occur. These are the result of the ending of the rest period. The problem is then to try to set up a timetable of events; those changes preceding the end of the rest period could conceivably be responsible for its ending. For example, the changes in dry weight of the embryonic axis and leaf primordia constitute an interesting problem. Obviously several changes in location and amounts of reserve materials must occur during after-ripening and early growth: 1) Reserves stored in the embryonic axis are utilized in respiration and/or converted to cell wall and protoplasmic materials, and 2) reserves in the cotyledons are used in the cotyledons for respiration and translocated to the growing cells for respiration and synthesis. Since the total dry weight of the leaf primordia is small relative to the axis weight and changes therein, we can, as a first approximation, consider only changes which occur in the embryonic axis. The data in figure 4 C show that the dry weight of the embryonic axis remains constant or drops during the 1st 4 weeks at both 25° C and 5° C, thereafter it remains constant at 25° C but rises steadily at 5° C. Obviously this increase in dry weight at 5° C must indicate translocation of enough materials from the cotyledons to the axis to supply both the requirements for respiration and for synthesis. Apparently much less translocation to the axis occurs at 25° C. Is a block to translocation of nutrients responsible for the rest period? Evidence in the literature (5) suggests that excision of parts of the cotyledons, while making the plants smaller, has no specific effect on breaking the rest period. However, this does not answer the question since it is possible that the stimulus directing translocation arises within the growing cells and that the rest period involves failure of this stimulus. It is equally possible that the failure of the translocation stimulus is just one of many secondary effects of a block elsewhere in metabolism. In other words, we cannot yet distinguish cause from result in this situation.

The most striking changes we have noted during after-ripening have been associated with the respiratory mechanism of the cells. The data show that the respiration rate of both the embryonic axis and the leaf primordia from seeds after-ripened at 5° C rises linearly with time of after-ripening; there is no obvious difference in response between the axis and leaf primordia. It is particularly interesting to note that the respiratory rate increases from the beginning of after-ripening, while the growth capacity rises only after a 4-week lag. Thus the increase in respiration appears to precede the removal of the rest period block. It is particularly interesting to examine the leaf primordia and the changes in respiratory capacity which occur within these 1st critical 4 weeks of after-ripening. At 5° C the respiratory rate and respiratory capacity rise together; this increase is accompanied by division of the equivalent of 51 % of the original cells. At 25° C the cells divide at the same rate, and the respiratory capacity rises almost as rapidly as at 5° C but the respiratory rate fails to increase at all. With further after-ripening the rate and capacity both climb at 5° C but the respiratory capacity declines while the respiratory rate remains constant at 25° C. These changes strongly suggest that the initial effect of after-ripening at 5° C is to increase the efficiency with which the respiratory enzyme system is utilized, hence increasing the supply of energy available for synthesis and growth. In contrast, at 25° C synthesis appears to start normally and then come to a halt for lack of available energy. A somewhat similar situation has been described by Evenari (4) for the lettuce seed.

What does the term "respiratory capacity" mean in the biochemical mechanisms of the cell? Dinitrophenol is usually considered to act by uncoupling mitochondrial oxidations from phosphorylation. If DNP acts to increase respiration, it may therefore be assumed that the rate-limiting step in untreated cells was associated with the supply of phosphate acceptors or their turnover rate. If this is the mechanism by which DNP increases the respiration of cherry embryonic leaf primordia, then the available data strongly suggest that 1 of the changes responsible for an increased respiratory rate in material after-ripened at 5° C is an increased supply of phosphate acceptors or an increased turnover rate. If the former is the

case, then it may be reasoned that the effect of low temperature is to increase the synthesis of phosphate acceptors in the embryonic axis and/or the synthesis and translocation of these acceptors from the cotyledons. As in the case of dry weight changes there is question as to whether this change is cause or result. However, because it occurs earlier than other changes, it is perhaps more likely to be a key step in the breaking of the rest period.

The problem of finding a suitable basis on which to express physiological measurements is a perennial one. In this particular case it is complicated at the outset by the fact that the conversion of storage materials into protoplasmic constituents makes the use of dry weight or total nitrogen somewhat questionable as a basis for expressing data. We have calculated some of the data on a unit cell basis. This is open to question also, 1st because it does not consider the size of the cell and 2nd because it implies an equality among cells which obviously does not exist. The application of this technique to the leaf primordia is probably on a sounder basis than its application to the embryonic axis, since the leaf primordia are more uniformly meristematic and have a much higher proportion of cell divisions. In spite of the difficulties raised, some interesting conclusions result from calculating respiration rate and capacity on a cell basis. For example, assuming that treatment with DNP shifts the rate-limiting step in respiration to the chain of respiratory enzymes, we then have a rough measure of the quantity of enzyme present in the cells. As shown in figure 10, during the 1st 4 weeks after-ripening at 5° C the enzyme must be synthesized (or activated) in a quantity sufficient not only to supply the newly-formed cells but also to increase the quantity within these cells. This increase must continue through the period 4 to 8 weeks when no cell divisions occur. Then from 8 to 12 weeks, when more frequent cell divisions occur, the synthetic rate is not adequate to maintain the rate per cell constant and the respiratory capacity per cell drops. This does not mean that synthesis stops, because the increase in total respiratory capacity per primordium continues to increase at a more or less constant rate; it merely means that cell division and synthesis are not precisely linked.

The changes in respiratory capacity with after-ripening at 25° C are also interesting. Accompanying the cell divisions during the 1st 4 weeks, there would appear to be a considerable amount of synthesis. It is not clear however whether the subsequent decline indicates a destruction of enzyme, its conversion to an inactive form, or a shift to a new rate-limiting step in respiration. It is perhaps significant that the reduction is to, but not below, the original level in non-after-ripened seeds.

In considering the present status of our knowledge of the mechanism of the rest period, it is clear that rest may be considered to involve at least 1 primary block to growth which will have many secondary effects on the blocked cells. Regardless of whether this primary block is in the nature of a chemical inhibitor, a deficiency of some essential hormone or cofactor, or some other type of mechanism, this primary block must operate through the metabolism of the affected cells and, in turn, must be generated and removed by changes in cell metabolism. The problem then is to construct a timetable of metabolic changes and a flow sheet of such compounds as nutrients and hormones sufficiently precise to permit identification of the metabolic block, together with its mode of generation and removal.

Summary

The changes associated with after-ripening in resting cherry seeds have been investigated by utilizing a combination of "conventional" and "ultramicro" analytical methods. With these methods, it has been possible to determine changes in the whole seeds and, more important, in the potentially growing cells of the embryo. In seeds after-ripened at 5° C, the capacity for growth develops linearly with time after a lag of 4 weeks. This capacity for growth is paralleled in the embryonic axis by an increase in dry weight, indicating translocation of material from the cotyledons, but is preceded by cell divisions and a sharp increase in respiration rate. Using 2,4-dinitrophenol to obtain a measure of respiratory capacity, we find that the increase in respiration is paralleled by an increase in respiratory capacity. In contrast, in seeds after-ripened at 25° C growth capacity does not develop and little translocation to the potentially growing cells of the embryo is evident. The respiratory rate remains approximately constant but the respiratory capacity rises, accompanied by a significant number of cell divisions, within the 1st 4 weeks of after-ripening, but thereafter declines to its original level.

We interpret these results as suggesting that one cause of the breaking of the rest period may be the increased availability of energy to the embryo, possibly by an increase in the supply of phosphate acceptors. Whether this is the primary reaction responsible for breaking the rest period or just one of many secondary reactions which follow activation of the cells is not clear.

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FORMATION OF β-PHENYLGLUCOSIDE IN PLANT LEAVES[1, 2]

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The formation of phenol sulfate or phenylglucuronide and subsequent excretion of these products has been observed on feeding phenol to animals (1). This paper reports the formation of β-phenylglucoside on feeding traces of phenol to plant leaves. Recently similar results have been reported on the formation of other phenolic glucosides (3).

MATERIALS AND METHODS

Uniformly labeled glucose-C^{14} or sedoheptulose-C^{14} solutions with specific activity of 3.7 μc/mg of carbon were obtained by biosynthesis and isolated by paper chromatography (5). The solution of sedoheptulose-C^{14} contained 1.4 mg/ml and that of glucose-C^{14}, 0.7 mg/ml. Synthetic glucose 1-C^{14} and 6-C^{14} were obtained from the National Bureau of Standards, and solutions of these sugars were prepared by dissolving 5 mg of the sugar in 5 ml of water.

Synthetic phenylglucoside was obtained by the method described by Helferich and Schmitz-Hillebrecht (2). The position of phenylglucoside on the chromatograms was determined by placing the chromatogram over a calcium tungstate screen and irradiating it with ultraviolet light (2537 Å maximum). The phenylglucoside appeared as a blue-violet spot on the chromatogram and as a dark shadow on the screen.

Barley or wheat leaves were fed the solutions of labeled sugar by placing the leaf base in a tube with 100 μl of the sugar solution with or without phenol. After the leaf had taken up this solution, a continual supply of water was provided. At the end of the experiment, the leaves were ground in liquid nitrogen, and the powder was taken up in water and immediately heated in a boiling water bath. After separation of the supernatant liquid from the solid debris, the extract was concentrated, and its components separated by the chromatographic procedures previously used (4). The C^{14}-labeled compound suspected of being phenylglucoside was separated by these procedures and located on the chromatograms by radioautographic techniques. The area was cut out and eluted with distilled water. The eluate was then again chromatographed in an ethyl alcohol : 15 % acetic acid (8 : 1 by volume) solvent system in order to separate any contaminating phenol. The C^{14}-labeled compound (phenylglucoside) was then eluted from the chromatograms, and its ab-

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