evidence against this hypothesis is the fact that B-ab males when mated with either B-a or B-ab females sired B-a as well as B-ab daughters. This result is not expected under any simple hypothesis in which males are hemizygous for the gene under consideration. All of the observed results, on the other hand, are in agreement with expectation for single-locus, autosomal inheritance.

Genetic evidence supports the hypothesis that the gene producing enzyme B occurs as two alleles, the polypeptide products of which are here called the B^a and B^b subunits. The two single-band phenotypes, B-a and B-b, are considered to be homozygous forms and the three-band type, B-ab, the heterozygous form, with the intermediate band representing a "hybrid" molecule, B^{ab}.

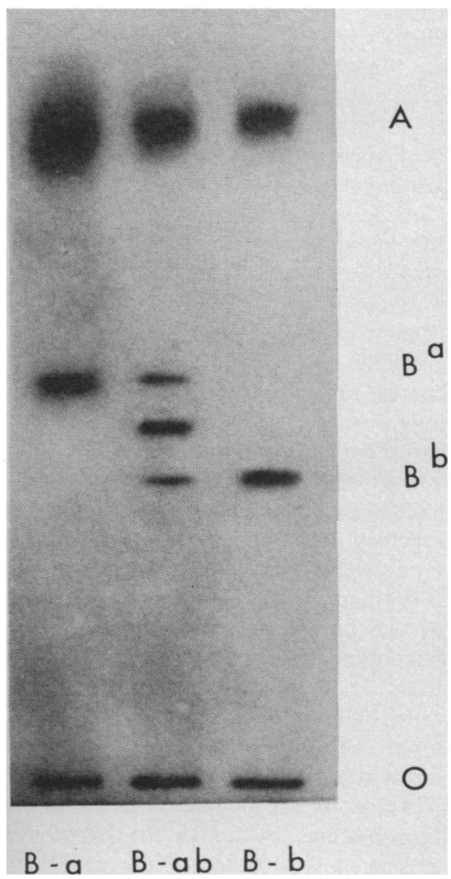

Fig. 1. Starch gel showing liver glucose-6-phosphate dehydrogenase of three animals. Anodal direction is upward. A and B enzymes are indicated, with the three different B types shown: B-a and B-b are the two homozygotes; B-ab is the heterozygote. Vertical electrophoresis was carried out for 18 hours at a gradient of 8 volt/cm at 40°C. The gel buffer was a tris-EDTA-borate mixture, *p*H 8.0, total molarity 0.05. The same buffer at 0.5*M* was used in the bridge boxes. Incubation mixture for demonstration of the enzyme activity contained per 100 ml: triphosphopyridine nucleotide, 70 mg; nitro-blue tetrazolium, 50 mg; phenazine methosulfate, 2 mg; NaCN, 5 ml, 0.1*M*; tris buffer, *p*H 6.8, 10 ml; glucose-6-phosphate, 5 ml, 1.0*M*; gels were incubated for 2 to 4 hours at 35°C.

The occurrence of a hybrid molecule in the heterozygote indicates that the B enzyme is probably a dimer composed of randomly associating subunits (*4*). In such a system, the hybrid dimer would theoretically be present in twice the amount of each of the homogeneous dimers in the heterozygote, and the increased intensity of the middle band in B-ab phenotypes fits this scheme. In the G-6-PD variants of human erythrocytes, heterozygotes do not have an intermediate, hybrid molecule; this is evidence that that enzyme and the B enzyme of *Peromyscus* are not homologous.

The fact that the B enzyme variation is not correlated with any alteration in the A enzyme indicates that the two molecules are controlled by different genetic sites. Whether the A enzyme is X-linked cannot now be determined. That *Peromyscus* erythrocytes contain only the A enzyme suggests that the A form might be homologous with the X-linked G-6-PD of human erythrocytes.

Both B-b and B-ab phenotypes were found in the stock of *P. m. bairdi*. The observed polymorphism, therefore, is present within this subspecies and is not entirely the result of bringing together genes from different subspecies by hybridization.

The enzyme 6-phosphogluconate dehydrogenase (6-PGDH), which catalyzes a subsequent metabolic step, sometimes produces a faint zone of activity in gels incubated with glucose-6-phosphate. To check the possibility that the B enzyme might be 6-PGDH, the specific substrate, 6-phosphogluconate, was used in place of glucose-6-phosphate. No activity occurred at the B position, but a strong zone developed at a position anodal to the A enzyme, an indication that 6-PGDH is distinct from both of the G-6-PD forms.

Tissue culture studies were done to investigate the applicability of these enzyme systems as genetic markers in cell culture. Diploid fibroblast lines were established from skeletal muscle of a B-a homozygote and from kidney of a B-b homozygote. Both A and B enzymes were present in the cultured cells, and the B polymorphism was clearly demonstrated.

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22 January 1965

Geoelectric Effect and Geotropic Curvature

Abstract. *A transverse electrical potential develops in many plant organs after they are reoriented from a vertical to a horizontal position. This phenomenon has been the basis for both a geoelectric hypothesis of geotropic curvature and its subsequent refutation. In both arguments a causal relation between the potential and curvature has been assumed. Curvature occurs in the absence of potential, as well as vice versa; it is concluded that both the hypothesis and its refutation are incorrect.*

A difference in electrical potential between the upper and lower sides of the shoot and root tips of many plant species develops after they are reoriented from a vertical to a horizontal position. This phenomenon is known as the geoelectric effect. In contemporary (*1, 2*) as well as earlier studies (*3*) experimenters have sought to relate this phenomenon causally to a lateral redistribution of the growth hormone, auxin, and thence to geotropic curvature. Recently, it was concluded that the geoelectric effect is a secondary consequence of the asymmetric distribution of auxin, and that the geoelectric hypothesis of geotropism is incorrect (*4*).

The refutation was based on an inversion of the cause and effect roles of the geoelectric effect and auxin distribution; it was concluded (*4*) that auxin is essential for the development of the transverse potential. Both the geoelectric hypothesis and its subsequent refutation relate the geoelectric effect and geotropic curvature to asymmetry of auxin distribution. The possibility that the geoelectric effect and geotropic curvature might not be causally related remained untested. We have therefore tested this possibility.

Intact corn (*Zea mays* cv Hybrid 64a × 22R, University of Wisconsin) seedlings were grown at 25°C on blotting paper soaked in tap water, under red light at an irradiance of about 1.6 mw/cm^2 (*5*). After 4 days, when the coleoptiles were 30 to 40 mm long, the seedlings were transferred to an "airtight" glass chamber. Rubber gloves permitted manipulation of objects in the chamber. Water-saturated air or nitrogen was passed through the chamber at a constant rate of flow. The flow rate of the nitrogen used for establishing anaerobic conditions was such that the oxygen concentration of the chamber was reduced by dilution to less than 0.01 percent before geotropic stimulation was applied. A corresponding flow rate of air was employed when seedlings were held under aerobic conditions. The gas pressure within the chamber was maintained slightly positive with respect to the surrounding atmosphere during the experiments. The temperature within the box was 26.5° ± 0.1°C, 1.5° higher than room temperature.

The glass chamber was kept in darkness, only phototropically inactive light being used during manipulations. One plant was placed vertically in an electrode holder and positioning assembly. Eight plants were placed adjacent to it in a similar vertical position on the bottom of the chamber. After 3 hours, electrode contact was established with the coleoptile by extruding saturated KCl solution in 1.5 percent agar from the tip of the electrode assembly. Contact was made 1 cm below the tip on diametrically opposite sides of the coleoptile. Contacts were linked through a calomel half-cell to an electrometer (input impedence 10^8 ohms). After an additional hour with the electrodes contacting the tissue, determinations of the transverse potential were made every 5 minutes for 4 hours when the gas used was air, and every 20 minutes for 8 hours when the gas was nitrogen.

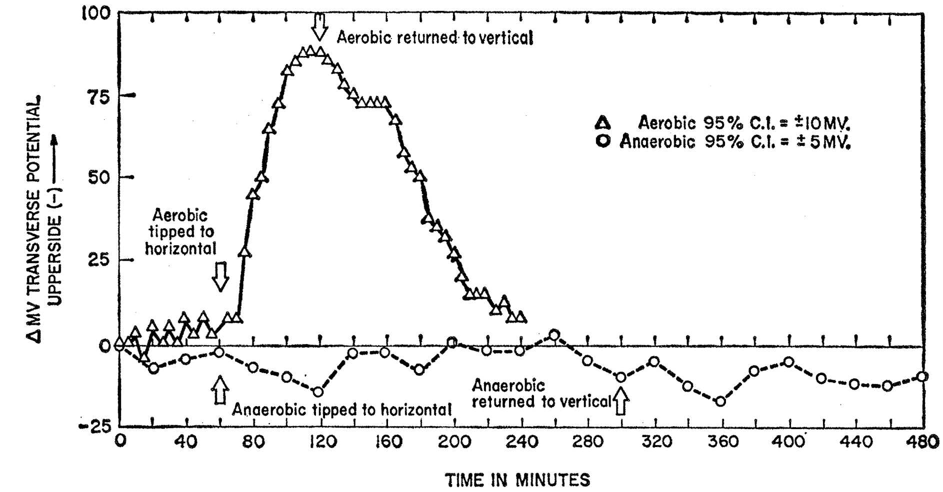

Fig. 1. The transverse geoelectric potential of *Zea mays* coleoptiles under aerobic and anaerobic conditions. Each point represents the average of the potentials of five separate plants. (*C.I.*, confidence interval.)

One hour after the initial measurement, all plants were reoriented to a horizontal position. Plants in air were returned to a vertical position after 1 hour. Plants in nitrogen were kept horizontal for 4 hours. At the end of the stimulation period, plants not used for measurements of potential were used for making shadowgraphs. The curvatures of these plants, as degrees, were determined by measuring the interior angle between a line projected through the central axis of the tip and a line projected through the central axis of the base of the coleoptile. The experiments were repeated five times. Average standard deviations and 95-percent confidence intervals of means for each treatment were determined from pooled estimates of error variance.

Our data for geoelectric potentials (Fig. 1) match almost exactly the published data (*1, 4*) obtained without direct electrode contact with the coleoptile. No geoelectric potential develops under anoxia.

Coleoptiles showed negative geotropic curvature regardless of their surrounding atmosphere. Those under anaerobic conditions were curved 17 ± 1 degrees after 4 hours of stimulation. Those under aerobic conditions curved 18 ± 1 degrees after 1 hour.

In earlier work (*1, 2*) it was established that removal of coleoptile tips virtually eliminated geotropic curvature without modifying materially the geoelectric potentials produced. This elimination demonstrated that the geoelectric effect occurs in the absence of geotropic curvature. We observed that curvature develops under anoxia, when no detectable potential gradient develops. Geotropism occurs in the absence of the geoelectric effect.

We conclude that our experiments together with those of Grahm and Hertz demonstrate a lack of either a causal or a direct relation between the geotropic and geoelectric effects. Auxin is undoubtedly required for both the tropic and electrical responses to gravity. But asymmetry of hormone distribution could well be a consequence of the action of the gravity sensor either on the reactivity to auxin of responsive cells, or on their ability to translocate auxin basipetally. Furthermore, a demonstration of asymmetric auxin distribution after geotropic stimulation under anoxia would show that geoelectric potential is not a mandatory consequence of auxin asymmetry.

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