GROSS NUTRITIONAL CONTRIBUTIONS OF MAIZE ENDOSPERM AND SCUTELLUM TO GERMINATION GROWTH OF MAIZE AXIS [1, 2]

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In 1890 Brown and Morris (8) showed that starch paste, when applied to the absorbing surface of the scutellum, could replace the endosperm during the germination of barley embryos. Andronescu (2) first demonstrated that the maize embryo (axis & scutellum) could develop into a normal plant when separated from its endosperm and supplied with sucrose. Most investigators, however, have emphasized the endosperm as a source of growth factors, either essential or stimulatory to the germination of the axis, rather than as a tissue serving singularly as a source of sugars and inorganic ions. Schander (26) considered the aleurone layer of the maize endosperm to be the source of an accelerator which is absorbed by the scutellum during germination. DeRopp (11) found that embryos which were allowed to remain in the intact grain during the early stages of germination exhibited more vigorous growth after excision than did embryos removed from the dry rye seed. He also interpreted this as due to the absorption by the scutellum early in germination of a growth factor from the endosperm. The "Z" factor of Robbins (25) and the "blastinin" hormone of Cholodny (10) are other compounds said to originate in the endosperm. The high percentage of bound auxin residual in the resting endosperm of maize (3, 4, 7, 13, 14), and the increasing discoveries of gibberellin-like substances in the seeds of higher plants (22, 24) also have made the contribution by the endosperm to the growth of the axis seemingly more complex than merely that of a sugar and inorganic ion source. Furthermore, Nason (19) found that the rate and extent of niacin accumulation in intact maize embryos were three times as great as that of excised embryos after 10 days of germination. His data suggest that this is related to the absorption of tryptophan from the endosperm rather than to the absorption of niacin itself.

The extent to which the scutellum can provide nutrition to the axis has received little attention, although it has been shown that lipids are rapidly lost from the maize scutellum during germination (18).

The object of this research was to determine the relative nutritional contributions of the scutellum and the endosperm of the maize kernel to the growth of the axis during germination.

METHODS AND MATERIALS

The material used was a non-waxy, inbred maize (*Zea mays* L.) line T854, secured from the Agricultural and Mechanical College of Texas. The endosperm of this line contain 85 % carbohydrate and 10 % protein on a dry weight basis (12). Oil is present in trace amounts in the aleurone layer, demonstratable by staining with Sudan IV. The predominate carbohydrates found in non-waxy maize endosperm during germination are starch, maltose, dextrins of various chain lengths, and a trace of glucose (29, 6). Amylose comprises about 25 % of the starch of non-waxy maize endosperm and the remainder is amylopectin (20, 21). The scutellum of the T854 line contain 27 % lipid,, 22 % protein, and 35 % carbohydrate on a dry weight basis (12). Sucrose is the principle sugar in the scutellum of germinating maize, and there is no detectable maltose in this organ (29).

The contributions of the endosperm and scutellum to axis growth were determined by comparing the axis growth of intact seeds with that of excised embryos and excised axes cultured on synthetic media. The germination period of this line of maize was arbitrarily defined as the 12 days following the initial imbibition of water by the seed. The stored nutritional reserves of this line will support a logarithmic rate of growth by the axis (as determined by dry wt) for this period in darkness at the temperature used. After 12 days this growth in darkness tapers off considerably. The initial imbibition of water by the seed which marks the commencement of germination was arbitrarily considered to begin as soon as the seeds were wetted.

The seeds were culled to a standard type kernel appearance and size. Those used to determine the growth rate of the axis in the normal germination of intact kernels (hereafter referred to as the intact-kernel culture) were disinfected by washing with "Tide" and rinsing with distilled water. These seeds were then germinated on agar in 25 ml micro-Fernback vessels, since these vessels were used in the in vitro cultures. In the cultures of excised axes the

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dissection effected a clean separation from the scutellum. In the cultures of entire embryos, the adhering endosperm was gently scraped away. In both cases the kernels were presoaked 1 hour, this in order to loosen the pericarp; neither the embryo nor the endosperm showed appreciable water imbibition. It was considered that there was no exchange of materials between the scutellum and the endosperm before excision. Normal aseptic procedures were employed. All cultures were grown at 21° C in total darkness.

The salt content of the medium was a modification of that of Randolph and Cox (23) (table I). In no case was the medium supplemented with amino acids, vitamins or other factors, but rather, deliberately maintained as minimal as possible. The medium was jelled with 0.75 % acid-washed Noble Agar and 10 ml added to each vessel. The pH of this medium was 5.0 regardless of the carbohydrate substrate used and was relatively stable throughout the germination period.

The following cultures were effected:

Culture designation	Composition of medium
Intact-kernel culture (entire kernels)	...
Axis culture (excised axis)	Salt solution, 2 % sucrose
Non-nutrient culture (entire embryo)	Agar only
2 % Maltose culture (entire embryo)	Salt solution, 2 % maltose agar
2 % Sucrose culture (entire embryo)	Salt solution, 2 % sucrose agar
5 % Maltose culture (entire embryo)	Salt solution, 5 % maltose agar
2 % Glucose culture (entire embryo)	Salt solution, 2 % glucose agar
2 % Soluble starch culture (entire embryo)	Salt solution, 2 % soluble starch, agar
Starch paste culture (entire embryo)	Salt solution on filter paper, soluble starch on scutellum

Table I

Salt Content of Media Employed in All Cultures Except Intact-Kernel and Non-Nutrient Cultures*

	mg/liter
$Ca(NO_3)_2 \cdot 4H_2O$	237.0
KNO_3	85.0
KCl	65.0
$NaH_2PO_4 \cdot H_2O$**	16.5
$MgSO_4 \cdot 7H_2O$	36.0
Fe citrate (chelate)	30.0
$MnSO_4 \cdot 4H_2O$	00.4

* Modified from Randolph and Cox (23).

** Substitution of $NaH_2PO_4 \cdot H_2O$ for "Calgon".

The choice of substrates for the cultures was based on the fact that A, the bulk of the endosperm is starch (17, 29); B, the degradation product of the amylase digestion of the endosperm starch is primarily maltose (5, 15, 20); C, the only sugars found in gross amounts in the embryo, and, thus, considered in transport form, are sucrose and glucose (17, 29). The starch paste culture was devised to simulate physically the intact-kernel endosperm with respect to endosperm starch. Ten discs of Whatman No. 1 filter paper were placed in circular vegetable dishes and saturated with 20 ml of the salt solution. Small glass microscope slide rings were packed with soluble starch paste and placed on the filter paper. The embryos were embedded in this simulated endosperm. The flat, external surface of the scutellum from which the axis emerges was exposed, allowing the shoot and root to grow out of the ring. This procedure eliminated the artificialities of root sugar absorption and of the substrate becoming limiting in the area of the absorbing surface of the scutellum.

The germination growth accomplished in the different cultures was determined by measuring the increase in fresh and dry weight of the axes. In each culture, 30 embryos or axes were used and samples removed for weight determinations at 3, 5, 8, 10, and 12 days after germination began. All experiments were repeated, and the results of the replications were within 10 % of the original culture. The data are averages of the two runs of each culture. Since the dry weight percentage of fresh weight for the axes was no different in any of the cultures, only axis dry weight values are given in the results.

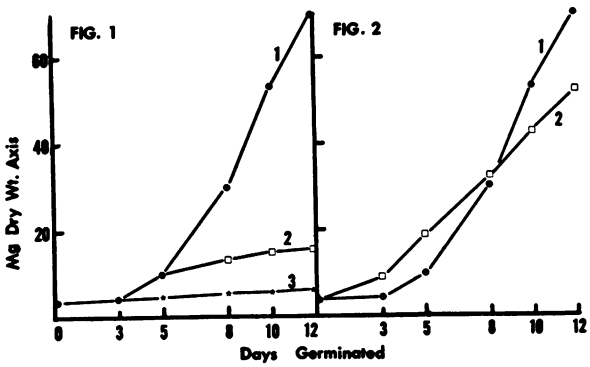
Fresh and dry weight changes in the scutellum were followed at the same intervals during germination in the intact-kernel culture and the other cultures which employed the entire embryo. Total nitrogen content of the scutellum was determined for the intact-kernel culture, the non-nutrient culture, and the 5 % maltose culture, and of the endosperm from the intact-kernel culture at these same intervals. Nitrogen was determined by the modified micro-Kjeldahl method of Jensen (16).

Lipid content of intact-kernel scutella was measured at 24-hour intervals during the first 6 days of germination. The scutella were sliced, oven dried, and the lipids extracted by repeated washings with an ether: ethanol (3:1) solvent at 45° C.

The respiratory quotient and total respiration of embryos dissected from intact kernels were also determined for the 12-day germination period, utilizing standard Warburg manometry. Gas exchange was followed for 2 hours in embryos removed from intact seeds germinating in the dark. The embryos were placed on a film of water in the Warburg vessels, but were not submerged. The uptake of O_2 was measured directly by absorbing the CO_2 evolved with 15 % KOH. CO_2 evolution was measured by the "direct method" as described by Umbreit et al (30).

Results

I. Comparison of Axis Growth Obtained in Intact-Kernel Culture With That in in Vitro Cultures (figs 1–5). The results of the intact-kernel culture (fig 1, curve 1) establish the growth pattern of the germinating axis in the intact seed and serve as a control to which the growth in other cultures is compared. Figure 1, curve 2, reveals the growth pattern of the non-nutrient culture. The growth of the axis supported by its scutellum alone equals the growth of the intact-kernel culture for the first 5 days. But after the 5th day this growth diminishes rapidly, and by the 12th day these axes have only 22 % of the intact-kernel culture dry weight. The results of the axis culture, (fig 1, curve 3) reveal that the maize axis without its scutellum is capable of very little growth on a sucrose and salt medium. After 12 days the dry weight of these axes is less than 10 % of that of the intact-kernel culture and only 41 % of that of the non-nutrient culture.



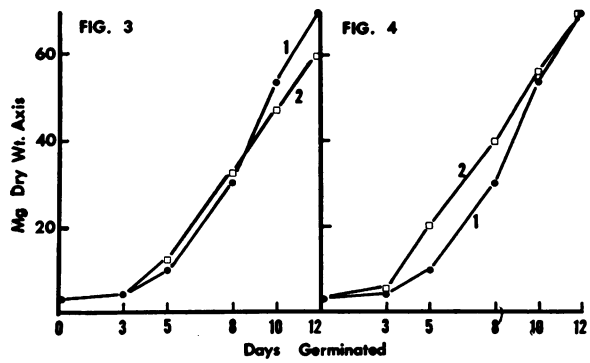


Fig. 1. Axis growth (dry wt increase) during germination. Curve 1, intact-kernel culture (control). Curve 2, non-nutrient culture. Curve 3, axis culture.

Fig. 2 Axis growth (dry wt increase) during germination. Curve 1, intact-kernel culture (control). Curve 2, average of 2 % maltose and 2 % sucrose cultures.

Fig. 3. Axis growth (dry wt increase) during germination. Curve 1, intact-kernel culture (control). Curve 2, 5 % maltose culture.

Fig. 4. Axis growth (dry wt increase) during germination. Curve 1, intact-kernel culture (control). Curve 2, 2 % glucose culture. (See text for details of culture conditions.)

However, when samples of the non-nutrient culture (axis & scutellum germinated on 0.75 % agar alone) are cultured in the light they develop into autonomous plants. Thus, the embryo with no nutrient other than what it contains in its scutellum can germinate successfully. Samples of the axis culture (dissected axis germinated on the salt and 2 % sucrose medium) will also develop into autonomous plants when cultured in the light. These excised axes in the light grow slowly, but their growth rate increases greatly when photosynthesis begins.

The 2 % maltose and the 2 % sucrose cultures can be discussed collectively since their growth rates are essentially identical (fig 2). These cultures reveal that sucrose, which is not found at maturity, nor throughout germination, in the endosperm (6, 17, 29), can be absorbed by the scutellum as readily as maltose. The data show that the axis growth on 2 % disaccharide media is more than 175 % of the intact-kernel culture growth by the 5th day. However, these media provide for only 75 % of the intact-kernel growth by the 12th day. This enhanced early growth is probably due to the ready availability of sugar in these cultures during the first few days of germination. A decreasing availability of sugar with time in the area of the scutellar absorbing surface is considered responsible for the tapering off of growth in these two cultures during the last phases of germination.

The axis growth in the 5 % maltose culture (fig 3) equals the growth found in the intact-kernel culture during the first 8 days, but it declines to 84 % by the 12th day. The axes of this culture in comparison to those of the 2 % disaccharide cultures show a lower initial growth rate, which could conceivably be due to the higher osmotic pressure of the medium. They exhibit less of a tapering off of growth during the last phases of germination, which may be explained by a greater availability of sugar at this stage than is afforded by a 2 % concentration.

The results of the 2 % glucose culture (fig 4) demonstrate that this sugar provides the most satisfactory sugar substrate of those used, since growth in this culture equals the intact-kernel growth after 12 days. A comparison of this culture with the intact-kernel growth after 5 days shows again that greater early growth can be correlated with sugar being readily available for absorption by the scutellum during the first days of germination.

The 2 % soluble starch culture (fig 5 curve 3) gives only 50 % of intact-kernel growth after 12 days. This poor growth may result from a poor diffusion of starch and, hence, an increasing sugar gradient at the scutellar surface. The starch paste culture (fig 5 curve 2) essentially follows the intact-kernel growth pattern throughout the germination period, including the early lag phase, when compared to embryos cultured on sugars.

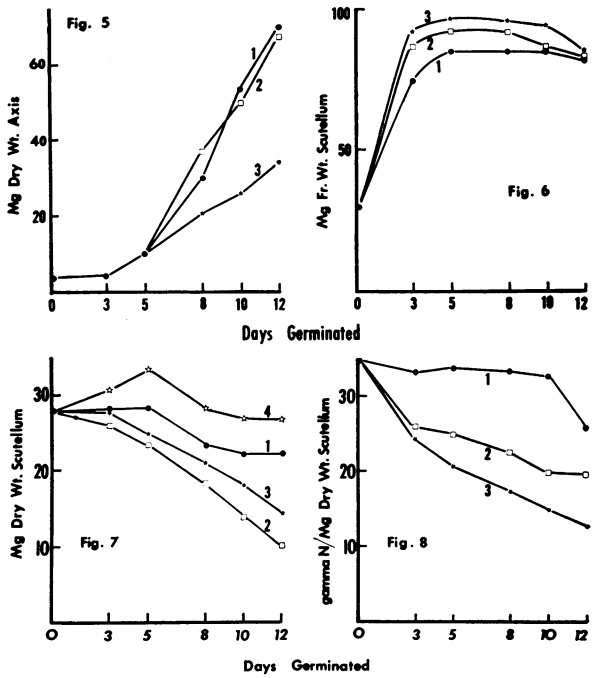


FIG. 5. Axis growth (dry wt increase) during germination. Curve 1, intact-kernel culture (control). Curve 2, starch paste culture. Curve 3, 2 % soluble starch culture.

FIG. 6. Fresh weight of the scutellum during germination. Curve 1, intact-kernel culture (control). Curve 2, average of non-nutrient, 2 % sucrose, 2 % maltose, 2 % soluble starch cultures. Curve 3, Average of 5 % maltose, 2 % glucose, starch paste cultures.

FIG. 7. Dry weight of scutellum during germination. Curve 1, intact-kernel culture (control). Curve 2, non-nutrient culture. Curve 3, average of 2 % maltose, 2 % sucrose, 2 % soluble starch cultures. Curve 4, average of 5 % maltose, 2 % glucose, starch paste cultures.

FIG. 8. Scutellum nitrogen during germination. Gamma of nitrogen per mg. dry weight. Curve 1, intact-kernel culture (control). Curve 2, non-nutrient culture. Curve 3, 5 % maltose culture. (See text for details of culture conditions.)

II. SCUTELLUM WEIGHT CHANGES (figs 6, 7). There is a constant rise in the fresh weight of the scutella of intact kernels up to the 5th day, and the weight then reached is maintained throughout the experimental period (fig 6). The dry weight of the intact-kernel scutellum remains constant for the first 5 days of germination and then decreases gradually (fig 7).

The results of the other cultures fall into three groups. In the non-nutrient culture the scutellar fresh weight approximates that of the intact-kernel culture, but its dry weight decreases much faster. Thus, if no other source of nutrition is available, the demands of the axis deplete the scutellum to a greater degree. In the 2 % disaccharide and soluble starch cultures, the scutellar fresh weights also approximate those of the intact-kernel culture, and the scutella lose more dry weight than do the intact-kernel scutella, although not to the extent that those grown on the non-nutrient culture do. Hence, on these substrates, at this concentration, the scutellum is depleted somewhat more than it is in the intact seed. In the 5 % maltose, the 2 % glucose, and the starch paste cultures, all of which approximate the intact-kernel growth of the axis, both scutellar fresh weight and dry weight exceed that of intact-kernel scutella. Apparently, these substrates at these concentrations are absorbed faster than they are transported to the axis and thereby increase the scutellar dry weight, and, at the same time, increase the osmotic pressure of the scutellum, which results in a greater water holding capacity and a higher fresh weight.

III. SCUTELLUM AND ENDOSPERM NITROGEN (figs 8–10). Total nitrogen content of the scutellum was determined for the intact-kernel, non-nutrient, and 5 % maltose cultures. In figure 8, nitrogen is expressed as micrograms per milligram dry weight. The data reveal that during germination in the non-nutrient and the 5 % maltose cultures nitrogen is lost from the scutellum faster than dry weight is lost. It is presumed that this loss represents a transfer to the axis. The fact that nitrogen per milligram dry weight is lost faster in the 5 % maltose culture than in the non-nutrient culture is deceiving, since the non-nutrient culture is not replenishing its dry weight by absorption from the medium, and therefore its dry weight is much lower than is that of the 5 % maltose culture. Nevertheless, the data show that much more nitrogen is absorbed from the endosperm than is available in the medium, since scutella from the 5 % maltose culture are depleted of nitrogen to a greater extent than are the intact-kernel scutella. A better evaluation of the loss of nitrogen from the scutellum during germination is obtained by plotting micrograms of nitrogen per scutellum (fig 9). This plot shows that by the 12th day the scutella cultured in the non-nutrient medium have lost 79 % of their nitrogen, those cultured on 5 % maltose have lost 59 %, while the intact-kernel scutella have lost but 27 %.

In figure 10, micrograms of nitrogen in the endosperm of the intact-kernel culture are plotted per milligram dry weight. After an initial lag period, there is a steady loss of nitrogen from the endosperm from the 3rd to the 10th day of germination. By the 10th day the endosperm has lost 71 % of its nitrogen per milligram dry weight, but loses little more (73 %) after the next 2 days. No logical explanation for the initial rise in nitrogen content can be given.

IV. LIPID AND RESPIRATION RESULTS (fig 11, table II). The results of the lipid extractions from intact-kernel scutella are presented in Table II, and are in agreement with similar work on maize by Malhotra (18). The data show that the lipid content of the scutellum decreases from 27 % to 12 % (dry wt basis), during the first 3 days of germination. During the next 3 days, the lipid content remains

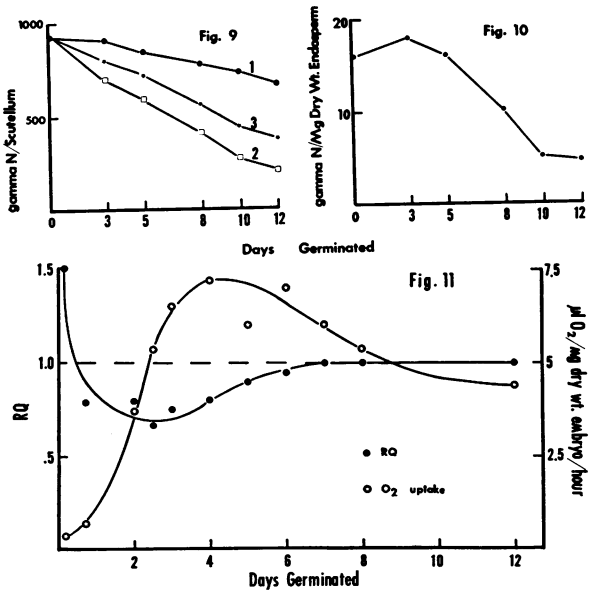


FIG. 9. Scutellum nitrogen content during germination. Gamma of nitrogen per scutellum. Curve 1, intact-kernel culture (control). Curve 2, non-nutrient culture. Curve 3, 5 % maltose culture. (See text for details of culture conditions.)

FIG. 10. Endosperm nitrogen content during germination. Gamma of nitrogen per mg dry weight.

FIG. 11. Respiratory quotient and oxygen uptake of intact-kernel embryos during germination. Dashed line is reference line denoting respiratory quotient of unity.

constant, indicating that lipids and material contributing to the dry weight of the scutellum are being lost at an equal rate.

The uptake of O_2 per milligram dry weight of the intact-kernel embryo over the 12-day germination period is given in figure 11. The total respiration of the embryo rises continually, but in relation to the increase in dry weight of the embryo a definite peak in respiration is reached on the 4th day. The decrease from the 4th to the 12th day may be caused by a gradual depletion of available carbohydrates. The respiratory quotient values for the intact-kernel embryo over the germination period are also presented in figure 11. The first respiratory quotient, that for 4 hours, shows that part of the embryo respiration at this early stage is still anaerobic, but by 18 hours the influence of lipid breakdown is such that the respiratory quotient is well below unity. The rise back to unity caused by the depletion of lipids and the increasing availability of carbohydrate is achieved by the 7th day at this temperature (21° C). This respiratory quotient pattern is in agreement with that of cereal germination in general (27).

DISCUSSION AND CONCLUSIONS

From this study concerning the nutritional contributions of the maize endosperm and scutellum to axis growth during germination, the following conclusions may be drawn.

First, the early germination growth of the axis (the first 5 days in this instance) apparently is dependent upon the scutellar food reserves. This conclusion is supported by the data in figure 1. The data show that the growth of the axis, when the embryo is cultured in the non-nutrient medium, is identical with the growth of intact-kernel axes for these first 5 days.

Second, this early growth of the axis is produced at the expense of the scutellar lipid reserves. This conclusion is supported by the data from figure 11 and table II. Table II shows that a rapid loss of lipid from the scutellum occurs during the first 3 days of germination, and the respiratory quotient values of the embryo (fig 11) are well below unity during this period, which suggests a predominately lipid catabolism. This view is further substantiated by the fact that there is very little amylase activity in the endosperm at 3 days and very little reducing sugar (12). It seems unlikely that a substantial amount of endosperm carbohydrate reaches the axis before the 5th day of germination at this temperature. Furthermore, the axes from the intact-kernel and the starch paste cultures show a definite growth lag during these first 5 days, when compared to axes from the 2 % maltose, 2 % sucrose, and 2 % glucose cultures. This growth lag may be caused by the time required for the scutellum to secrete amylases into the endosperm and absorb the sugars produced from the amylolysis of starch, which must occur in the intact-kernel and starch paste cultures, but not in the sugar cultures. It is during this period, in which the endosperm nutrients are becoming mobilized, that the lipid catabolism in the scutellum apparently provides the axis with the energy and synthesis substrates for its initial growth in the germination of intact seeds.

Third, the function of the endosperm during germination seems to be confined to that of a source of carbohydrate and inorganic ions. This conclusion is supported by the following facts, A, Growth in

TABLE II

LIPID CONTENT OF SCUTELLUM DURING GERMINATION*

UNGERMINATED	DAYS GERMINATED					
	1 DAY	2 DAYS	3 DAYS	4 DAYS	5 DAYS	6 DAYS
27	16	14.5	12	11	11	11

* Expressed as percent of scutellar dry weight.

two cultures, 2 % glucose and starch paste, both equalled the growth found in the intact-kernel culture, while growth in a third culture, 5 % maltose, closely trailed it (figs 3, 4, & 5). The growth on lesser concentrations of disaccharides tended to lag only after the 8th day. B, the results of the axis culture and the non-nutrition culture demonstrate that the embryo without additional nutrition can grow into an independent plant; and the axis alone, if provided with sucrose and inorganic ions, can grow into a self-sustaining plant, if, in both cases, they are allowed to photosynthesize.

Unquestionably, many compounds other than sugars and inorganic ions are absorbed by the scutellum during germination (19), but their role as essential and non-synthesizable metabolites is questionable in light of these results.

Fourth, in these experiments the axis growth of excised embryos is essentially independent of the carbohydrate supplied. Nutrient media that contained 5 % maltose, 2 % glucose or starch paste in addition to inorganic salts were approximately equal to the endosperm of intact kernels in supporting axis growth (figs 3, 4, & 5). As shown by figure 2, axis growth of embryos cultured in media containing 2 % maltose or 2 % sucrose was exceeded by that of intact kernels only after the 8th day of germination.

Concerning the different substrates used in these cultures, the superiority of glucose for isolated cereal tissue has been shown by Burström (9), Almestrand (1), and others. Yet the growth sustained by glucose in this study is surprising for two reasons. A, only trace amounts of glucose are formed in the amylolysis of starch (15, 5), and this only from the occasional splitting of maltotriose into maltose and glucose by alpha amylase. B, if sugar absorption involves a phosphorylation at the cell surface (28, 31), a disaccharide (maltose in this case) should be a more efficient substrate than a monosaccharide such as glucose. The reaction of a hexoglucoside with inorganic phosphate requires little energy, whereas the phosphorylation of glucose alone is dependent upon ATP.

Sucrose is, in general, the best carbohydrate source for plant tissue culture (31), but the fact that it supports the same rate of growth as does an equal concentration of maltose in these experiments is also surprising. There is no trace of sucrose in the endosperm of germinating maize (6, 17).

Summary

The gross nutritional contributions to the germination growth of the maize axis by the scutellum and the endosperm were investigated by germinating excised embryos and excised axes on different carbohydrate substrates and following their growth. The results indicate that the mature embryo depends on the endosperm only for a source of carbohydrate and inorganic ions, and that it does not require hormones, vitamins, or other factors originating in the endosperm for successful germination.

Respiratory quotients for the embryo and the loss of lipid from the scutellum were determined at intervals during germination, and these data coupled with the results of the culture studies suggest that the early germination growth of the axis is dependent upon the lipid of the scutellum.

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SITE OF ORIGIN AND EXTENT OF ACTIVITY OF AMYLASES IN MAIZE GERMINATION[1,2]

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The means by which the endosperm starch of the cereal grain is mobilized and made available to the embryo during germination have received considerable attention. The enzymes involved in the degradation of starch and their specific functions have been extensively reviewed (1, 2, 4, 12, 18, 20, 24, 30). Of these enzymes, alpha and beta amylase are responsible for the bulk of the starch hydrolysis in the cereal endosperm. Other hydrolytic enzymes, Z, D, and R enzymes (1, 3, 24, 25), that cleave the 1-6 alpha glucosidic linkage and other rare and anomalous linkages, provide relatively small amounts of soluble carbohydrates.

The reports concerning the origin of the amylase enzymes within the tissues of the cereal grain itself present a somewhat confused and incomplete picture. Many investigators propose that the amylases originate, at least in part, in the endosperm itself, and ascribe to the endosperm the capability of autodigestion. Other workers conclude that no such capability exists in this tissue [reviewed in Miller (19)]. The finding of beta amylase activity in the subaleurone layers of the endosperm of resting wheat, barley, and rye grains (9) suggests the in situ origin of at least some amylolytic activity during germination.

Many workers consider the cells of the scutellar epithelium to be the source of the amylases, which are thought to be secreted by these cells into the endosperm during germination. The evidence for this secretion has been: A, light microscope observations of the behavior of the scutellar epithelium and of the apparent migration of particles from it into the endosperm during germination (13, 21, 28, 31, 32), B, the isolation of particles from the scutellum that contain amylolytic activity (11), C, the fact that excised cereal embryos will exhibit good germination growth on boiled endosperm and on starch paste (6, 8).

Bernstein (5) has suggested a separate origin for each of the amylases, based on genetic studies. He proposes that the inheritance of alpha amylase is an embryo characteristic (hence, secreted from the scutellum) and that the inheritance of beta amylase is a more maternal characteristic (formed in situ in the triploid endosperm). Most investigators agree on a scutellar origin of some or all of the amylolytic activity found in the cereal endosperm during ger-

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