DIFFERENTIAL RATES OF DEVELOPMENT OF HETEROTIC AND NONHETEROTIC YOUNG MAIZE SEEDLINGS, I. CORRELATION OF DIFFERENTIAL MORPHOLOGICAL DEVELOPMENT WITH PHYSIOLOGICAL DIFFERENCES IN GERMINATING SEEDS*†

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Heterosis, the evident superiority of a hybrid to its parents, has not been defined in precise genetic terms. The reasons given for heterosis are for the most part rather general and vague. A recent statement is made that heterosis is the phenotypic result of gene interaction and occurs because the heterozygote either masks different detrimental recessive genes which are homozygous in the parents, or is adaptively superior to both parents.1 About 30 years ago, East2 suggested that "physiologically active" genes control speed of reactions responsible for heterotic expression.

Admittedly, the elucidation of the genetic mechanism(s) responsible for heterotic expression is not as yet possible. Much work needs to be done to describe precisely the required genetic constitution of heterotic individuals, especially when these are compared to the nonheterotic ones. And the time is ripe for such work to be done.

It is obvious that an alternative approach to the understanding of heterosis would be through studies of mechanisms or reactions responsible for growth and development in general, since heterosis, for the most part, is a manifestation of a superior growth rate of a hybrid over its parents.3–6 Information on the biochemistry and physiology of heterosis is scant mainly because much of the effort has been directed toward the elucidation of the genetic constitution required for heterotic expression. Cherry and co-workers have shown, however, that a maize hybrid generally had higher quantity of embryonic axis nucleotides than did the inbreds and that RNA was synthesized at a much faster rate in the hybrid, and suggested that hybrid vigor involved the plant as a whole and that reaction speeds were intimately involved.7 However, the study of the reactions per se would not be sufficient. Rather, the agents controlling these reactions should be elucidated. It is felt that then we would be taking a big step in the direction of the answer to the question, "How is heterosis accomplished?"

The present paper is one of a series of reports8–13 intended to elucidate a physiological-biochemical basis for heterosis. It is concerned with early postgermination physiological changes occurring in the endosperm and seedlings of hybrid corn. Observations showing differences in respiration, liberation of reducing sugars, and amylase activity are presented in an attempt to shed light on the mechanism of hybrid superiority in terms of growth. A possible means of control of this mechanism is suggested.

Materials and Methods.—Germinating seeds and seedlings of several maize single crosses and their parents were used throughout this study. The strains used were the dent inbreds Ohio 43, Ohio 45, West Virginia 5, and their single crosses W. Va. 5/Ohio 43, and Ohio 43/Ohio 45 (supplied by M. W. Johnson, West Virginia University).
Seeds were sterilized with 1% hypochlorite, thoroughly rinsed in distilled water, and germinated at 25°C on filter paper moistened with deionized water. Samples were collected at 12-hr intervals from 0 to 60 hr after the start of incubation. The collected material was immediately frozen at −23°C. The seeds (including the embryo) were lyophilized and ground for 2 min in a fine-screened Wiley mill. The ground material was again lyophilized and then stored in the cold under vacuum until needed. Portions of the ground material were used for determination of sugars and amylase activity.

Sugars were determined by the method described by Hassid.14,15 The dried material (0.15 gm) was extracted 16 hr with 80% ETOH in a Soxhlet extractor, and the reducing sugars of 5 ml aliquots were then determined by the ferricyanide-ferrocyanide method using 0.1% Setopaline C (Unitech Chemical Manufacturing Co.) as indicator. This method permits the reduction of ferricyanide ion to ferrocyanide ion by the reducing sugars and then measures the amount of ceric sulfate required to reoxidize ferrocyanide to ferricyanide. Sucrose was calculated as the difference between total reducing sugars (after acid inversion of an aliquot of the original extract) and reducing sugars prior to inversion.

To assay the amylolytic activity of the ground endosperm + embryo, 1 gm of lyophilized material was extracted in the cold (0°C) with 22 ml Tris buffer, 0.2 m, pH 7.15, and the homogenate was centrifuged 10 min at 22,000 × g. The procedure described by Bernfeld16 was followed. One ml of the homogenate was incubated 3 min at 20°C with 1 ml of 1% soluble starch solution. After addition of 2 ml 3,5-dinitrosalicylic acid reagent, the optical density of the reduction product was determined photometrically at 525 mμ. The readings were converted into milligrams of maltose from a calibration curve established with maltose. Results are presented in terms of amylase activity, i.e., milligrams of maltose liberated in 3 min at 20°C by 1 ml of the enzyme solution, even though, in the case of α-amylatic action, the actual reaction products are dextrins rather than maltose.

Respiratory activity of germinating seeds was measured by standard manometric techniques17 in a refrigerated Warburg apparatus. Seeds were allowed to germinate at 25°C, were transferred at appropriate times to Warburg flasks with distilled water, and were allowed to equilibrate; the readings were then taken for a period of 1 hr. With first visible signs of germination (radicle emergence), care was exercised to use seeds of similar stages of development.

All experiments were repeated at least three times, and each individual determination was done in duplicate.

Results and Discussion.—Respiratory activity of germinating seeds is shown in Figure 1. It can be noted that 5/43, after 60 hr of incubation, shows the highest uptake of oxygen. However, it does not differ significantly from oxygen uptake by W. Va. 5 or 43/45 at 60 hr.

Reducing sugar content of dried kernels (endosperm + embryo) is plotted in Figures 2 and 3. As shown in Figure 2, there is a striking increase in reducing sugars in 5/43 from 48–60 hr of germination. The parent 5 increases to some extent, but does not attain a level similar to that of 5/43. Inbred 43 shows no appreciable change in reducing sugars from 0 to 60 hr germination.

Figure 3 presents the reducing sugar levels during germination of hybrid 43/45 and its parents. In contrast to the behavior of 5/43, 43/45 does not differ appre-
Changes in sucrose levels of germinating seeds are shown in Figures 4 and 5. In Figure 4, hybrid 5/43 and its parents are presented, with the hybrid showing a sharp decline in sucrose beginning at the 24th hr of germination and continuing through the 60th. The parents show a similar trend, but do not drop as sharply and beginning at 48 hr show an increase in sucrose. In Figure 5 the patterns of sucrose levels in germinating 43/45 and its parents do not demonstrate any appreciable changes during germination of these 3 varieties. The hybrid is not different from the parents and, in fact, at 60 hr, has the least amount of sucrose.

In order to relate these metabolic patterns to early growth and development, the germination behavior of the genotypes studied is shown in Table 1.
Hybrid 5/43 germinates earlier than the other strains, parent 43 starting visible germination 24 hr later and 12 per cent of parent 5 germinating 12 hr later than 5/43. This superiority of 5/43 is maintained through the 60 hr where the entire sample has fully germinated. The other strains do not show complete germination at this hour. Hybrid 43/45 does not show such a striking difference with respect to its parents. At 36 and 48 hr it does show superiority over its parents in terms of radicle and coleoptile emergence, but at 60 hr this advantage drops. Inbred W. Va. 5 shows a noticeable advantage over 43 and 45 as well as over the hybrid 43/45.

A fact of utmost importance bearing on the elucidation of the nature of heterosis in the light of the germination and physiological data presented is that hybrid 5/43 is an extremely heterotic hybrid, expressing heterosis not only at germination, but at maturity in terms of height and yield of grain. On the other hand, hybrid 43/45 is almost completely nonheterotic, even at maturity, and frequently is indistinguishable from its parents. The germination data, as well as the reducing sugars and sucrose data, support this information. Additional support is provided by the behavior of inbred 5 which is a very vigorous inbred when compared with 43 and 45. This vigor is shown by its germination pattern beginning with 36 hr (Table 1) and by the levels of reducing sugars and sucrose (Figs. 1–4).

Mobilization of starch reserves from the endosperm and their subsequent availability to the metabolizing embryo is in part a result of the activity of amylases. To show that amylolytic activity was present in our germinating seeds, amylase determinations were made as described under Materials and Methods. α-Amylase activities are shown in Figures 6 and 7. Activity of β-amylase was not detectable in our samples. Again, 5/43, beginning at 24 hr, shows a remarkable rise in α-amylase activity (Fig. 6), while 43/45 does not. When α-amylase was tested in 60-hr kernels, 5/43 had the highest level of activity of all strains.

The results presented thus far can be readily interpreted from the point of view of physiology of the phenomenon of heterosis in plants. Morphologically, heterosis is expressed 48 hr after incubation of seeds (Table 1). The 5/43 seedlings were
observed to elongate more rapidly, both the radicle and the shoot. Another heterotic hybrid has been shown to behave in this manner also. The vigorous hybrid 5/43 and the inbred 5 and, to some extent, 43/45 utilized more oxygen from 36–60 hr of germination (Fig. 1). Presumably, increase in reducing sugars in the heterotic hybrid would enable more rapid metabolic activity and thus elongation of the germinated embryo. High $\alpha$-amylase activity provided a means of efficient breakdown of endosperm starch, and the drop in sucrose levels of 5/43 (Fig. 4) could be attributed to enhanced respiration and/or growth of the hybrid seedling.

These conclusions, however, do not readily explain the nature of heterosis. They merely suggest well-known facts of plant physiology and biochemistry. But observation of differential rates of physiological and morphological activity make it imperative that we go one step further in our study of heterosis and describe mechanisms or agents controlling these activities.

On the basis of the information presented below a specific mechanism or agent can now be tentatively suggested. The observations all point to the involvement of differential levels of gibberellic acid (GA) in germination and early postgermination development of a heterotic hybrid and its parents.

Some of the observations on physiology of heterotic hybrids are summarized as follows:

1. Hybrids germinate earlier than parents (24 hr).
2. Hybrid seedlings elongate faster, both roots and shoots.
3. Hybrids respire at a higher rate than parents.
4. Hybrid endosperm liberates much higher amounts of reducing sugars.
5. Germinating hybrid kernels have higher $\alpha$-amylase activity.
6. Hybrid seedlings (12 days) fix significantly more CO$_2$ per unit fresh or dry weight.
7. Hybrid seedlings (12 days) have less chlorophyll per unit fresh or dry weight.
GA has been shown to accelerate germination.\textsuperscript{19, 20} Exogenous application of GA stimulated cell division and elongation of cells and plant parts.\textsuperscript{19, 21–26} GA was shown to increase seed respiration.\textsuperscript{27, 28} $\alpha$-Amylase activity and liberation of reducing sugars was increased in seeds treated with GA,\textsuperscript{29–32} and it has been suggested recently that mobilization of endospermal reserves in germination of barley kernels is initiated by an endogenous gibberellin secreted into the endosperm by the embryo.\textsuperscript{33} GA has been suggested by some workers to enhance photosynthetic CO$_2$ fixation and was shown to promote the reduction of chlorophyll content.\textsuperscript{26}

Our observations, coupled with information presented in the previous paragraph, strongly suggest differential levels of endogenous gibberellic acid as being, at least partially, responsible for the observed differences. Experiments are in progress to elucidate further the possibility that endogenous GA is an agent of primary importance in controlling seedling heterosis.

\textit{Summary.}—Germination patterns of heterotic and nonheterotic maize hybrids and their parents have been studied. It was shown that during germination the heterotic hybrid (endosperm + embryo) increases in content of reducing sugars, while the nonheterotic hybrid and the parents do not. The heterotic hybrid dropped sharply in sucrose content during germination, while the nonheterotic hybrid and the parents did not. Germination data indicated that the heterotic hybrid exhibited an earlier onset of radicle emergence. In respiration of the germinating seeds, the heterotic hybrid exceeded its parents and the nonheterotic hybrid, indicating a faster rate of metabolic activity. The results are discussed with reference to the involvement of higher levels of gibberellic acid as instrumental in germination of heterotic seeds and subsequent development of the seedlings.

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THE RIBOSOMAL ASSOCIATION OF THE DISSIMILAR POLYPEPTIDE CHAINS OF TRYPTOPHAN SYNTHETASE*

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The tryptophan synthetase of Escherichia coli can be considered as an example of that class of proteins whose functional unit is composed of dissimilar polypeptide chains. Biochemical studies have revealed that this enzyme is reversibly dissociable, in vitro, into two components termed A and B.1, 2 Furthermore, the genetic mapping of many mutants for the chromosomal region concerned with the structure of tryptophan synthetase is consistent with this view since the genetic loci for components A and B are distinct though adjacent.3, 4

On the basis of these facts, the formation of the active enzyme can be visualized to occur as a result of the association of components A and B either at the synthetic site or, alternatively, subsequent to the release of the components from the synthetic sites. In addition, it should be noted that the adjacency of the structural genes for the two components along with the current conceptual views for the synthesis of the messenger RNA5-7 makes it feasible to consider the possibility that both components of tryptophan synthetase are synthesized on the same synthetic site.

Our approach was to study tryptophan synthetase (or components A and B) associated with ribosomes, on the assumption that the activity associated with isolated ribosomes represents a terminal stage in the synthesis of this protein. Evidence concerning the nature of tryptophan synthetase associated with ribosomes will be presented and discussed in relation to the above issues.

Materials and Methods.—Component A, component B, and the AB complex were measured for their ability to catalyze the indole + serine — tryptophan reaction. Component A is inactive in this reaction except in the presence of excess component B. Component B alone has activity in this reaction which is enhanced about 30-fold following its conversion to the AB complex by the addition of excess component A.6

The bacterial strains7 used were E. coli T3, B8, and A2. T3 is wild type with regard to tryptophan synthetase while B8 and A2 are mutant for component B and component A, respectively. The altered component in the mutants is inactive enzymatically and essentially free of cross reactivity to antibodies specific for the normal components.5 The bacteria were grown in medium Ea supplemented with limiting amounts of indole or tryptophan8 and harvested when the tryptophan synthetase activity had been derepressed. Ten gm cells were ground with 30 gm alumina and