

ing high isophane numbers, reflecting boreal climates with severe winter cold; and those of smaller body size are from stations with low isophane numbers, reflecting mild or austral climates, occasionally with severe summer heat. A similar relationship between body size and climate is found in many native species of birds.

South of latitude 28°N in North America, other selective factors tend to override the effects of selection for body size as described by Bergmann's ecogeographic rule. Although mean body weight in the sample from Oaxaca City does not fall far from an expected position along the regression line based on data from samples taken in the United States and Canada, birds from Mexico City are surprisingly light in weight and those from Progreso, Texas, are unexpectedly heavy. That these differences reflect real variation in body size and not merely nongenetic variation in level of fat deposition is indicated not only by examination of the fat condition of the specimens but also by data on the length of the tarsus, which in house sparrows is closely correlated with body weight.

Current taxonomic practice gives formal nomenclatural recognition, at the subspecific level, to morphologically definable geographic segments of species populations. And it is obvious that the levels of differentiation achieved by the introduced house sparrow in the Hawaiian Islands and in a number of areas in North America are fully equivalent to those shown by many polytypic native species. Although application of subspecific trinomial to certain New World populations of sparrows would be fully warranted, we are not convinced that nomenclatural stasis is desirable for a patently dynamic system. Nomenclatural considerations aside, the evolutionary implications of our findings are apparent. Current estimates of the minimum time normally required for the evolution of races in birds range upward from about 4000 years (14), and nowhere is there a suggestion that such conspicuous and consistent patterns of adaptive evolutionary response to environments as we have found in New World house sparrows are to be expected within a period covering not more than 111 generations. Actually, much of the differentiation in North American populations must have occurred in the present century, since sparrows did not reach Mexico City

until 1933 (15), and they were not present in Death Valley before 1914, or in Vancouver before 1900. Our findings are consistent with recent evidence of evolutionary changes in some other groups of animals, including mammals and insects (16), within historical times. Clearly, our thinking must not exclude the possibility of animals attaining to extremely rapid rates of evolution at the racial level.

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Labeled Oxygen: Transport through Growing Corn Roots

Abstract. *Oxygen, labeled with oxygen-18, is transported across wax membranes penetrated by growing corn roots. The rate of transport is a linear function of the number of penetrating roots. Other factors also influence the rate of transport.*

In a previous report, Jensen and Kirkham (1) observed that diffusion of O¹⁸-labeled oxygen through cores of moist soil increased as growing corn roots penetrated the cores. The increased diffusion could not be accounted for by changes in soil porosity, and Jensen and Kirkham assumed that the oxygen was moving through the intercellular spaces of the root for at least part of its path. However, when a nonporous layer (for example, paraffin, 6 mm thick) was placed below the soil core, transport of oxygen was barely detectable when corn roots pierced the layer. Results obtained by Ebert and Howard (2) suggested that tissue near the root tip may offer more resistance to the diffusion of oxygen than tissue farther back.

We repeated the wax layer experiment of Jensen and Kirkham with

modifications intended to test the effect of proximity of the root tips. Our experimental container was similar to that used previously (Fig. 1). The lower wax membrane was near the root tips; however, we increased the length of the soil column to 17.8 cm and added a 1.4-cm gap, 5.6 cm from the top of the column, which contained a second wax membrane. Roots growing through the upper membrane continued downward for 12.2 cm before reaching the lower membrane. Growth generally ceased when the root tips were 1 to 2 cm below the lower membrane. In this way, the mean distance of the membrane from the root tips that had penetrated it was greater for the upper membrane than for the lower. In addition, the upper membrane was nearer the foliage than the lower one.

The soil in the container was Yolo

silt loam, which had been air-dried, pulverized to pass a 2-mm screen, and then moistened again before it was packed into the container. Eighteen germinated corn seeds (*Zea mays* var. Golden Bantam) were planted 1.6 cm deep in the upper soil column. The height of the plants at the end of an experiment was about 15 cm.

The procedure for obtaining the air samples as the roots penetrated the upper membrane was as follows. Air with its oxygen slightly enriched in oxygen-18 (0.8 atom of O^{18} for every 100 atoms of O^{16}) was passed continuously (at 5 ml/min) into the container at *A* and exhausted at *B* (Fig. 1). Unenriched air (0.2 atom O^{18} per 100 atoms O^{16}) was passed in at *C* (at 2 ml/min), under the wax membrane, and exhausted at *D*. Air samples for mass spectrometric analysis were taken daily from the air streams entering *C* and leaving *D* and from the port *G* (otherwise closed) just above the wax membrane. The rate of oxygen

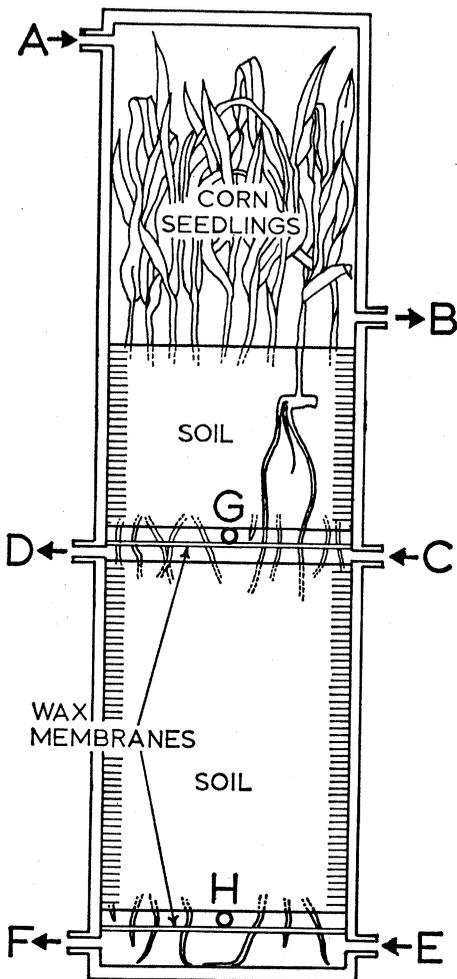


Fig. 1. Experimental container for the determination of oxygen transport across wax membranes through which corn roots are growing.

entry ρ^{10} at *C* was also measured precisely and the number of roots *R* piercing the membrane noted. The downward component, ρ , of oxygen transport across the membrane was calculated by using Eq. 2 of Jensen and Kirkham:

$$\rho = \frac{a_1 - a_0}{a_2 - a_1} \rho^{10}$$

where a_0 , a_1 , and a_2 are the ratios of O^{18} to O^{16} in the air entering at *C*, in the air leaving at *D*, and in the air just above the membrane (at port *G*), respectively. The units of ρ and ρ^{10} are given at normal temperature and pressure, in microliters per minute.

When several roots had penetrated the upper membrane and a few had reached the lower membrane, port *B* was connected to port *C* with tubing so that the enriched air passed beneath the upper membrane before being exhausted at *D*. The unenriched air was now passed in at *E* and exhausted at *F*. Measurements of oxygen transport across the lower membrane during root penetration were then made in the same way as for the upper membrane.

For the first experiment the membranes were prepared from Bareco wax, a microcrystalline wax slightly harder than paraffin. The upper membrane was 1.49 ± 0.01 mm thick. Most of the roots were unable to penetrate these membranes; therefore, for the remaining experiments, membranes were prepared by stretching cheesecloth on a frame, dipping in melted paraffin, and slowly rotating at a slight angle to horizontal while cooling. Roots readily penetrated these membranes. Membrane thicknesses for the second experiment were 0.40 ± 0.02 mm for the upper and 0.31 ± 0.03 mm for the lower membrane. For the third experiment, the values were 0.37 ± 0.02 mm for the upper and 0.38 ± 0.01 mm for the lower.

The results in Fig. 2 demonstrate that oxygen can be transported and released by corn roots. The rates are relatively high compared with those observed by Jensen and Kirkham (1); however, this may result from our use of thin wax membranes which reduce the diffusion path length of the oxygen. The points for only two membranes deviate significantly from a single regression line *B* ($\rho = -4.2 + 3.4R$). These are for an upper membrane, line *A* ($\rho = 0.8 + 9.9R$), and for a lower membrane, line *C* ($\rho = -1.7 + 1.3R$).

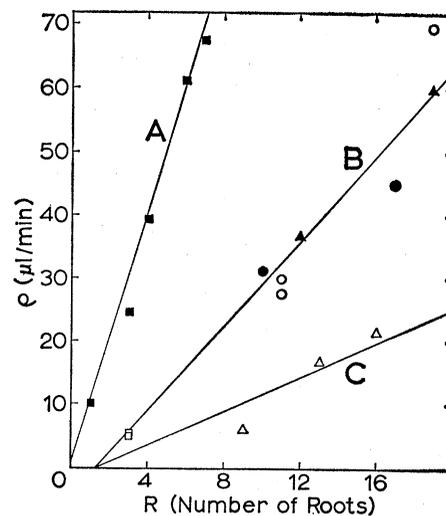


Fig. 2. Downward component, ρ , of oxygen transport across wax membranes penetrated by growing corn roots, *R*. Squares, first experiment; circles, second experiment; triangles, third experiment; solid symbols, upper membranes; open symbols, lower membranes.

We compared the rates of transport across the upper and lower membranes by calculating the mean rates per root, using the regression lines *A*, *B*, and *C*. The mean rate for the upper membranes was $2.8 \mu\text{l}/\text{min}$ per root greater than for the lower membranes. However, an analysis of variance did not show this difference to be significant (20 percent level) because of the large variation between the three experiments. Significance might result with further replications, but the variation shows that other important factors are present. The good fit to linear regression lines in Fig. 2 indicates that these factors affect all roots through the same membrane equally.

Such a pervasive factor might be the soil moisture content or the composition of the air around the roots. The concentration of oxygen below the membranes did not vary from that of the atmosphere but was not measured above the membranes. Carbon dioxide or traces of nitrous oxide (from soil denitrification) may have affected the roots. Rate of root growth may also be a factor; root growth was slower in the first experiment than in the second and third experiments. A related factor is the root respiration rate; Eq. 2 of Jensen and Kirkham does not correct for respiratory loss of oxygen within the root. Only by further experimentation will the pertinent factors be identified.

Oxygen transport and release may be of considerable importance to root

and soil aeration. For example, oxygen release from roots may be a factor in sustaining the "rhizosphere effect," the abundant microbial activity often observed on the surface of plant roots in soil.

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Hemoglobins A and F: Formation in Thalassemia and Other Hemolytic Anemias

Abstract. Rates of synthesis of hemoglobin A by erythroid cells from thalassemic subjects are markedly decreased. Formation of hemoglobin F, however, proceeds at similar rates in cells from subjects with thalassemia and other types of hemolytic anemias. A mechanism is suggested regarding the altered patterns of hemoglobin synthesis under conditions of erythropoietic stimulation in subjects with and without thalassemia.

The thalassemia syndromes include a group of inherited diseases which are characterized by a marked decrease in the concentration of hemoglobin A and, in many instances, an increase in the concentration of hemoglobin F. Various theories have been advanced to explain the mechanism of this disorder. Recently, hypotheses which presume a primary defect in the rates of synthesis of the globins (1-4) have been emphasized. Itano (1) and Ingram and Stretton (2) postulated that the defect in hemoglobin A synthesis in thalassemia is associated with the formation of a structurally abnormal hemoglobin which is made at a rate slower than normal. However, analysis of the amino acid content of individual tryptic peptides of hemoglobin from the thalassemias have as yet revealed no structural abnormalities (5). An alternative proposal (1, 2) is that the defect in thalassemia involves the

genetic regulatory system resulting in an altered rate of synthesis of structurally normal hemoglobin A.

Ribosomes characterized by sedimentation coefficients greater than 100S (polyribosomes) are the site of protein synthesis in erythroid cells of both thalassemic and nonthalassemic subjects (4). However, polyribosomes in cells from thalassemic patients have a significantly lower capacity to incorporate leucine than polyribosomes in cells from nonthalassemic persons (4). Leucine is in all peptide chains of hemoglobins A and F. Isoleucine, which is in hemoglobin F but not in hemoglobin A, is incorporated by polyribosomes in cells of thalassemic and nonthalassemic patients in at least comparable amounts. These initial observations suggested that in thalassemia the defect in protein synthesis involves a selective impairment in the capacity of polyribosomes to form hemoglobin A. In populations of erythroid cells synthesizing hemoglobin A and hemoglobin F, this hypothesis could be tested further by determining the rate of formation of these proteins.

Blood was obtained from patients with thalassemia major, sickle cell anemia, and hemolytic anemias without primary abnormalities in hemoglobins. Cells were separated from the blood, suspended in a modified Krebs-Ringer bicarbonate medium (6) that contained C¹⁴-leucine (17.1 $\mu\text{C}/\mu\text{mole}$) and C¹⁴-valine (20.3 $\mu\text{C}/\mu\text{mole}$), and incubated at 37°C. At intervals up to 80 minutes, portions of the cell suspension were removed to determine the amount of radioactivity incorporated into acid-insoluble material (7). At 80 minutes the cells were recovered from the cell suspension by centrifugation and then lysed by a short exposure to a hypotonic solution (7). The hemolysate was made free of ribosomes by centrifugation at 200,000g for 2 hours. Hemoglobin concentrations were determined and ribosomes were analyzed (4). Hemoglobin fractions were separated from ribosome-free hemolysates by using an amberlite IRC-50 synthetic cation-exchange resin with developer No. 2 (8). The amount of nonheme protein contaminating the hemoglobin fractions was determined by DEAE (diethylaminoethyl) chromatography (9).

The proportion of the hemoglobin which was hemoglobin F was generally higher in the subjects with thalassemia major than in the patients with sickle cell anemia or chronic hemolytic

anemia (Table 1). In all subjects studied, however, the concentrations of hemoglobin were similar, and the percentage of hemoglobin F was above normal (Table 1). The normal concentration of hemoglobin F is below 2 percent.

The rate of incorporation of amino acid into hemoglobin A by cells from thalassemic subjects was strikingly lower than that by cells from subjects with the other types of hemolytic anemia (Table 1). By contrast, the incorporation into the fraction containing hemoglobin F was not markedly different in cells from the thalassemic compared with the nonthalassemic subjects. In no experiment did the incorporation of radioactive amino acid into nonheme protein exceed 6 percent of the total amino acid incorporated. The decreased rate of amino acid incorporation into hemoglobin A cannot be attributed to a failure of saturation of the ribosomes in thalassemic cells. In all instances examined, the radioactivity associated with ribosomes reached a plateau value within 5 minutes. The incorporation of the C¹⁴-amino acids into the supernatant protein continued to increase at a linear rate during the 80-minute period of incubation. In three patients with thalassemia (Table 1, thalassemia subjects 1, 3, and 4) the relative concentration of hemoglobin F to hemoglobin A was smaller than the ratio of amino acid incorporated into these fractions, respectively. A possible explanation for this finding is that prior to these studies these patients were regularly transfused. The other patient with thalassemia (Table 1, thalassemic subject 2) had never been transfused. In this case, the relative concentration of hemoglobins F to A was higher than the ratio of their rates of synthesis. This could be due to a lower rate of turnover of hemoglobin F in vivo, as suggested by Gabuzda *et al.* (10).

The present results indicate a selective decrease in the capacity of ribosomes of erythroid cells of thalassemic subjects to synthesize hemoglobin A, which is composed of two alpha- and two beta-peptide chains. The rate of amino acid incorporation into hemoglobin F, composed of two alpha- and 2 gamma-peptide chains, was similar in cells of both thalassemic and nonthalassemic subjects. Therefore the primary defect in these subjects with thalassemia major may be a decreased capacity to synthesize beta chains. In normal subjects, more than 90 percent of the protein formed by erythroid cells is