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Respiratory and enzymatic activity in corn seeds in relation to viability

Glyn O. Throneberry
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RESPIRATORY AND ENZYMATIC ACTIVITY IN
CORN SEEDS IN RELATION TO VIABILITY

by

Glyn O. Throneberry

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Plant Physiology

Approved:

In Charge of Major Work

Head of Major Department

Dean of Graduate College

Iowa State College

1953
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INTRODUCTION

The germination process in seeds like any other type of growth must depend on respiratory metabolism. It is likely, therefore, that lack of ability to grow, or germinate, may be due to some failure in the respiratory mechanism, which is an integrated combination of a number of specific enzymes. Perhaps the strongest evidence for such a relationship between respiratory activity and ability to grow is the success often found in estimating viability with the tetrazolium test. The tetrazolium reaction is known to be dependent on activity of dehydrogenases, essential enzymes in the respiratory mechanism. Therefore, the failure to reduce tetrazolium would indicate some sort of respiratory deficiency. The respiratory metabolism of germinating seeds has not received intensive enough investigation, however, to determine the extent to which overall respiration or particular respiratory enzymes limit the ability to germinate. A study of this type, therefore, is not only of basic interest in respect to the role of respiratory activity in controlling seedling growth but could provide a systematic basis for development of quick tests of viability based on tests of respiratory activity. In order that a quick test be practical, it must depend on the fact that the breakdown of respiratory or enzymatic activity controlling growth occurs at an early stage in the germination process so that an estimate of viability could be determined before visible growth or complete germination. The only extensive work in the past has dealt primarily with the relation of catalase and peroxidase to viability. However, for the most
part the results have been unsatisfactory, particularly because these two enzymes were shown to be active in seeds incapable of germination.

The present investigation was planned to determine the extent to which respiratory activity and the activity of certain respiratory enzymes measured at an early stage in the germination process would correlate with ability to germinate. Manometric measurements of respiratory activity were made to investigate whether respiratory activity is generally limiting in the germination process or whether other metabolic processes were more often limiting. In addition, it was hoped that measurements of both oxygen consumption and carbon dioxide evolution would reveal whether the mechanisms involved would be differentially inactivated in the loss of viability. If so, such information would offer clues to selection of other enzymes for investigation. Malic and alcohol dehydrogenase have been shown to be especially active in the reduction of tetrazolium by corn embryos. This information, together with the success often achieved in estimating viability by the tetrazolium test, suggests that these two enzymes may be critical in the maintenance of viability. Cytochrome oxidase has been suggested to be the principal terminal oxidase in corn embryos, as well as in other plant tissue, particularly embryonic tissue. Therefore, this enzyme and the two dehydrogenases were selected for investigation in relation to viability.

Corn was particularly suitable for this investigation in that the embryos are large and readily accessible. Since lots having a range of germination percentages were desired, field collected corn was given
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The severe of the study I have conducted to investigate the pattern of increase of seedling growth. These factors therefore were the subject of further research. These factors therefore were important because in determining the response and size of the seedlings used, it is obvious the seedling growth of the seedlings for further investigation of the seedling growth. In contrast, the methods of growth were employed in an experiment on the nature of seedling growth.

The severe of the seedling growth methodology was tested in experiments with corn seedlings. These factors were introduced for comparison with other experiments. The test was based on the seedlings growth. By monitoring the growth rate, it was observed that seedling growth was dependent on water. In contrast, it was observed that growth rate was opposed to growth rate. The seedling growth methodology was tested in experiments with corn seedlings. These factors were introduced to determine the

methodology that under favorable conditions for seedling growth. After testing the seedling growth methodology and method of growth, a greater emphasis was placed on seedling growth conditions. These conditions were introduced to determine the growth rate. With the method of growth, the growth rate is determined. By monitoring the growth rate, it was observed that seedling growth is dependent on water. In contrast, it was observed that growth rate is opposed to growth rate. The seedling growth methodology was tested in experiments with corn seedlings. These factors were introduced to determine the

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*Future production and marketization was expected to approach these conditions which were encountered.
in respiratory and enzymatic activity up to and beyond the 18 hour stage of development used in the viability study, not only as a matter of basic interest but also as a means of better characterizing the 18 hour stage.
The relation between enzymatic activity and viability of seeds in general was reviewed by Bennet (6) and Gadd (15). For corn in particular, the literature is limited. McHargue (31) reported the presence of catalase but not peroxidase in seed incapable of germination and concluded that the latter would better correlate with viability. However, Bennet (6) was unable to demonstrate such a correlation. Nemec and Duchon (33) claimed that catalase activity correlated with viability of corn but Gracinin (19) found catalase activity in nonviable seed and could establish no relation between catalase activity and viability.

Bennet (6) and Gadd (15) also reviewed previous work on quick tests for viability, including the tetrazolium test. Since that time, Isely (20) and Favilli (12) have further reviewed reports on the tetrazolium test. Various workers have reported satisfactory results in using this test on corn (4, 6, 18, 22, 29, 35), although Bennet (6) and Goodsel (18) found difficulty with freshly frozen corn in that tetrazolium tests gave unusually high results. Lakon (22) and Baird et al. (4) maintained that the central area of the scutellum, in addition to the plumule and scutellar node, must be stained to indicate ability of corn to produce a normal seedling, whereas Favilli's criterion (12) does not include this area of the scutellum.

References to the respiratory activity of the cereals are extensive but are largely related to storage problems such as moisture content, heating and effects of mold and insect damage. Corn respiration has been investigated relatively little, even in relation to these problems.
Bailey (3) reported a 400 percent increase in the respiratory rate of corn when the moisture content was raised from 15 to 17 percent. Recently, Ragai (36) found a logarithmic increase in respiration of corn as the moisture content was increased, but the rate of increase of respiration diminished at approximately 20 percent moisture. Stoward (45), as early as 1908, investigated the relative respiratory rates of seed parts of corn soaked approximately 28 hours. He found that, on a fresh weight basis, excised corn embryos respired 13, 31 and 167 times as fast as intact seed, seed residues and endosperm, respectively. On a per seed basis, excised embryos respired three times faster than intact seed and six times faster than seed residues. He concluded that seed residue respiration was largely due to the aleurone layer, although he reported a measurable rate for endosperm. Stiles and Leach (44) and Leach (23) reported that the respiratory quotient was near unity for germinating corn up to 33 hours after contact with water. After 50 hours, the respiratory quotient dropped to 0.73 and then increased to reach unity again at 150 hours. They attributed the initial respiratory quotient to utilization of a small amount of hexose of some type present in the embryo, the subsequent lower value to the action of lypolytic enzymes and the utilization of fats, and the final rise to action of starch hydrolyzing enzymes furnishing carbohydrate substrate from the endosperm. Smith (38) reported respiratory quotients near unity for corn up to 8 hours after contact with water.

Picklum (34) found that embryo cells of corn in wet Sphagnum moss were turgid in 12-18 hours and that mitotic activity did not take place
until after this period. He also stated that rupture of the seed coat was due to enlargement of existing cells rather than to meristematic activity.

Rather extensive work on the respiratory nature of other cereals has been reported. Leach (24) found the respiratory rates of wheat grain after five hours in contact with water were 1000 times that of dry grain. He postulated three respiratory periods for germinating wheat. The first was one of slowly rising respiration, after the sudden increase due to imbibition of water. The second period, from 10 to 22 hours, entailed an initial increase followed by a decrease which corresponded to mobilization of new substrates from the endosperm and subsequent saturation of the oxidation system. The third period, from 22 hours on, was one of increasing respiratory rates due to the development of new respiratory centers in conjunction with meristematic activity. He further stated that dry grain apparently had a sufficient complement of oxidative enzymes and substrates so that initial respiration depended only on imbibition of water. Merry and Goddard (32) reported oxygen consumption rates of 0.1 microliter per gram per hour for dry barley seeds. This rate increased 200–300 times after seed were exposed to water for 1 hour. Oxygen consumption rates increased approximately three times from 1 hour to 9 hours. The respiratory quotients were 0.74, 2.02 and 0.99 at 1, 9 and 26 hours, respectively. They attributed the high value at 9 hours to limitation by oxygen diffusion and to resulting aerobic fermentation. As the coleorhiza broke through the seed coat, the situation was remedied and the respiratory quotient decreased. Brown (10) reported similar
results for barley seed. He found the respiratory quotient to be 1.15 up to 3.5 hours after contact with water and 2.0-3.0 at 12 hours. Up to 12 hours there was a gradual rise in oxygen consumption and a greater rise in carbon dioxide evolution. Excised embryos had respiratory quotients of 0.8-0.9 at 3.5 hours and 1.0-1.25 at 12 hours. He believed that the low relative oxygen consumption rates of intact seed was due to low permeability of the seed coat to oxygen. In a previous paper (9), he reported that carbon dioxide diffused through the inner seed coat of cucumber four times as fast as oxygen.

The effects of oxygen tension on seed respiration have been described for oats (1), barley (32) and wheat (8, 27). Albaum (1) measured oxygen consumption of oats which had been previously aerated with oxygen-nitrogen mixtures containing 2.5, 5, 10, 20, 50 and 100 percent oxygen. Rates increased with oxygen tension up to 50 percent and then decreased. On the other hand, Merry and Goddard (32) reported increased oxygen consumption of barley seeds in pure oxygen as compared to air; carbon dioxide evolution increased also but to a lesser extent. The stimulation was not so striking in "stripped" seeds (lemma, palea, ovary wall and seed coat removed). Respiratory quotients were lower in oxygen than in air and were lower for "stripped" seeds in air or oxygen than for whole seeds under similar conditions. Their conclusion was that this phenomenon was due to limitation by oxygen diffusion. Wheat seed were shown by Mack (27) also to have higher respiratory rates in higher oxygen concentrations. Brown and Goddard (8) found that the oxygen consumption of wheat embryos was 29 percent less in 5 percent oxygen than in air.
The literature on respiration of slices of plant material is abundant, although none was found for corn embryo slices. Therefore, only literature pertaining to the effects of oxygen tension on slice respiration will be referred to here. Boswell and Whiting (7) reported no limitation of oxygen consumption by oxygen diffusion in potato tuber slices up to 0.78 mm. thick. However, when 0.75 mm. thick potato slices were aerated with 3.8 percent oxygen as compared with air, Steward et al. (42) reported a decrease in carbon dioxide evolution. Levy and Schade (25) found 2.5, 15 and 37 percent inhibition of oxygen consumption by 0.3 mm. thick potato tuber slices in 10, 5 and 2.5 percent oxygen, respectively, as compared with air. However, they were able to show that cytochrome oxidase activity was insensitive to oxygen concentrations down to 2.5 percent in the gas phase. Marsh and Goddard (28) found lowered oxygen consumption rates by 0.5 mm. thick carrot slices at decreased oxygen tensions. Stiles and Dent (43), who worked with other slices as well as carrot, concluded that respiratory rates of slices were a function of "specific surface", or that as tissue was sliced thinner and thinner, a greater proportion of the cells were subjected to nonlimiting oxygen concentrations. The literature on effects of washing tissue slices shows variable results. Stiles and Dent (43) reported a more rapid rise of respiratory rates of slices of plant material if unwashed than if washed, whereas other workers (7, 37) reported a greater increase in washed tissue slices than in unwashed slices. Link and Goddard (26) could find no difference due to washing in tomato stem slices and Barron et al. (5) stated that they did not wash slices of tomato stem to avoid possible damage to cell membranes and loss of soluble coenzymes.
The presence of α-difluoromethyl oxazole in corn emulsions was found to increase in carbon dioxide of corn. The germination was reported by Strickler (21) and further by Powers et al. (31) to be accompanied by the formation of oxazaxa. This increase in α-difluoromethyl oxazole during germination of corn was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. The increase was due to the formation of oxazaxa rather than enamine. 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MATERIALS AND METHODS

Source and Treatment of Seed and Measurement of Viability

Hand-harvested ears were collected from open pollinated B6 x W22 and B6 x L289 hybrids in 1951 and from open pollinated Ia. 4297 hybrid in 1952. Moisture levels at time of harvest ranged from 32 to 44 percent. Husks were removed and the ears were dried either at room temperature or at elevated temperature, with and without previous freezing. An oven adapted for air circulation was used for drying at higher temperatures. These treatments were designed to lower viability in part of the lots and to obtain a wide range of germination percentages. When the moisture levels had dropped to 8-12 percent, the ears were hand-shelled and the lots stored at 7°C until used. Lots were designated by two numbers, the first referring to the lot within the season and the second referring to the season collected. The letters a and b refer to varieties B6 x W22 and B6 x L289, respectively. A tabulation of treatments listed by lot numbers is included in Table 1 in the section on experimental results.

For germination tests, two 100 seed samples per lot were placed on a tray covered with blotters and then covered with paper towels. A jar of water was inverted on each tray to supply moisture and the trays were placed in an alternating 20-30°C germinator. The number of germinated seedlings were recorded daily and the seedlings discarded. A normal seedling was considered to be one containing a normal shoot and adventitious roots.
The cold tests consisted essentially of exposing seed to wet soil containing microorganisms at 10\(^{\circ}\) C. for 1 week and then allowing germination to proceed at a higher temperature. The details of the method are according to Svien (47). The substrate consisted of one-third soil obtained from a field on which corn had been grown the previous season and two-thirds sand. Seeds, 100 per tray, were placed on half of the soil and covered with the remaining half. Enough water was added to bring the moisture content of the soil to 60 percent saturation and the closed trays were kept at 10\(^{\circ}\) C., for 1 week. Trays were then transferred to a temperature near 30\(^{\circ}\) C. and seedlings were counted after 5 days. One 100 seed sample per lot in the 1951 group and two 100 seed samples per lot in the 1952 group were tested.

Tetrazolium tests were made on a single 100 seed sample from each lot. Seed were soaked 18 hours at 30\(^{\circ}\) C., bisected longitudinally, and placed cut side down in 0.05 percent 2,3,5-triphenyltetrazolium chloride. Dishes were kept in the dark and samples examined after two hours. Areas of the embryo considered essential for production of a normal seedling and which must be stained to indicate viability were the plumule, scutellar node and the central area of the scutellum. This criterion is that reported by Lakon (22). Seed were classified as viable when all three of these areas were stained and nonviable when one or more were unstained or stained very lightly.

In an effort to determine to what extent malic and alcohol dehydrogenase were responsible for the reduction of tetrazolium by the corn embryo, tetrazolium ratings were calculated. These ratings are approximations of the relative amounts of tetrazolium reduced by the 100 seed
in each test and were calculated in the following manner. Preliminary
determinations showed that on a fresh weight basis these two dehydro-
genases were approximately 2.3 times as active in the embryo axis as
in the scutellum. Furthermore, the scutellum weighed about four times
as much as the axis. Therefore, it was estimated that the scutellum
contributed 1.8 times as much dehydrogenase activity per embryo as did
the axis. The embryo axis was arbitrarily divided into three areas,
the plumule, the scutellar node and the radicle. The scutellum was
similarly divided into three approximately equal sections, the upper,
central and lower areas. Values of 0, 1 and 2 were assigned each of
these six areas depending upon whether the area was unstained, stained
a medium red color or stained a dark red color. Values were then added
separately for the axis and the scutellum in each embryo and the total
values were obtained for 100 seed on this basis. The scutellum rating
was then multiplied by the factor 1.8 obtained above and the resulting
value added to the rating for the 100 embryo axes, thus giving a total
rating for 100 embryos. For example, 100 seed having embryos completely
stained a dark red color would have a rating of 1,680 for 100 x 6
+ 1.8 (100 x 6). For simplification, the values listed in the table
are the actual values divided by 1,000.

Vigor ratings to express velocity of germination were calculated
in the following manner. At each days counting of normal seedlings
during the germination tests, the number obtained per 100 seed sample
was multiplied by the reciprocal of the time, in days, that the seed
had been in the germinator. For example, 50 seedlings counted on the
third day would contribute 16.67 (or 50 x 1/3) to the total vigor rating for that sample. When germination tests were completed at the end of 7 or 8 days, calculations for each day were added to give a total vigor rating.

Enzymatic Measurements

Seeds were soaked half submerged, embryo side down, in Petri dishes for 18 hours to initiate the germination process. This period of soaking was selected to correspond to that used for tetrazolium tests and was used throughout except for the experiments on stages of germination in which dry seed and seed soaked 9 and 27 hours also were used. After 18 hours soaking, embryos showed visible swelling but rarely any emergence of the radicle. Embryos were excised with a small spoon-shaped spatula and kept on moist blotters in covered dishes until further treatment.

Embryos from 50 seed per lot were bisected and sliced to a thickness of approximately 0.5 mm. Slices were kept in an ice bath prior to washing and blotting dry. The sample was then mixed thoroughly and divided into four approximately equal subsamples. One of these was used for enzyme determinations. This sampling procedure was used throughout except for the experiments on stages of germination where embryo slices from 10 seed were used. Slices were ground cold in 0.02 M pH 8.0 phosphate in a Dounce glass homogenizer to make a 10 percent homogenate on a fresh weight basis. Malic and alcohol dehydrogenase activities were determined on the supernatant after centrifuging cold at 1,000 times gravity for 3 minutes to remove cellular debris.
An aliquot of the supernatant was further centrifuged cold at 20,000 times gravity for 1 hour to obtain a particulate fraction which was resuspended in 0.02 M pH 7.0 phosphate. This fraction rather than the whole homogenate was used for cytochrome oxidase determinations since the latter contained sufficient dehydrogenase substrates to cause nonlinear rate curves. Both the supernatant and the resuspended particulate fraction were stored in an ice bath until used.

Homogenates were assayed for enzymatic activity by a colorimetric method employing 2,6-dichlorobenzenoneindo-3'-chlorophenol. The method for malic and alcohol dehydrogenase was developed in earlier work (48) and the cytochrome oxidase method was essentially that developed by Ginter and Smith for corn root tips (17).

Optimum concentrations of reactants for the assay of malic dehydrogenase were the following: 0.032 M pH 8.0 phosphate, 0.04 M pH 8.0 malate, 2.9 \times 10^{-4} M coenzyme I, 1.08 \times 10^{-5} M oxidized dye, 0.1 M cyanide (to bind the oxalacetate formed), and sufficient diaphorase to saturate the system. Total volume including appropriate amounts of diluted homogenate was 2.5 ml. Coenzyme I was obtained from Schwartz, dye was obtained from Eastman, and diaphorase was prepared from pork hearts (48), as adapted from Straub (46). Diluted homogenates were warmed to 30° C. and used within 3-4 minutes, since the enzyme kept longer at this temperature exhibited a slow initial reaction rate.

Reactants and their optimum concentrations for the measurement of alcohol dehydrogenase activity were the following: 0.032 M pH 8.0
phosphate, 0.14 M ethyl alcohol, 0.08 M pH 8.0 semicarbazide (to bind the acetaldehyde formed), 2.9 x 10^{-4} M coenzyme I, 1.08 x 10^{-5} M oxidized dye and sufficient diaphorase to saturate the system. Total reaction volume was 2.5 ml., including diluted homogenate. These dilutions always exhibited a slow initial reaction rate, for which no explanation can be given.

The complete system for measurement of cytochrome oxidase activity contained the following reactants: 0.04 M pH 7.0 phosphate, 7.2 x 10^{-5} M reduced dye, 1.4 x 10^{-5} M cytochrome c and sufficient catalase to destroy peroxide present. Total reaction volume, including appropriate amounts of the resuspended particle fraction was 2.5 ml. The dye was reduced by hydrogenating in the presence of 0.04 mg. palladinized asbestos per 18 micromoles of dye, then filtered and stored under hydrogen to keep it in a reduced state. Aliquots of the resuspended particle fraction were warmed to 30°C. and used within 1-2 minutes, since cytochrome oxidase was somewhat unstable at this temperature.

Rates for all three enzymes were determined at 30°C. on four concentrations from each homogenate and plotted to obtain a proportionality curve. From these curves, it was not only possible to determine an average rate but also possible to correct for blanks by extrapolating to zero concentration. Rates, as changes in optical density per minute, were converted to a Q_{O2} basis of equivalent microliters oxygen uptake per milligram dry weight per hour by the following formula:
\[ Q_{O_2} = \frac{OD \times 2.5 \times 11.2 \times 60}{28.0 \times v \times C \times D_h} \]

- **OD** = change in optical density per minute
- **2.5** = total reaction volume in milliliters
- **11.2** = microliters oxygen equivalent to 1 micromole of dye
- **60** = minutes per hour
- **28.0** = extinction coefficient times light path
- **\( v \)** = volume in milliliters of diluted homogenate used
- **\( C \)** = milligrams dry weight per milliliter of 10 percent homogenate before the first centrifugation
- **\( D_h \)** = dilution of homogenate used (e.g. 1/5)

**Respiratory Measurements**

Preparation of slices, handling procedure and division of the samples were described in relation to enzymatic measurement. Respiratory rates for the viability study were determined on slices suspended in 0.05 M pH 6.0 phosphate. Oxygen and carbon dioxide exchange in oxygen and carbon dioxide evolution in nitrogen were measured for each sample. Preliminary investigation showed that the slices respired at a faster rate in oxygen than in air; therefore, oxygen was used. Duplicate determinations were made on each lot of corn.

Experiments concerning the factors affecting respiratory measurements of seed parts were conducted using embryo slices, bisected and whole embryos, whole seeds, seed with pericarp removed from the embryo side, seed residues and endosperm tissue. The latter was obtained by
slicing the pericarp and sufficient tissue to include the aleurone layer from seed residues. Pieces of tissue 2-4 mm. on a side and 2-3 mm. thick were obtained.

Respiratory measurements on embryo tissue were made both in 0.05 M pH 6.0 phosphate and without liquid present. When tissue was not immersed, the air in the flasks was saturated with water vapor by a moist filter paper in the bottom for slice experiments and by a wet, folded filter paper in the side arms for bisected and whole embryo experiments. Ten embryos per flask were used. Sample sizes varied between 10 and 25 seed per flask for measurements on seeds and seed residues, and moisture was supplied by a wet filter paper in the side arms.

Embryo slices from dry seed and from seed soaked 9, 18 and 27 hours were used to obtain respiratory rates as a function of soaking time. Each flask contained 10 sliced embryos, suspended in 0.05 M pH 6.0 phosphate. Oxygen consumption and carbon dioxide evolution were measured both in air and in oxygen as well as carbon dioxide evolution in nitrogen.

All measurements were made at 30°C with a standard Warburg apparatus, using flasks of approximately 50 ml. capacity. Oxygen consumption was determined by placing 20 percent potassium hydroxide in the center well, and aerobic carbon dioxide evolution was measured by the two-flask direct method (11). For rates under an atmosphere of oxygen, flasks were flushed approximately 15 minutes with tank oxygen. Similarly, for anaerobic measurements, flasks were flushed with tank nitrogen passed through two alkaline pyrogallol towers. Flasks were equilibrated to bath temperature for approximately 15 minutes, and readings were made
at 10 minute intervals for 1 hour. Rates were linear throughout the period measured. After several experiments, there was found to be no difference between initial and final bound carbon dioxide, so this determination was omitted in following experiments. Tissue was placed in weighing bottles at completion of runs, and dry weights were determined after 24 hours at 100°C.

Correlation coefficients and other statistical values were determined according to Snedecor (40).
EXPERIMENTAL RESULTS

Treatment of Lots and the Effects on Viability

The freezing and drying conditions which were used to produce a range of viability in the various lots are listed in Table 1 with the resulting germination, cold test and tetrazolium test percentages and with the vigor ratings. The conditions to which the 1951 collection were exposed were somewhat unsatisfactory in that germination percentages included few intermediate values. Lots 1-5la and 2-5lb, both frozen in the field, and 24-51a, which became heavily contaminated with mold while drying, were the only lots in the intermediate range. It was apparent that laboratory freezing conditions had been too severe and drying conditions not severe enough to secure lots having a suitable spread of germination percentages. Therefore, conditions were altered accordingly for the 1952 collection, and several lots in the intermediate range were obtained.

Cold test results were not appreciably different from germination percentages except in the lots which were dried at elevated temperatures. In these lots, cold test values were consistently lower, whether or not they were given previous freezing treatments. Lots with these lower values did not have correspondingly lower vigor ratings, so that the difference in their behavior under favorable and unfavorable conditions did not appear to be due to growth rate.

Estimates of viability obtained by tetrazolium tests were in many cases higher than by germination tests in the lots which germinated below
<table>
<thead>
<tr>
<th>Lot</th>
<th>Treatment</th>
<th>Germ. (%)</th>
<th>Cold test (%)</th>
<th>Tetra. (%)</th>
<th>Vigor rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-51a</td>
<td>R. D. (room dried)</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>25.4</td>
</tr>
<tr>
<td>23-51b</td>
<td>R. D.</td>
<td>100</td>
<td>100</td>
<td>100 (89)</td>
<td>19.4</td>
</tr>
<tr>
<td>11-52</td>
<td>R. D.</td>
<td>100</td>
<td>98.5</td>
<td>100 (100)</td>
<td>27.8</td>
</tr>
<tr>
<td>7-51a</td>
<td>R. D.</td>
<td>100</td>
<td>97</td>
<td>99 (99)</td>
<td>26.9</td>
</tr>
<tr>
<td>12-51a</td>
<td>65 hrs., 40° C.</td>
<td>100</td>
<td>68</td>
<td>100 (100)</td>
<td>27.3</td>
</tr>
<tr>
<td>3-51b</td>
<td>R. D.</td>
<td>99.5</td>
<td>100</td>
<td>100 (100)</td>
<td>24.9</td>
</tr>
<tr>
<td>7-52</td>
<td>18 hrs., 2° C.; R. D.</td>
<td>99.5</td>
<td>99.5</td>
<td>100 (100)</td>
<td>23.2</td>
</tr>
<tr>
<td>4-51a</td>
<td>R. D.</td>
<td>99.5</td>
<td>98</td>
<td>100 (100)</td>
<td>21.5</td>
</tr>
<tr>
<td>15-51b</td>
<td>R. D.</td>
<td>99</td>
<td>99</td>
<td>100 (100)</td>
<td>20.8</td>
</tr>
<tr>
<td>8-51b</td>
<td>R. D.</td>
<td>99</td>
<td>96</td>
<td>99 (98)</td>
<td>22.8</td>
</tr>
<tr>
<td>12-52</td>
<td>68 hrs., 50° C.</td>
<td>98.5</td>
<td>91</td>
<td>100 (100)</td>
<td>27.4</td>
</tr>
<tr>
<td>6-52</td>
<td>R. D.</td>
<td>98</td>
<td>100</td>
<td>99 (99)</td>
<td>23.1</td>
</tr>
<tr>
<td>16-51a</td>
<td>65 hrs., 40° C.</td>
<td>97.5</td>
<td>21</td>
<td>99 (98)</td>
<td>22.3</td>
</tr>
<tr>
<td>11-51b</td>
<td>68 hrs., 40° C.</td>
<td>95.5</td>
<td>42</td>
<td>100 (98)</td>
<td>17.8</td>
</tr>
<tr>
<td>13-51b</td>
<td>65 hrs., 40° C.</td>
<td>90.5</td>
<td>31</td>
<td>100 (93)</td>
<td>16.2</td>
</tr>
<tr>
<td>1-52</td>
<td>4 hrs., -7° C.; R. D.</td>
<td>89</td>
<td>88.5</td>
<td>95 (95)</td>
<td>19.7</td>
</tr>
</tbody>
</table>

"51a" indicates 1951 collection of B6 x W22; "51b" indicates 1951 collection of B6 x L289; "52" indicates 1952 collection of Lf. 4297.

*Mean of two 100 seed samples.*

*One 100 seed sample for 1951 collections; mean of two 100 seed samples for 1952 collection.*

*One 100 seed sample; figures in parentheses based on complete embryo stain.*
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Lot</th>
<th>Treatment</th>
<th>Germ.</th>
<th>Cold test</th>
<th>Tetra.</th>
<th>Vigor rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-52</td>
<td>72 hrs., 50° C.</td>
<td>72</td>
<td>2.5</td>
<td>98 (93)</td>
<td>17.2</td>
</tr>
<tr>
<td>24-51a</td>
<td>R. D.</td>
<td>55</td>
<td>45</td>
<td>75 (66)</td>
<td>15.0</td>
</tr>
<tr>
<td>1-51a</td>
<td>Frozen in field; R. D.</td>
<td>51.5</td>
<td>55</td>
<td>62 (51)</td>
<td>10.7</td>
</tr>
<tr>
<td>14-52</td>
<td>8 hrs., -7° C.; 100 hrs., 50° C.</td>
<td>44.5</td>
<td>25.5</td>
<td>60 (52)</td>
<td>11.3</td>
</tr>
<tr>
<td>3-52</td>
<td>12 hrs., -7° C.; R. D.</td>
<td>43</td>
<td>38</td>
<td>60 (60)</td>
<td>11.3</td>
</tr>
<tr>
<td>2-51b</td>
<td>Frozen in field; R. D.</td>
<td>39.5</td>
<td>41</td>
<td>54 (52)</td>
<td>8.6</td>
</tr>
<tr>
<td>9-52</td>
<td>16 hrs., -7° C.; R. D.</td>
<td>39</td>
<td>44.5</td>
<td>67 (67)</td>
<td>11.5</td>
</tr>
<tr>
<td>10-52</td>
<td>16 hrs., -7° C.; 100 hrs., 45° C.</td>
<td>30</td>
<td>7.5</td>
<td>60 (49)</td>
<td>7.5</td>
</tr>
<tr>
<td>8-52</td>
<td>18 hrs., 2° C.; 110 hrs., 50° C.</td>
<td>29</td>
<td>7</td>
<td>50 (44)</td>
<td>5.2</td>
</tr>
<tr>
<td>13-52</td>
<td>8 hrs., -7° C.; R. D.</td>
<td>25.5</td>
<td>26</td>
<td>33 (31)</td>
<td>7.8</td>
</tr>
<tr>
<td>4-52</td>
<td>12 hrs., -7° C.; 114 hrs., 50° C.</td>
<td>18</td>
<td>1.5</td>
<td>45 (22)</td>
<td>3.2</td>
</tr>
<tr>
<td>2-52</td>
<td>4 hrs., -7° C.; 144 hrs., 50° C.</td>
<td>9.5</td>
<td>0</td>
<td>40 (10)</td>
<td>1.7</td>
</tr>
<tr>
<td>5-51b</td>
<td>16 hrs., -7° C.; R. D.</td>
<td>1</td>
<td>0</td>
<td>0 (0)</td>
<td>0.2</td>
</tr>
<tr>
<td>10-51b</td>
<td>16 hrs., -7° C.; 74 hrs., 40° C.</td>
<td>1</td>
<td>0</td>
<td>0 (0)</td>
<td>0.1</td>
</tr>
<tr>
<td>18-51b</td>
<td>65 hrs., 40° C.</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
<td>0</td>
</tr>
</tbody>
</table>
90 percent. In the tetrazolium tests, staining of the plumule, scutellar node and the central portion of the scutellum was considered essential to indicate the production of a normal seedling. The figures in parentheses show that tetrazolium tests agreed more closely with germination percentages when a complete embryo stain was the basis for estimating viability. However, even with this altered criterion, tetrazolium tests were still higher than germination tests by as much as 20 percent. These data indicate that the tetrazolium test gives unsatisfactory results for corn which has been exposed to certain freezing and drying conditions.

Vigor ratings on the basis of velocity and extent of germination were calculated for each lot to determine whether such an index of growth rate correlated with other indices of physiological activity. The data in Table 1 show these ratings to be more closely correlated with germination percentages than with cold test or tetrazolium percentages. However, the considerable variations in the lots germinating 98 percent or higher indicate that germination proceeded at a slower rate in some lots.

**Respiratory Capacity and Enzymatic Activity in Relation to Viability**

Respiratory capacity and enzymatic activity were measured on each lot to determine the extent to which lowered viability might be related to lowered respiratory activity. The means of duplicate determinations of these activities are presented in Table 2, along with germination percentages, respiratory quotients, ratios of anaerobic to aerobic carbon dioxide evolution (I/N) and tetrazolium ratings. The possibility of differences in metabolic activity due to variety differences was considered.
Table 2. Relation of respiratory and enzymatic activity to germination and tetrazolium rating.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Germ.</th>
<th>Malic dehydrogenase</th>
<th>Alcohol dehydrogenase</th>
<th>Cytochrome oxidase</th>
<th>Q'O</th>
<th>Q'CO₂</th>
<th>Q'CO₂</th>
<th>R. Q.</th>
<th>I/N</th>
<th>Tetra.</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-51a</td>
<td>100</td>
<td>20.7</td>
<td>33.3</td>
<td>0.59</td>
<td>4.24</td>
<td>2.34</td>
<td>1.63</td>
<td>0.55</td>
<td>0.70</td>
<td>-</td>
</tr>
<tr>
<td>23-51b</td>
<td>100</td>
<td>20.9</td>
<td>45.3</td>
<td>0.42</td>
<td>3.75</td>
<td>1.82</td>
<td>1.87</td>
<td>0.49</td>
<td>1.03</td>
<td>1.59</td>
</tr>
<tr>
<td>11-52</td>
<td>100</td>
<td>23.6</td>
<td>45.9</td>
<td>0.99</td>
<td>3.86</td>
<td>2.14</td>
<td>1.67</td>
<td>0.55</td>
<td>0.78</td>
<td>1.68</td>
</tr>
<tr>
<td>7-51a</td>
<td>100</td>
<td>21.2</td>
<td>44.1</td>
<td>0.73</td>
<td>4.08</td>
<td>2.26</td>
<td>1.63</td>
<td>0.55</td>
<td>0.72</td>
<td>1.66</td>
</tr>
<tr>
<td>12-51a</td>
<td>100</td>
<td>17.4</td>
<td>41.3</td>
<td>0.54</td>
<td>3.51</td>
<td>1.97</td>
<td>1.66</td>
<td>0.65</td>
<td>0.84</td>
<td>1.68</td>
</tr>
<tr>
<td>3-51b</td>
<td>99.5</td>
<td>22.4</td>
<td>58.4</td>
<td>0.53</td>
<td>3.66</td>
<td>1.84</td>
<td>1.95</td>
<td>0.50</td>
<td>1.06</td>
<td>1.66</td>
</tr>
<tr>
<td>7-52</td>
<td>99.5</td>
<td>20.2</td>
<td>41.0</td>
<td>0.72</td>
<td>4.49</td>
<td>2.59</td>
<td>1.86</td>
<td>0.58</td>
<td>0.72</td>
<td>1.68</td>
</tr>
<tr>
<td>4-51a</td>
<td>99.5</td>
<td>25.7</td>
<td>44.3</td>
<td>0.54</td>
<td>4.33</td>
<td>2.77</td>
<td>2.17</td>
<td>0.64</td>
<td>0.78</td>
<td>1.68</td>
</tr>
<tr>
<td>15-51b</td>
<td>99</td>
<td>21.8</td>
<td>41.0</td>
<td>0.28</td>
<td>4.11</td>
<td>2.24</td>
<td>2.08</td>
<td>0.54</td>
<td>0.93</td>
<td>1.68</td>
</tr>
<tr>
<td>8-51b</td>
<td>99</td>
<td>17.9</td>
<td>55.3</td>
<td>0.61</td>
<td>3.24</td>
<td>1.69</td>
<td>1.83</td>
<td>0.52</td>
<td>1.08</td>
<td>1.65</td>
</tr>
<tr>
<td>12-52</td>
<td>98.5</td>
<td>19.5</td>
<td>45.4</td>
<td>0.82</td>
<td>3.07</td>
<td>1.92</td>
<td>1.48</td>
<td>0.62</td>
<td>0.77</td>
<td>1.68</td>
</tr>
<tr>
<td>6-52</td>
<td>98</td>
<td>22.9</td>
<td>42.3</td>
<td>0.67</td>
<td>3.85</td>
<td>2.13</td>
<td>1.71</td>
<td>0.55</td>
<td>0.80</td>
<td>1.66</td>
</tr>
</tbody>
</table>

a "51a" indicates 1951 collection of B6 x W22; "51b" indicates 1951 collection of B6 x L289; "52" indicates 1952 collection of La. 4297.

b Mean of two 100 seed samples.

c Enzymatic activities are expressed as equivalent microliters oxygen uptake per milligram dry weight per hour and are means of two determinations.

d Respiratory rates are expressed as microliters gas exchange per milligram dry weight per hour and are means of two determinations.

e R. Q. = Q'CO₂/Q'O; I/N = Q'CO₂/Q'CO₂.

f Described in Materials and Methods.
Examination of the data, however, revealed such differences only in the case of cytochrome oxidase. For purposes of individual comparisons, scatter diagrams including regression lines and correlation coefficients are presented.

Seeds were not surface disinfected prior to soaking to avoid the possibility that chemicals used for treatment would be present in or on the embryo tissue and thus affect the respiratory and enzymatic activities. This, therefore, raises the question whether mold contributed to the metabolic activities measured. However, even lots which were known to be heavily contaminated did not have significantly higher respiratory and enzymatic activities than those which were not contaminated or only slightly so and which had comparable germination percentages. The only exception was lot 24-51a, which had visible mold damage in the embryo itself before soaking. These observations indicate that mold activity was not a significant factor in the measurements.

The data in Table 2 not only provide information relating to levels of respiratory activity in lots of varying viability but also, with proper comparisons, allow some judgement of the levels in individual seeds. It is of interest to know the activity of viable seeds (i.e., those capable of germination) and to know to what extent this activity may decrease before the ability to germinate is lost. Furthermore, the respiratory activity, if any, of nonviable seeds (i.e., those incapable of germination) is also of interest. Careful examination of the relationships shown when metabolic activities of lot means are plotted against germination should allow some judgement of this kind.
The relationship between oxygen consumption and germination is illustrated in Figure 1. As would be expected, the graph shows an essentially linear correlation, but with certain deviations. Lots 3-52, 9-52 and 13-52, indicated in the graph, had a large number of seeds which showed initiation of growth but failed to produce seedlings. Since the oxygen consumption rates for these lots were higher than would be expected from the germination percentages, the abnormal seeds apparently were respiring at 18 hours. The rate for lot 24-51a was high also, probably due to mold respiration. With these exceptions, the data indicate that seeds will not germinate if their respiratory activity is greatly decreased. On the other hand, nonviable seeds apparently have very little or no respiratory activity. The data in Table 2 show no marked changes in either the respiratory quotient or the ratio of anaerobic to aerobic carbon dioxide evolution as germination percentages became lower. This indicates relationships of aerobic and anaerobic carbon dioxide evolution to germination very similar to that shown by oxygen consumption.

Figure 2 shows that malic dehydrogenase activity is related to germination in an essentially linear fashion, with the exception of the lots described above. However, malic dehydrogenase activity did not decrease to the same extent as germination percentage. This is shown by the lots which had significant enzyme activity but very low germination percentages. It would appear, therefore, that seed could not have significantly decreased malic dehydrogenase activity and still remain viable whereas nonviable seed retained significant activity.
Figure 1. Relation of oxygen consumption rate to germination percentage.

(Each point represents the mean of duplicate determinations.)
GERMINATION %

Q₂ UPTAKE (Q₀₂)

- B6 x w22
- B6 x L289
- la. 4297

r = 0.92
Figure 2. Relation of malic dehydrogenase activity to germination percentage.

(Each point represents the mean of duplicate determinations.)
A somewhat similar relationship is shown in Figure 3 for alcohol dehydrogenase activity and germination, although certain exceptions are obvious. In the first place, the lots germinating below 2 percent did not have significant alcohol dehydrogenase activity. In the second place, three lots had much lower activity than could be predicted by germination. One of these lots was 24-51a, which had very high malic dehydrogenase activity. Lots 3-52, 9-52 and 13-52, as in the case of malic dehydrogenase, had somewhat higher alcohol dehydrogenase activity than could be expected from the germination percentages. The data show, therefore, that viable seed may be very low in alcohol dehydrogenase activity, and on the other hand nonviable seed may retain significant activity.

To relate the relative activities of malic and alcohol dehydrogenase as viability decreased, the two are compared in Figure 4. The graph shows greater relative decreases for alcohol dehydrogenase activity than for malic dehydrogenase activity. Several lots in particular had significant malic dehydrogenase activity but practically no alcohol dehydrogenase activity. This indicates that of the two, alcohol dehydrogenase was the more susceptible to conditions used to lower viability. As shown earlier, these extreme decreases in alcohol dehydrogenase activity had no appreciable effect on germination.

Figure 5 illustrates the relationship between cytochrome oxidase and germination. Extreme variations in cytochrome oxidase activity were very much in evidence, particularly in the lots having high germination percentages. This can partly be explained by variety differences, since a significant difference in cytochrome oxidase was found between
Figure 3. Relation of alcohol dehydrogenase activity to germination percentage.

(Each point represents the mean of duplicate determinations.)
ALCOHOL DEHYDRO. (QO₂)

GERMINATION %

- B6 x w22
- B6 x L289
- la. 4297
r = 0.82

24-51a

0 10 20 30 40 50 60 70 80 90 100

0 10 20 30 40 50 60 70

9-52
3-52
13-52
Figure 4. Relation of malic dehydrogenase activity to alcohol dehydrogenase activity.

(Each point represents the mean of duplicate determinations.)
A scatter plot shows the relationship between Malic Dehydro. ($Q_{O_2}$) and Alcohol Dehydro. ($Q_{O_2}$). The plot includes three sets of data points:

- Black circles: B6 x w22
- Black dots: B6 x L289
- Open circles: la.4297

The correlation coefficient ($r$) is 0.70.
Figure 5. Relation of cytochrome oxidase activity to germination percentage.

(Each point represents the mean of duplicate determinations.)
The graph shows a positive correlation between cytchrome oxidase (QO2) and germination percentage (GERMINATION %). The correlation coefficient (r) is 0.57. The data points are differentiated by the following symbols:

- B6 x w22
- B6 x L289
- la. 4297

Key points on the graph include:
- 3-52
- 24-51a
- 13-52
- 9-52

The graph suggests that as the germination percentage increases, the cytchrome oxidase activity also increases.
the 1952 collection and either of the 1951 varieties in lots germinating 98 percent or better. The high activity in lots 24-51a, 3-52 and 13-52 could be explained by the reasons given earlier. It is apparent that seeds may have extremely low cytochrome oxidase activity and yet be viable.

The various indices of metabolic activity might have been expected to show a higher correlation with cold tests or tetrazolium tests than with germination tests. However, an inspection of the data in Tables 1 and 2 revealed no marked decreases in metabolic activity in lots which had lower cold test values than germination percentages. Lots which had high tetrazolium tests in comparison with germination percentages also had relatively high dehydrogenase activities. This would be expected if tetrazolium reduction is limited by dehydrogenase activity. It is likely, therefore, that the dehydrogenase relationship may explain the difference between tetrazolium and germination response.

It also seemed reasonable to expect that some of the various indices of metabolic activity might show a closer relationship to rates of growth than to ability to germinate. The data in Tables 1 and 2, particularly in the lots which had high germination percentages and variable vigor ratings, show that only in the case of cytochrome oxidase was there such a trend. However, when cytochrome oxidase activity was graphically compared with vigor ratings (Figure 6), it did not show a higher correlation with vigor ratings than with germination. In general, the evidence is insufficient to say that cytochrome oxidase limits rate of growth more than ability to germinate.
Figure 6. Relation of cytochrome oxidase activity to vigor rating.

(Each point represents the mean of duplicate determinations.)
CYTOCHROME OXIDASE (Q_o2)

VIGOR RATING

- B6 x w22
- B6 x L289
- la. 4297
r = 0.65
The data in Table 2 also provide an opportunity to compare enzymatic activity with respiratory activity to determine the extent to which the various enzymes limit respiration. Figure 7 shows the relation between cytochrome oxidase and oxygen consumption. A wide range of activity of this enzyme was found in lots having high oxygen consumption rates (2.5 to 4.5 microliters per milligram per hour). At lower rates, cytochrome oxidase activity decreased as respiratory activity decreased. This shows oxygen consumption to be essentially independent of cytochrome oxidase activity, at least until low enzyme activity is reached. In almost all lots, cytochrome oxidase activity measured was insufficient to support more than half of the total oxygen consumption. This indicates that cytochrome oxidase is not the major terminal oxidase in corn embryos and might explain the almost complete independence of oxygen consumption and cytochrome oxidase activity.

Figure 8 shows a high degree of correlation between malic dehydrogenase and oxygen consumption. The latter rates, however, were very low in several lots which had significant malic dehydrogenase activity. Residual activity of this enzyme in seeds which did not germinate was evident in Figure 2. The data in Figure 8 indicate that malic dehydrogenase is an essential part of the oxygen consumption mechanism of corn embryos and that it may be limiting until its activity reaches a minimum level where other factors become limiting. Of course, the possibility also remains that activity of malic dehydrogenase is merely closely correlated with that of some other enzyme which controls the oxygen consumption rate.
Figure 7. Relation of oxygen consumption rate to cytochrome oxidase activity.

(Each point represents the mean of duplicate determinations.)
Figure 8. Relation of oxygen consumption rate to malic dehydrogenase activity.

(Each point represents the mean of duplicate determinations.)
- B6 x w22
- B6 x L289
- la. 4297
r = 0.91
Though alcoholic fermentation has not been reported in corn embryos, it seemed of interest to compare alcohol dehydrogenase activity and anaerobic carbon dioxide evolution. Figure 9 indicates a general relationship between the two. However, there were three lots which had relatively high anaerobic carbon dioxide evolution rates but very low alcohol dehydrogenase activity. Also, several lots had higher alcohol dehydrogenase activity than would be expected from the respiratory rates. It must be concluded that while alcohol dehydrogenase participates in anaerobic carbon dioxide evolution in corn, it is probably not usually the limiting factor.

It seemed desirable to estimate the extent to which the two dehydrogenases were involved in the reduction of tetrazolium. Therefore, the sum of the activities of malic and alcohol dehydrogenase were compared with tetrazolium ratings (Figure 10). The lots which had high tetrazolium ratings had quite variable dehydrogenase activity. At lower ratings, dehydrogenase activity decreased with decreasing amounts of tetrazolium reduced. Lots 1-5la, 2-51b and 24-51a, as shown in the figure, had unexplained low alcohol dehydrogenase activity and did not follow the expected pattern. Although activity of these two dehydrogenase apparently decrease with tetrazolium reducing ability, the data indicate that other dehydrogenase are also involved. Similar comparison of tetrazolium ratings with malic and alcohol dehydrogenase activity individually showed no closer correlations than obtained with the sums of the two. Tetrazolium ratings were only rough approximations of the amount of tetrazolium reduced. A closer correlation might have been
Figure 9. Relation of anaerobic carbon dioxide evolution rate to alcohol dehydrogenase activity.

(Each point represents the mean of duplicate determinations.)
Figure 10. Relation of tetrazolium rating to malic and alcohol dehydrogenase activity.

(Each point represents the mean of duplicate determinations.)
found if quantitative determination of the amounts of tetrazolium reduced could have been made.

**Respiration of Seed Parts and Whole Seed**

Aerobic respiratory capacity measurements in the preceding experiments were made by the standard technique involving slices immersed in buffer in an atmosphere of oxygen. Slices were used to minimize limitation in rates by gas diffusion and preliminary experiments had shown that the slices respired at a greater rate in oxygen than in air. However, since no reports on corn embryo respiration were found in the literature, it was desirable to investigate factors effecting the rate of gas exchange, with particular emphasis on tissue thickness and oxygen tension. This study also included measurements on whole seeds and dissected seeds to determine the relative respiratory capacities of various seed parts.

To study the effect of tissue thickness on respiration, measurements were made on whole embryos, bisected embryos and embryo slices. The data on immersed embryo respiration in Table 3 show that tissue thickness had a marked effect on oxygen consumption but none on carbon dioxide evolution of embryos. In comparison with the rates for whole embryos, rates of oxygen consumption were slightly higher for bisected embryos and more than twice as high for embryo slices. The largest difference, between bisected embryos and sliced embryos, corresponded to the greatest reduction in tissue thickness. There was no similar increase in carbon dioxide evolution as tissue thickness was reduced from that of whole
Table 2. Effects of tissue thickness and immersion on respiration.

<table>
<thead>
<tr>
<th>Seed Part</th>
<th>Condition</th>
<th>H, q</th>
<th>O2, %</th>
<th>CO2, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole seed</td>
<td>Hot immersed</td>
<td>0.075</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>Whole seed</td>
<td>Not immersed</td>
<td>0.147</td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>Seed less percentage</td>
<td>Hot immersed</td>
<td>0.295</td>
<td>0.295</td>
<td></td>
</tr>
<tr>
<td>Seed less percentage</td>
<td>Not immersed</td>
<td>0.157</td>
<td>0.157</td>
<td></td>
</tr>
<tr>
<td>Blasted empires</td>
<td>Hot immersed</td>
<td>0.595</td>
<td>0.595</td>
<td></td>
</tr>
<tr>
<td>Blasted empires</td>
<td>Not immersed</td>
<td>0.207</td>
<td>0.207</td>
<td></td>
</tr>
<tr>
<td>Sticked, blasted empires</td>
<td>Hot immersed</td>
<td>3.972</td>
<td>3.972</td>
<td></td>
</tr>
<tr>
<td>Sticked, blasted empires</td>
<td>Not immersed</td>
<td>2.687</td>
<td>2.687</td>
<td></td>
</tr>
</tbody>
</table>

Note: Data per hour are averages of two to five determinations. Weight per hour are averages of two to five determinations.
embryos. It therefore appears that oxygen diffusion was limiting at the greater thicknesses, whereas carbon dioxide diffusion was not. Another factor which might have contributed to the high oxygen consumption rates of thinner tissue was wound stimulation which might be expected to increase with the greater extent of cutting. However, the absence of similar changes in carbon dioxide evolution casts doubt on this possibility. Respiratory quotients increased from 0.78 for slices to 1.80 for whole embryos, a change due entirely to decrease in oxygen consumption. The higher respiratory quotients seemed to indicate a partial shift to anaerobic respiration under conditions in which oxygen diffusion was limiting. However, on this basis it is difficult to see why carbon dioxide evolution did not increase in the thicker tissue preparation (e. g., whole embryos).

The effects of tissue thickness on embryo respiration when tissue was not immersed were somewhat different. Only a slight increase in oxygen consumption rates of slices was found as compared to rates for bisected embryos and no increase was found for bisected as compared to whole embryos. This indicates that oxygen diffusion was not limiting to the same extent as when tissue was immersed, probably due to the higher concentration of oxygen to which tissue was exposed when no liquid was present. Again, it is also possible that the slightly higher rates for slices were due to wounding, although no similar increase in carbon dioxide evolution was found. As when tissue was immersed, decreased tissue thickness did not result in increased carbon dioxide evolution rates. The small changes in respiratory
respiration in such a way as to reduce carbon dioxide evolution.

nation is that the phosphate buffer used as a suspending medium altered the respiration rate.

Therefore, the other factors were involved. It becomes apparent that if the surrounding tissues did not allow carbon dioxide escape, it became under similar conditions. Also, there is no reason to believe that the rates of carbon dioxide evolution for tissue of different thicknesses was not a factor in carbon dioxide evolution as shown by the constant diffusion or concentration of carbon dioxide, that rate of diffusion or extraction caused by immobilization can hardly be expected by the rate of pronounced as tissue thickness increased. The decrease in carbon dioxide when the rate of tissue was increased by the increase in thickness would become more apparent. Further, rates in nonimpressed tissues. Furthermore, a decrease in the thickness of tissue, the muscle accounted for the nonimpressed tissues was expected to an oxygen partial pressure approximated the air at the time that the surrounding tissues were exposed to an oxygen partial pressure approximated the air. A decrease in the thickness of the tissue was expected by the oxygen concentration to which the tissue was subjected or measured. The thickness of tissue between oxygen concentrations was less than 0.1 mm. The thickness difference between oxygen concentrations of 0.1 millimolar carbon dioxide evolution rates were also higher for nonimpressed tissues, however, the difference was constant for all thicknesses of tissues. The thickness difference for the tissues decreased with increasing thickness, with the tissue being more when the tissue was not exposed to an oxygen partial pressure approximated the air at the time that the surrounding tissues were exposed to an oxygen partial pressure approximated the air. The effect of immobilization, therefore, is evident in the data on respiration.

The type of respiration in nonimpressed as compared to impressed tissues.

the results with tissue thickness indicated much less effect.
phosphate might also have been involved in the effect of immersion on oxygen consumption. However, no evidence could be found in the literature to support such an effect of phosphate on slice respiration. So until this possibility is studied further, it seems doubtful that it explains the immersion effect.

Comparison of the respiratory rates for whole seeds with those for seed in which the pericarp had been removed from the embryo side showed the importance of the pericarp in restricting gas exchange. Oxygen consumption was increased two and a half times by removing this much of the pericarp, whereas carbon dioxide evolution was increased only one and a half times. This seems to indicate that the pericarp limits oxygen diffusion more than carbon dioxide diffusion. The respiratory quotient for whole seeds with intact pericarp (1.38) was high enough to indicate a considerable amount of anaerobic respiration.

Table 3 indicates further that seed residues had a low but significant respiratory rate which on a dry weight basis was only about 2 percent of that for whole embryos not immersed. That the respiration of seed residues was not due to endosperm was shown when respiratory measurements were made on the latter tissue alone. Total oxygen consumption for 1.3 g. of endosperm was only about 10 microliters an hour, which was considered too small to establish a significant rate with the apparatus used. Apparently other tissues in the seed residues, probably the aleurone layer, were responsible for residue respiration.

On a per seed basis, seed residue respiration was less than a fourth of that of seeds with the pericarp removed from the embryo side. Rates
of the latter were less than the sum of the rates for the two component parts, the embryo plus the seed residue. This would indicate that gas diffusion was limiting in whole seeds, even with part of the pericarp removed.

Results from preceding experiments made it evident that respiration of embryo slices was affected by oxygen tension. To further establish this fact, respiratory measurements were made on slices in air and in oxygen, both immersed and nonimmersed. This provided four different partial pressures of oxygen to which tissue was exposed, the two in buffer calculated from the concentration of oxygen in water exposed to air and to oxygen. The respiratory rates obtained are summarized in Table 4. Oxygen consumption increased when the oxygen partial pressure was raised from 3.7 to 18.5 mm. (of mercury) but did not between 18.5 and 152 mm. A further increase was found when the partial pressure was raised to 760 mm. Aerobic carbon dioxide evolution, on the other hand, increased throughout the range. Respiratory quotients remained relatively constant except for the value at 18.5 mm. This was lower but it agreed with those obtained previously for slices under these conditions. The two respiratory quotients for slices in air, immersed and not immersed, agreed closely with those reported in Table 3. The observed results are difficult to explain purely on the basis of oxygen tension, particularly since oxygen consumption was the same at 18.5 and 152 mm. Again it is apparent that the rates were not only affected by oxygen tension, but also by some factor inherent in immersion.
Table 4. Effects of oxygen tension and immersion on respiration of corn embryo slices. (Sample 6-52, seed soaked 18 hours.)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Oxygen partial pressure</th>
<th>O$_2$</th>
<th>CO$_2$</th>
<th>R. Q.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slices immersed, air in flasks</td>
<td>3.7 mm. Hg</td>
<td>2.23</td>
<td>1.74</td>
<td>0.78</td>
</tr>
<tr>
<td>Slices immersed, oxygen in flasks</td>
<td>18.5 mm. Hg</td>
<td>3.90</td>
<td>2.26</td>
<td>0.58</td>
</tr>
<tr>
<td>Slices not immersed, air in flasks</td>
<td>152 mm. Hg</td>
<td>3.91</td>
<td>3.35</td>
<td>0.86</td>
</tr>
<tr>
<td>Slices not immersed, oxygen in flasks</td>
<td>760 mm. Hg</td>
<td>4.86</td>
<td>3.85</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*Rates are expressed as microliters gas exchange per milligram dry weight per hour and are averages of two or three determinations.

R. Q. = $Q_{CO_2}/Q_{O_2}$. 
Respiratory and Enzymatic Activity As a Function of Stage of Germination

The data presented so far were obtained from seed soaked 18 hours. It was desirable, therefore, to know how rapidly respiratory and enzymatic activity were changing at this stage of germination particularly since no previous work of this kind is available on the metabolic activity of corn during germination. This was done by measuring these activities in embryos from dry seed and seed soaked 9, 18 and 27 hours. Embryos in seed soaked 9 hours showed visible swelling while those in seed soaked 18 hours were more completely imbibed and had occasional emergence of the radicle. After 27 hours, radicles were approximately 1 cm. in length and coleoptiles were 1-2 mm. in length.

It must be emphasized that rates summarized in Table 5 do not measure the respiration of whole embryos in the seed, but are measures of the relative respiratory capacities present at the various stages of germination. This distinction is particularly significant for determinations on embryos from dry seeds. The respiration rate of dry embryos is far too low to measure over a short period of time with the apparatus used. Therefore, slices from dry embryos, as well as embryos at the other stages, were suspended in phosphate buffer for respiratory measurements. In addition, slices from dry embryos were kept in an ice bath until placed in the respirometer flasks, just as other slices were. That this additional soaking time did not increase the respiratory rates of slices from dry embryos was shown when this procedure was omitted. Dry embryo slices, therefore, were exposed to water for about 1 hour before readings were started. Respiratory rates must have increased
Table 5. Effect of time of soaking on respiratory capacity of immersed embryo slices and on enzymatic activity of embryos. (Sample 6-52)

<table>
<thead>
<tr>
<th>Hours soaked</th>
<th>Dry wt. per 10 embryos&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Q&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Q&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R. Q.&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Q&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Q&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>R. Q.&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Malic dehydrogenase&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Alcohol dehydrogenase&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Cytochrome oxidase&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>237 mg.</td>
<td>1.08</td>
<td>1.10</td>
<td>1.02</td>
<td>1.44</td>
<td>0.93</td>
<td>0.65</td>
<td>15.9</td>
<td>35.4</td>
<td>0.41</td>
</tr>
<tr>
<td>9</td>
<td>255 mg.</td>
<td>1.78</td>
<td>1.35</td>
<td>0.76</td>
<td>2.30</td>
<td>1.34</td>
<td>0.58</td>
<td>16.2</td>
<td>34.2</td>
<td>0.71</td>
</tr>
<tr>
<td>18</td>
<td>269 mg.</td>
<td>2.23</td>
<td>1.74</td>
<td>0.78</td>
<td>3.90</td>
<td>2.26</td>
<td>0.58</td>
<td>16.6</td>
<td>38.9</td>
<td>0.75</td>
</tr>
<tr>
<td>27</td>
<td>279 mg.</td>
<td>2.34</td>
<td>2.13</td>
<td>0.91</td>
<td>5.32</td>
<td>2.85</td>
<td>0.54</td>
<td>24.7</td>
<td>47.4</td>
<td>0.81</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dry weights are averages of 15 determinations except at 0 hours, which is an average of 5 determinations.

<sup>b</sup>Respiratory rates are expressed as microliters gas exchange per milligram dry weight per hour and are averages of three determinations.

<sup>c</sup>R. Q. = Q<sub>CO<sub>2</sub></sub>/Q<sub>O<sub>2</sub></sub>.

<sup>d</sup>Enzymatic activities are expressed as equivalent microliters oxygen uptake per milligram dry weight per hour and are averages of three determinations.
many fold during this period when the cells were becoming imbibed. This increase is assumed to be due to activation of existing enzymes rather than formation of new ones; so the "0 hour" activities measure the potential enzymatic activity in the dormant seed. It was also apparent that increase in rates due to imbibition in this period was essentially complete in the first hour since rates were linear over the second hour during which they were measured.

The data in Table 5 show that respiratory activity increased as length of time of exposure of seeds to water was increased. Oxygen consumption and carbon dioxide evolution in air, as well as anaerobic carbon dioxide evolution, increased approximately twofold at 27 hours. The greatest rate of increase, particularly in oxygen consumption, was in the period between 0 and 9 hours. It is probable that the rate of imbibition was greatest in this period also. Since essentially no growth was observed at the 18 hour period, it would appear that the higher respiratory activity at this stage was due to more complete imbibition of the tissue. Assuming that imbibition was essentially complete at 18 hours, the increased rate at 27 hours could be attributed to growth and the accompanying formation of new enzymes. Up to 18 hours, increases in respiratory activity were probably due only to activation of existing respiratory capacity as a result of water uptake. Respiratory quotients in air were initially near unity and then decreased to about 0.77 at 9 and 18 hours. They were higher again at 27 hours. This pattern indicates that respiration was first dependent on carbohydrates and later on fats. The higher respiratory quotient at 27 hours
suggests a shift back toward pure carbohydrate metabolism, probably due to translocation of sugars from the endosperm. Relative increases of oxygen consumption and carbon dioxide evolution with time were greater in oxygen than in air. This seems to indicate that as respiratory capacity increases with time of soaking the limiting effect of oxygen diffusion becomes greater.

Enzymatic activities showed somewhat different patterns during the early stages of germination. Malic and alcohol dehydrogenase activities were relatively high in dry seed and remained at approximately this level through the 18 hour stage. Significant increases in activity were found only at 27 hours. At all stages there was more than sufficient dehydrogenase activity to account for oxygen consumption. These data indicate that dry seed have a large amount of these two enzymes and that activation depends only on imbibition of water. The increase at 27 hours is probably due to the formation of new enzymes in the process of growth.

Cytochrome oxidase activity, on the other hand, was relatively low in dry seeds and appeared to increase only in the first 9 hours. The most striking results are the very low rates obtained, particularly when compared with the dehydrogenase rates and the oxygen consumption rates. In no case was cytochrome oxidase activity sufficient to account for total oxygen consumption. Generally, only about a third of the oxygen consumption could be attributed to cytochrome oxidase activity. This indicates that either the method measured only a fraction of total cytochrome oxidase present or that other terminal oxidases were present.
Peroxidase was found to be approximately 200 times as active as cytochrome oxidase and presents one possibility of another terminal oxidase system.

Although the data show small increase in dry weight during each 9 hour interval, these cannot be considered significant. In the first place, the figure given for dry seeds is low due to inability to obtain all the scutellar tissue when embryos were excised, even though seeds were soaked cold overnight to facilitate excision. The figure for 9 hours is probably slightly low also, as excision was difficult in a few seeds even at this stage. There were also no increases in protein nitrogen through the 27 hour period, since determinations showed that the level remained at approximately 25 mg. per gram dry weight of embryo. Because of the lower respiratory and enzymatic activities of the scutellum in comparison with those of the embryo axis, the effect of loss of scutellar tissue on the activity for whole embryos was considered. Calculations based on the differences in activities and the estimated amount of scutellum not excised showed that the respiratory and enzymatic activities would have been not more than 5 percent less in the dry seed. Therefore, this loss of relatively inactive tissue did not seriously influence the high activities found for whole embryos from dry seeds.
DISCUSSION

Growth of any type must be dependent on respiratory metabolism so it seems likely that loss of ability of seeds to germinate may be due to some type of deficiency in the respiratory mechanism. The close agreement frequently observed between germination tests and tetrazolium tests, which are dependent on dehydrogenase activity, strongly supports this possibility. For this reason, the general objective of this investigation was to determine the extent to which ability to grow, or germination, was related to respiratory activity in the early stages of growth. It was hoped that this information would provide a more systematic basis for the development of quick tests for viability based on indicators of respiratory activity or the activity of particular enzymes or groups of enzymes.

The comparison of rate of oxygen consumption at 18 hours with germination revealed a relatively high linear correlation. The principal deviations were due to lots which had a high proportion of seeds which initiated growth but failed to germinate. Such seeds may very well have had near normal respiration at the 18 hour period but have been limited in their growth by respiratory failure or some other type of metabolic breakdown at a later stage. Since there were no characteristic changes in the respiratory quotients or I/N values, there was no evidence to indicate that loss of viability was associated with differential inactivation of oxygen consumption or carbon dioxide evolution processes. In general, the measurements of overall respiratory activity indicated that loss of viability may in most cases be
due to respiratory failure. However, this type of analysis alone does not rule out the possibility that respiratory failure is merely closely correlated with some other type of metabolic breakdown.

The relation of malic and alcohol dehydrogenase to viability was of particular interest since these two enzymes have been shown to be especially active in tetrazolium reduction in corn embryos (39). The failure of tissue to reduce tetrazolium, however, cannot be assumed to be due to absence of dehydrogenase activity. It is also possible that preceding steps in the respiratory mechanism are inactive and thus fail to produce the necessary substrates for dehydrogenase activity. It would, therefore, be valuable to compare malic dehydrogenase activity to both germination and tetrazolium tests. Examination of the comparison of malic dehydrogenase with ability to germinate showed roughly the same relation as in the case of overall respiration and germination. Lots having seeds with limited ability to grow were relatively high in malic dehydrogenase activity just as they were high in respiratory activity. The principle difference was that there was significant malic dehydrogenase activity in seeds incapable of growth. The fact that malic dehydrogenase activity, at least down to a certain level, was highly correlated with oxygen consumption indicates that this enzyme may limit oxygen consumption and, therefore, may also limit growth. Alcohol dehydrogenase was similar to malic dehydrogenase in that high activity was present in lots with seeds showing limited growth. The difference is that there was no evidence of appreciable alcohol dehydrogenase activity in seeds incapable of germination.
In addition, alcohol dehydrogenase activity was very low in some lots having a high percentage of viable seeds, which would indicate that alcohol dehydrogenase probably does not limit growth.

The relation of these two dehydrogenase activities to germination seems to offer a possible explanation for the differences observed between tetrazolium tests and germination tests. The fact that tetrazolium tests were generally higher may be due to some lots containing seeds having high respiratory activity but limited ability to grow and also to the presence of malic dehydrogenase in seeds which do not germinate. An attempt was made to determine the extent to which tetrazolium reduction is dependent on malic and alcohol dehydrogenase activity by comparing the activity of these two enzymes with tetrazolium ratings. The latter were based on the estimates of the total amount of tetrazolium reduced rather than on the staining of critical embryonic parts on which tetrazolium tests were based. However, no significant difference was observed between alcohol and malic dehydrogenase in their relation to tetrazolium rating. And, although there was a rough correlation between the combined activities of these two enzymes and tetrazolium ratings, it must be concluded that this kind of data could not establish whether these two dehydrogenases are primarily responsible for tetrazolium reduction in corn embryos. It is possible that other dehydrogenases are also important and that other parts of the respiratory mechanism involved in the production of dehydrogenase substrates might be limiting rather than the dehydrogenases themselves.
The results presented here would indicate that tetrazolium tests give unsatisfactory estimates of germination capacity in corn subjected to freezing and high temperature drying. Bennet (6) earlier reported similar results for corn which had been frozen. However, the treatments given the lots in the present work were probably more severe than would ordinarily be encountered by seed in production and marketing. Therefore, these results do not disprove the value of tetrazolium tests for quick estimates of viability.

The reason for selecting cytochrome oxidase for comparison with ability to grow was that there was some evidence that it might be the principal terminal oxidase in corn seedlings. Also, growth of corn seedlings is commonly considered to be dependent on aerobic processes. Cytochrome oxidase activity showed much greater variability in relation to germination than did the activity of the two dehydrogenases. Only in the case of the lots having very low germination percentages did it appear that cytochrome oxidase might limit ability to germinate. A similar relationship was found between cytochrome oxidase and oxygen consumption. The extreme variability of this enzyme was not related to variations found in rate of germination, expressed by the vigor ratings. The very low cytochrome oxidase activities found even in lots having maximum germination was surprising. Not only was the oxidase activity low in comparison with oxygen consumption but it was only about a third of that found by Maxwell (30) at approximately the same stage of germination. There is reason to believe, however, that differences in variety and method may be largely responsible for this lack of
agreement. Until a more complete investigation of the terminal oxidases in corn is undertaken, it may be concluded that oxidases other than cytochrome oxidase are present in corn embryos.

Since cold tests are a measure of the ability of corn to germinate under adverse conditions, lots having low cold tests might be expected to be less vigorous than lots having high cold tests but similar germination percentages. Furthermore, such cases of reduced vigor might be a result of reduction in activity of particular processes in respiratory metabolism. Another possible explanation, however, is that cold tests might be lower than germination percentages because of damage to the pericarp, resulting in less protection from microorganisms. The results obtained in this investigation showed no marked reduction in either respiratory or enzymatic activity in lots having lower cold tests than germination percentages. It would appear then that the differences between cold tests and germination tests might be due to some type of pericarp damage in these lots. However, the possibility remains that the differences may have been due to decreased metabolic activity of some type not measured.

Maximum respiratory rates can only be achieved when oxygen concentration at the sites of respiratory activity and rate of removal of carbon dioxide from these sites are not limiting. When possible, respiratory capacity measurements are usually determined on thin slices of plant material so that the effects due to oxygen and carbon dioxide concentration are minimized. Of the two, the effect of oxygen concentration is commonly considered to be more important. With these
principles in mind, embryo slices rather than whole embryos were selected for determination of respiratory rates in relation to viability. In addition, rates were determined with an oxygen gas phase to further minimize the effect of oxygen diffusion. However, subsequent investigation revealed that although rates under these conditions were greater than if either whole embryos or air in the gas phase had been used, they were still greater when the gas phase was oxygen and the slices were not immersed in buffer. These results indicate that oxygen diffusion to the respiratory centers was limiting in the immersed slices, even when the gas phase was oxygen. The limitation of oxygen consumption by decreased oxygen tension has been reported for slices of various other plant tissues of similar thicknesses (25, 28, 43). Carbon dioxide evolution seemed to be virtually independent of tissue thickness but was markedly reduced by immersion of the slices in buffer. This effect of immersion on respiration, particularly carbon dioxide evolution, cannot be explained on the basis of the present data. The overall results show that tissue thickness, oxygen concentration in the gas phase and the presence of buffer are critical factors in the measurement of maximum respiratory rates.

The respiratory rates of seed parts are in general agreement with those reported by Stoward (45) for seed at approximately the same stage of development. The tissue in his experiments were suspended on a layer of water and the conditions, therefore, were not exactly the same as used here. In fact, the rates obtained for immersed rather than non-immersed whole embryos agree with his rates for whole embryos. It is
not inconceivable that sufficient water was present in his experiments to virtually surround the excised embryos. The relative rates of whole embryos in comparison with those of intact seed and seed residues also agree with those found by Stoward. The respiratory quotient found for whole seeds was well above unity, whereas Stiles and Leach (44) reported respiratory quotients slightly below unity for corn at a similar stage of germination. This lack of agreement could possibly be due to pericarp condition since it was found in the present work that seeds with pericarp removed on the embryo side had a respiratory quotient below unity. It has also been reported that the respiratory quotient of germinating barley is well above unity until the seed coat is ruptured by emergence of the coleorhiza (10, 32). This phenomenon was attributed to anaerobic respiration caused by limitation of oxygen diffusion through the seed coat. Therefore, it seems likely that a similar condition may be true in corn with undamaged pericarp. The corn used in this investigation was hand-shelled and had very little or no pericarp injury.

Embryos from dry corn were found to have a relatively high capacity for respiratory and enzymatic activity, indicating that a normal supply of oxidative enzymes and substrates were present in the dry state and that the resumption of respiratory activity depended entirely on imbibition of water. This is consistent with the results reported by Leach (24) for respiration of germinating wheat. The increase in respiratory rate up to 18 hours was probably due only to imbibition of the tissue. Picklum (34) reported that cells of embryos from corn under comparable soaking conditions were turgid at this period and that mitotic activity
was not evident until afterwards, specifically, until after the coleorhiza had emerged. Therefore, growth and the synthesis of new protoplasm were apparently not a factor in the respiratory activity obtained at 18 hours. The lack of significant increases in either dry weight or protein nitrogen per unit dry weight also are evidence that water uptake was the primary factor in the respiratory rates at 18 hours. Although no increase was found in total protein nitrogen at the 27 hour stage, the increases in malic and alcohol dehydrogenase activity at this period indicated synthesis of these enzymes, as a result of growth. The low cytochrome oxidase activity and the early increase in activity, as compared with the dehydrogenases, is puzzling and requires further investigation before an explanation could be attempted. The change in respiratory quotients with time observed here for embryo slices in air is similar to that reported by Stiles and Leach (44) for whole seed, in that the respiratory quotient was initially near unity, then subsequently decreased and finally increased to approach unity again. This would indicate, as was their conclusion, that initial respiration depends on carbohydrates in the dry embryo and that later respiration depends first upon fat utilization and finally on carbohydrates translocated from the endosperm. However, the shifts in type of respiration observed here came at a much earlier stage than those found by Stiles and Leach (44). This difference is puzzling but might be partly explained by the different conditions under which respiration was measured, since excised embryo slices were used in one case and
whole kernels in the other, and by possible differences in stages of development at the various times.

Although this investigation has shown that a close relationship existed between respiratory activity measured at an early stage and ability to grow, the data were not of such nature as to permit an accurate evaluation of the factors responsible for loss of viability in individual seeds. In addition, the results obtained from the lots having seeds with limited ability to grow show that seeds may have near normal respiration at an early stage in the germination process but still fail to germinate. The extent to which such seeds occur naturally limits the validity of using measurements of respiratory activity made at an early stage as indices of germination potential. As was the case with the respiratory measurements, the data from the three respiratory enzymes were not of such nature as to prove that activity of any of the three was a limiting factor in growth. Different factors may be responsible for lack of ability to grow in different individual seeds. It is also possible that other parts of the metabolic mechanism or other enzymes may be more critical in the growth process.

Therefore, it seems evident that a different technique may be required to effectively study the metabolic limitations of ability to grow in seeds. Pooled samples of seed, as employed here, do not permit an adequate evaluation of variation among individual seeds. It is conceivable that a more intensive statistical analysis involving more replicates of pooled samples with less seeds per replicate would offer more information of this type. However, it seems more likely that the
development of methods for comparing metabolic or enzymatic activity with ability to grow on individual seeds may be necessary to obtain the desired information. In particular, it seems evident from the topographic nature of the tetrazolium test that measurements of metabolic activity of critical embryonic parts are required for an accurate evaluation of the factors which limit ability of seeds to germinate. This type of an investigation would involve using one half of a seed for determinations of metabolic activities and the other half for determination of ability to grow. The former could probably be done by scaling down the existing analytical methods. While there is some evidence that the latter is possible with corn, the possibility has not been sufficiently investigated. Another possible technique is the development of treatments which would insure more uniform damage to all seed within a lot, enabling a comparison of metabolic activity of one seed or embryonic parts of one seed with the growth response of another seed. This approach also would require extensive investigation before it could be considered of merit. Determinations of metabolic activity on individual seeds, especially half seeds, would be particularly valuable in investigating the factors responsible for tetrazolium reduction by seeds. The value, however, would depend on the ability to measure quantitatively the amount of tetrazolium reduced by very small amounts of tissue. While these suggested approaches to a more vigorous investigation of the metabolism of germinating seeds seem worthy of consideration, it must nevertheless be realized that they represent technically formidable and very time-consuming tasks.
SUMMARY

Overall respiratory activity and the activity of three respiratory enzymes in corn seeds were measured at an early stage in the germination process to determine the extent to which these indices of metabolism were related to ability to grow. The correlations between respiratory activity and germination percentage were sufficiently high to indicate that loss of viability was due to respiratory failure in most cases. However, there were a few lots in which seed initiated growth but failed to germinate and in which respiratory rates were near normal at the 18 hour period. There was no evidence that the oxygen consumption and carbon dioxide evolution mechanisms were differentially inactivated in lots of lowered viability.

Malic dehydrogenase activity was highly correlated with oxygen consumption rate and roughly correlated with germination percentage, although activity of this enzyme was retained in seeds incapable of respiration and growth. Alcohol dehydrogenase activity was roughly correlated with both anaerobic carbon dioxide evolution rate and germination percentage, although there was evidence that this enzyme was not limiting in either case. There was no apparent relationship between cytochrome oxidase activity and either oxygen consumption rate or germination percentage, except perhaps in lots which had low oxidase activity. In addition, cytochrome oxidase activity was insufficient in almost all lots to account for more than half of the total oxygen consumption.
No evidence was found that respiratory metabolism was a factor in the differences between germination tests and cold tests or was responsible for the differences in growth rates. Tetrazolium tests were in many cases higher than germination tests, probably due to the fact that dehydrogenase activity was present in seed incapable of germination. However, the data were insufficient to show that malic and alcohol dehydrogenase were primarily responsible for tetrazolium reduction.

Additional investigation showed that tissue thickness and oxygen tension were important factors in oxygen consumption rate of embryo tissue. In addition, some factor inherent in immersion caused reduced respiratory rates. The pericarp restricted gas exchange of whole seeds, and seed residues had significant respiratory rates. Dry seeds had a high capacity for both respiratory and enzymatic activity. Increases in respiratory rates up to 18 hours were probably due to imbibition rather than formation of new enzymes. Increases in dehydrogenase activity were found only at a stage where considerable growth was evident. Changes in respiratory quotients of embryo slices with time of soaking were such as to indicate an initial oxidation of carbohydrates, a shift to oxidation of fats and then a shift back to oxidation of carbohydrates.
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