from 1.0 to 20.0, in t from 0.0 to 20.0, and which include several generating curve shapes, at varying distances from the coiling axis. The resulting array will be an extension of Fig. 2 and may find application to a variety of problems of gastropod evolution and ecology. For example, the problem of the efficiency with which a given snail uses skeletal material may be studied by this means, because shell geometry has a pronounced effect on the amount of skeletal material necessary to produce a given volume of living space (*4*).

DAVID M. RAUP

Department of Geology,
Johns Hopkins University,
Baltimore 18, Maryland

References and Notes

1. D. M. Raup, *Proc. Natl. Acad. Sci. U.S.* **47**, 602 (1961).
2. D'A. W. Thompson, *On Growth and Form* (Macmillan, New York, 1942), pp. 778–779; see pp. 748–849 for a general review of the literature on shell coiling.
3. The same approach can be applied to coiled cephalopods and to the individual valves of pelecypods and brachiopods. In general form, these differ from snails only in values of the four parameters.
4. I am indebted to all those who gave helpful advice and criticism at various stages of this study. In particular, I wish to thank Erle G. Kauffman, Lynton S. Land, Harald A. Rehder, and Aaron C. Waters for reading early drafts of the manuscript, M. Patricia Power for aid in computer methods, John S. Spurbeck for preparing the illustrations, and Mary Gill for typing the manuscript.

1 June 1962

Two-Dimensional Gel Electrophoresis

Abstract. Improved resolution of serum protein mixtures is effected by electrophoresis, first in a 5 percent acrylamide gel following which a strip of the resolved pattern is embedded in 8 percent gel and subjected to a second electrophoresis separation at right angles to the first. Lactic dehydrogenase enzymes appear as small rectangular spots lying on an oblique straight line passing through the point of initial application.

Two-dimensional electrophoresis, employing paper medium in the first direction and starch gel in the second direction, has been effectively applied by Poulik and Smithies (*1*) to the resolution of serum proteins. Hermans, McGuckin, McKenzie, and Baird (*2*) described a similar technique employing paper in the first direction and cyanogum gel in the second direction. Ashton (*3*) also used agar in the first direction combined with starch gel in the second direction with good resolution.

Experimental studies of the factors affecting electrophoretic migration and resolution in acrylamide gels, now in progress in this laboratory, have demonstrated significant nonlinear effects of gel concentration on the electrophoretic mobility of various proteins. These studies suggest that two-dimensional gel electrophoresis employing a low-concentration gel in the first direction followed by a high-concentration gel in the second direction, will produce separations analogous to those obtained in two-dimensional paper chromatography. The resolution should be obtained without change in *p*H, composition, ionic strength, or electrophoretic conditions, inasmuch as they depend solely on the concentration of the gel itself.

The procedure employed in the experiments reported here was as follows. A 5 percent concentration of acrylamide gel was prepared in *p*H 9.0 tris buffer by the method of Raymond and Nakamichi (*4*) and then poured into the vertical gel electrophoresis cell described by Raymond (*5*). A sample of fresh human serum 0.04 to 0.06 ml in volume was applied to a 2-cm wide slot in the top of the gel. Electrophoresis was effected at 300 v, 50 to 150 ma for 1½ hours at which time the albumin (rendered visible by prestaining with a small crystal of bromphenol blue) had migrated approximately 10 cm. The gel slab was removed from the apparatus and a 1-cm wide strip was excised longitudinally through the pattern. This strip was replaced transversely in the gel compartment of the cell. The compartment was then filled with an 8 percent acrylamide gel solution in the same buffer. The solution gelled within 30 min, with the 5 percent slab embedded across the top of the gel. Electrophoresis was again effected at 300 v and 50 to 150 ma, increasing the time to 3 hr to compensate for the reduction in average migration velocity of the migrating components.

Following electrophoresis the entire gel slab can be stained for protein and other components in the usual way. It is particularly effective to use multiple stains in sequence. Figure 1 shows lactic dehydrogenase isozymes stained by the procedure of Latner (*6*), which form a linear series of spots that do not coincide with any of the protein components demonstrated by amidoblack stain. The straight line through the lactic dehydrogenase spots passes through the point of initial application, suggesting that these isozymes all have the same molecular size but differ by equal increments of charge. The components of the haptoglobin complex, on the other hand, fall on a smooth curved line which is asymptotic to the 5 percent direction, suggesting a constant ratio of charge to molecular size with increasing size of the molecule.

The initially thin layer of sample applied in the first direction diffuses as the components migrate through the gel so that the resolved components form spots 1 to 3 mm long (in the direction of travel) at completion of the first electrophoresis. (Albumin, because of its greater concentration, spreads 10 to 15 mm). At the start of

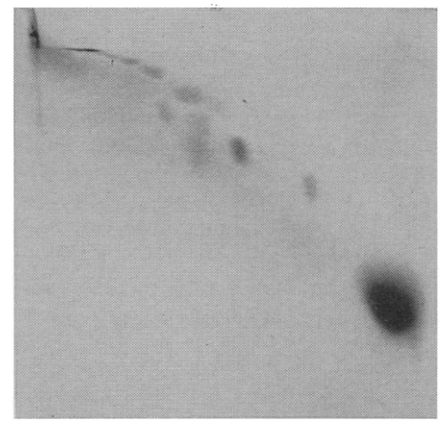

Fig. 1. Two-dimensional electrophoresis in acrylamide gel, 5 percent and 8 percent, tris buffer *p*H 9.0. Three rectangular spots on an oblique line near the center are lactic dehydrogenase isozymes, others are proteins stained with Light Green SF. Origin at upper left, 5 percent direction across, 8 percent direction down.

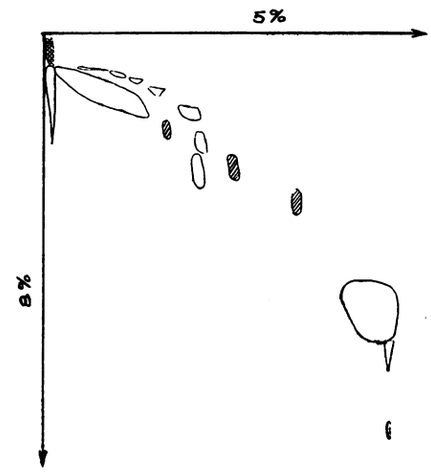

Fig. 2. Tracing of the gel shown in Fig. 1. Cross-hatched spot is origin, diagonal-hatched spots are lactic dehydrogenase isozymes.

the second electrophoresis, all components are uniformly 1 cm long, the width of the applied gel strip. The spots are, however, compressed as they enter the 8 percent gel by a factor which is the ratio of the respective migration rates in the two gels. The lactic dehydrogenase isozymes, for example, appear as rectangular spots 3 mm (5 percent direction) by 5 mm (8 percent direction).

SAMUEL RAYMOND
BARBRO AURELL

William Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Philadelphia

References and Notes

1. M. D. Poulik and D. Smithies, *Biochem. J.* **68**, 636 (1958); O. Smithies and M. D. Poulik, *Nature* **177**, 1053 (1956).
2. P. E. Hermans, W. F. McGuckin, B. F. McKenzie, E. D. Baird, *Proc. Staff Meetings Mayo Clinic* **35**, 792 (1960).
3. G. C. Ashton, *Nature* **180**, 917 (1957).
4. S. Raymond and M. Nakamichi, *Anal. Biochem.* **3**, 23 (1962).
5. S. Raymond, *Clin. Chem.* **8**, 455 (1962).
6. A. L. Latner and A. W. Skillen, *Lancet* **1961-1**, 1287 (1961).
7. This work was supported by grants from the National Institutes of Health and the Institute for Cooperative Research of the University of Pennsylvania.

4 June 1962

Anion-Exchange Properties of Plant Root Surfaces

Abstract. Anion-exchange properties of plant root surfaces were demonstrated by using negatively charged anion dyes. The site of the positive charges may be amino groups which become positively charged in the presence of hydrogen ions but are neutralized under alkaline conditions.

Cation-exchange properties of root surfaces have been demonstrated by Williams and Coleman (*1*). Further experimentation has shown the (i) nature of the exchange sites, (ii) the cation exchange capacities of different plant species, and (iii) how differences in capacities can be used to explain differences in uptake of monovalent and divalent cations. Plant roots contain many —COOH groups whose negative charge can adsorb positive ions, such as methylene blue$^+$, Ca^{++}, NH^+_4, and H^+. Plant cells also contain considerable quantities of protein (*2*) whose amino groups (NH_2) become positively charged under acidic conditions. These groups on root surfaces could be a potential source of positively charged spots for anion-exchange reactions. Plant roots placed in negative dyes, such as eosin or acid fuchsin, become highly colored, especially on the tips and on meristematic tissue. If this adsorption is on positively charged spots, then anion exchange can take place on the root surface.

An experiment was performed in which eosin dye previously adsorbed on root surfaces was replaced by a series of sodium salts—NaF, NaCl, NaBr, NaI, $NaNO_3$, Na_2SO_4, Na_2HPO_4, $NaHCO_3$, Na_2CO_3, and NaOH. (Figure 1 shows the results.) A different quantity of dye is released by each anion, and the amount released increases with increasing salt concentration. A lyotropic series of replacement is observed in which the order of replacement of dye is: $F^- < Cl^- < Br^- < I^- < NO_3^- < SO_4^{--}$, $< HPO_4^{--}$, CO_3^{--}, HCO_3^-, and OH^-.

The replacing power of neutral salts increases slowly as the concentration of salt increases from 0.0001*N* to 0.1*N*. The shape of the curves resembles cation exchange curves for replacement of methylene blue by chloride salts (*1*). A peculiarity in the shape of the curve is observed with the alkaline salts Na_2CO_3, $NaHCO_3$, Na_2HPO_4, and NaOH. In 0.0001*N* solutions slightly more eosin was replaced by these salts than was replaced by neutral salts. However, in 0.001 to 0.1*N* solutions, a plateau of release was reached and further increases in concentration did not release more eosin. The data suggest that positive exchange spots are neutralized by basic salts, thus causing adsorbed eosin to be released. A possible adsorption site that would be affected in this manner is the NH_2 group of the protein molecule. The group could react as follows:

$$\begin{matrix} R\text{-}NH_2 \\ | \\ COOH \end{matrix} + H^+ \longrightarrow \begin{matrix} R\text{-}NH_3^+ \\ | \\ COOH \end{matrix}$$

$$\begin{matrix} R\text{-}NH_3^+ \\ | \\ COOH \end{matrix} + \text{eosin}^- \longrightarrow \begin{matrix} R\text{-}NH_3^+\ \text{eosin}^- \\ | \\ COOH \end{matrix}$$

Addition of OH^- to eosin-saturated groups would bring about neutralization of the positively charged H-ion, thus forming water and neutral NH_2 groups and causing the release of eosin to the solution:

$$\begin{matrix} R\text{-}NH_3^+\ \text{eosin}^- \\ | \\ COOH \end{matrix} + 2\,Na^+OH^- \longrightarrow$$

$$\begin{matrix} R\text{-}NH_2 \\ | \\ COONa \end{matrix} + Na^+\ \text{eosin}^- + 2H_2O$$

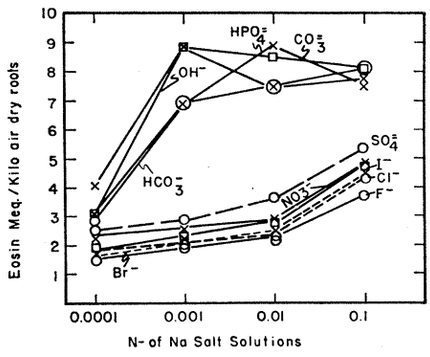

Fig. 1. Exchange of eosin from intact barley roots by salts as a function of concentration.

If this reaction takes place, adsorption of anion dyes should be affected by the *p*H of the medium. A number of NaCl solutions were prepared in which the *p*H was varied from 1 to 12 with HCl and NaOH. Three Algerian oat plants grown in Hoagland solutions for 2 weeks were washed in distilled water, placed in 0.1*N* HCl for 1 minute, rinsed five times in 200 ml of distilled water, and then placed in eosin at *p*H 7 for 30 seconds. The dyed roots were then rinsed five times in water and placed in 50 ml of 0.1*N* NaCl (adjusted to *p*H values from 2 to 12) for 30 seconds. The concentration of eosin in the solutions was determined with a colorimeter (Klett Summerson) with a blue filter (No. 42). (It was later observed that a No. 54 green filter, with eosin adjusted to *p*H 5.5, gave more reproducible readings.) The results plotted with *x*'s are shown in Fig. 2. The data indicate that acid solutions were quite effective in keeping amino groups hydrogen-saturated, thus allowing eosin to remain tightly bound.

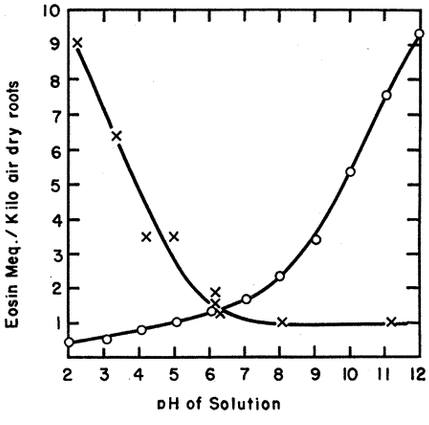

Fig. 2. The effect of the *p*H of solutions upon the amount of eosin removed from oat roots: (*x*) *p*H of saturating eosin solutions; (*o*) *p*H of displacing NaCl solutions.