of replicate colonies (*8*). A single hydranth is removed from a hermit crab shell, where it occurs naturally, and is placed on a microscope slide in standing sea water. In 2 or 3 days the hydranth attaches to the slide, which can then be transferred to a glass slide holder in an aquarium. After 1 month at 23°C, the colony reaches a size that permits using it as a source for about 75 replicate colonies.

To feed the 20 colonies that fit into one glass slide tray, it is only necessary to immerse the tray in a finger bowl of hatched eggs of *Artemia salina* for 10 minutes, after which the hydranths have ingested as many brine shrimp as they can. Each day the *P. carnea* colonies were fed for 10 minutes and the filtered sea water was changed. The water in a 15-liter aquarium is constantly aerated and is stirred by means of a glass propeller (*9*).

To determine the time course of the appearance of sexual zooids, each of nine similar colonies was photographed almost every day for a month. (The daily growth of colony 11, clone W, is typical, Fig. 2.) Stolon lengths and hydranth numbers were calculated from enlargements. Initial growth of stolons and increase in hydranth numbers were exponential. After the second week density made it no longer possible to calculate stolon lengths from the photographs. During the third week, the rate of increase of asexual hydranths fell off sharply; simultaneously, small medusa-bearing zooids began to appear in the central part of the colony.

Thus, *P. carnea*, although continuing to propagate nutritive hydranths, initiates its second hydranth differentiation at a time when the rate of increase in number of the first form declines. In the hydra, too, the onset of sexuality coincides with the cessation of logarithmic growth. Another similar situation is that described by Braun (*10*) for *Brucella abortis*, a bacterium that is sensitive to one of its own metabolic products, D-alanine. After a period of logarithmic growth, the rate of increase of the bacterium declines; at this time selection pressure encourages the reproduction of a mutant form less sensitive to the inhibiting substance.

Experiments are in progress to determine the mechanism of asexual hydranth inhibition and sexual hydranth inception and to determine what relations, if any, exist between the two. Detailed reports on the determination of hydranth-to-hydranth distance, the pattern of growth in single hydranths, the effect of CO_2 on sexuality and the mechanism of this effect, and descriptions of the five clones being maintained in this laboratory (*11*) are in preparation (*12*).

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9. That sea water—an unknown factor—is the medium is an objection that has been raised by C. Fulton, *Science* **132**, 473 (1961), but which can hardly be considered seriously regarding any experimental setup that requires the feeding of live food of unknown chemical composition. What is important in such studies is that one variable at a time can be changed and controlled. The totality of a defined milieu is no more precisely defined than its least known quantity.
10. W. Braun, *Am. Naturalist* **86**, 355 (1952).
11. The Stazione Zoologica, Naples, Italy, has agreed to maintain cultures of five clones of *P. carnea* and to make them available to anyone wishing to use the animal either in Naples or elsewhere. Since the clones differ considerably in rate of growth, rate of sexuality, and stolon-hydranth ratio, it would be advantageous for investigators wishing to work on this species to use the same clones, rather than to isolate new ones.
12. This research was carried out while I was a NATO post-doctoral fellow at the Stazione Zoologica, Naples. Thanks are due to Dr. Peter Dohrn and the staff of the Stazione Zoologica for their cooperation during the course of these experiments. The use of *P. carnea* was suggested by Dr. Howard Schneiderman while I was a student in the invertebrate course at the Marine Biological Laboratory, Woods Hole, Mass.

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Iron Chelates in Soybean Exudate

Abstract. Soybean exudates contain iron compounds which can be separated electrophoretically into anodic bands and eluted with water. Chromatography of the water extracts separated iron from chelating agents which were identified as malic acid and malonic acid. Hence organic acids seem to function in the translocation of iron in plants.

Although much has been done to characterize metal chelates of organic acids in simple systems (*1*), very little is known about these compounds in the aqueous systems of plants. However, a considerable amount of information concerning metal binding and translocation is being accumulated from studies with expressed fluids and exudates from plants. Schmid and Gerloff (*2*) have reported a negatively charged iron compound in tobacco stem exudate, but unfortunately did not identify the chelating agent involved. Jones (*3*) has recently shown that organic acids (especially malic acid) may be responsible for the absorption and translocation of aluminum.

Several studies in this laboratory (*4–6*) have provided information leading to the identification of iron compounds in plant exudates. The data may be summarized as follows: (i) large amounts of iron absorbed from synthetic chelate were found in exudate (*4, 5*); (ii) exudate from controls of several species bound iron in vitro and held it soluble against heat and filtering at high *p*H [the solubility of iron was attributed to natural chelators (*4*)]; (iii) iron compounds in the exudate migrated electrophoretically as anions and had mobilities different from those of the chelates that supplied iron to the roots (*5*); and (iv) tests for iron protein (*6*) by dialysis, centrifugation, heat, and high salt treatment, combined with electrophoresis, failed to reveal iron protein but indicated, rather, that the iron carriers were small, stable, anodic molecules. The study reported here shows that the iron-binding agents were organic acids.

The methods used, discussed in detail elsewhere (*5*), are briefly as follows: Hawkeye seedlings, 6 days old, were bound in groups and transferred to a standard nutrient (*5*) containing $10^{-6}M$ ferric ethylenediamine di(*o*-hydroxyphenylacetate) (FeEDDHA). This level of iron produced green plants and prevented a build-up of iron in the roots. On the 16th day from germination each plant group was transferred to standard nutrient (1 liter) labeled with $10^{-6}M$ Fe^{59}EDDHA (526 count/sec ml). The tops of the plants were cut off, and exudate from the stems was delivered by plastic tubing into vials in vacuum jars that were packed with ice.

Electrophoresis of the iron compounds was accomplished by means of 1,3-iminodiproprionitrile-acetic acid-formamide buffer (*p*H 5.9) (*7*) and a Misco ionophoresis apparatus (*8*). By running the negatively charged dye amaranth (*7*), 9 cm from the origin,

the iron compounds of malate and malonate could be separated on the paper; the mobilities were closely reproducible. A pattern of exudate iron applied as a band is shown after electrophoresis (Fig. 1, path 1). After electrophoresis the papers were dried and radioautographed to locate the Fe^{59} bands. These areas were then cut out and dipped in acetone to free the papers of buffer. The paper strips, rolled and inserted into plastic tubing, were eluted with water. Assays of the solvents revealed no activity in the acetone but apparent complete solubility of the iron compounds in the water. The concentrated water extracts containing the electrophoretically separated iron compounds were subjected to reruns by electrophoresis and to paper chromatography. Figure 1, path 2, shows a rerun of a water extract of Fe^{59} (previously separated as a band, path 1).

Chromatographic identification of exudate compounds was accomplished by means of a swamping acid solvent (*9*) consisting of 1-pentanol and 5*M* formic acid (1/1). In this solvent the Fe^{59} was separated at the origin from two exudate components which were identified chromatographically as malic and malonic acids. The malic acid was at a much higher concentration than the malonic aid.

In view of these results, additional electrophoretic tests were run with Fe^{59} bound to known malic and malonic acids, and the results were compared with those for the labeled exudate. Figure 1 shows ferric malate (path 3) and ferric malonate (path 4). Path 5 shows the inorganic Fe^{59} control remaining at the origin. Path 6 shows the mobility of the chelate, FeEDDHA, which was supplied to the roots. This confirms previous findings (*5*) by showing that the iron in the exudate (path 1) and the iron supplied to the roots (path 6) are in different forms.

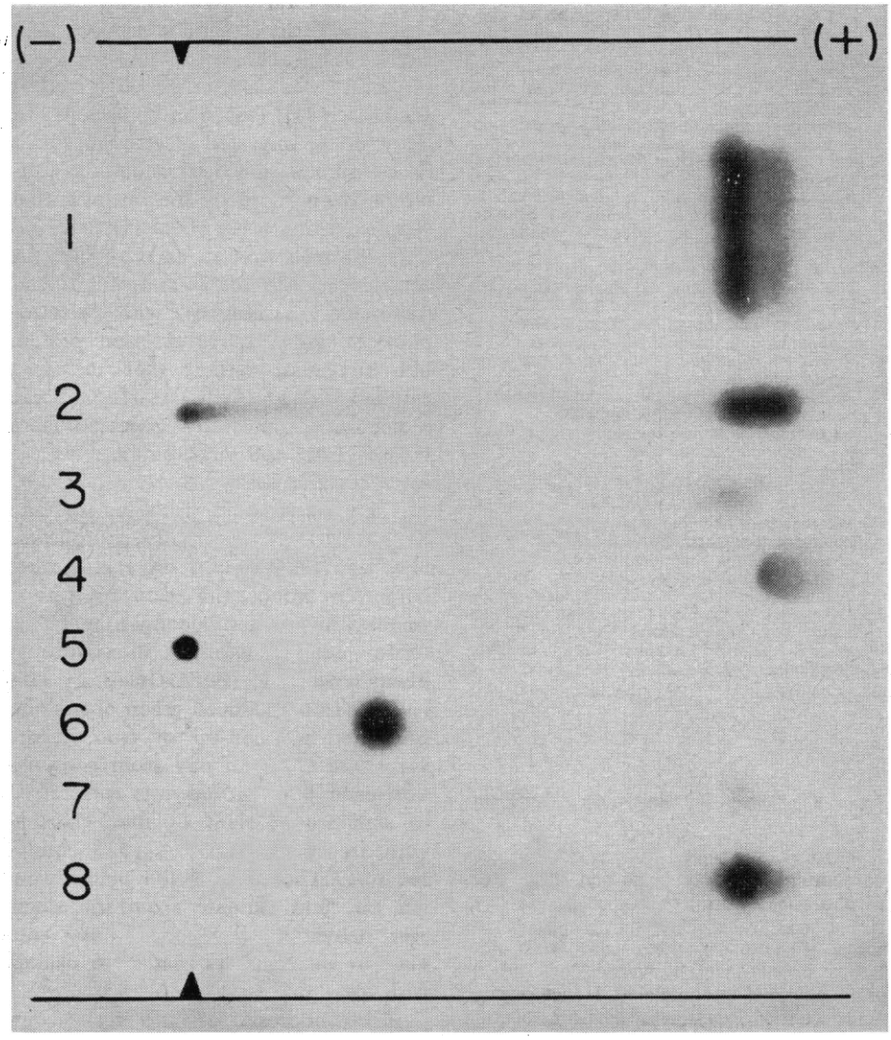

Fig. 1. Radioautographs of iron-labeled compounds after electrophoresis. Numbered paths show Fe^{59} patterns for the following solutions applied initially at the origins: 1, soybean exudate; 2, rerun of water extract of iron previously separated, as on path 1; 3, ferric malate; 4, ferric malonate; 5, inorganic iron control; 6, FeEDDHA chelate supplied to roots; 7, soybean exudate from green plants precultured in a nutrient of low iron concentration; and 8, soybean exudate from chlorotic plants precultured in a nutrient without iron.

In a related experiment four groups of Hawkeye soybeans were grown for 16 days in nutrients containing different levels of iron; the exudates were then collected. The nutrients for three of these groups contained FeEDDHA in concentrations 10^{-5}, 5×10^{-6}, and $10^{-6}M$, respectively; the fourth group was grown without iron. After treatment with Fe^{59}EDDHA and collection of exudate for 20 hours, the exudates were assayed for Fe^{59}, with results as follows: group 1 ($10^{-5}M$), activity of 22 count/sec ml; group 2 ($5 \times 10^{-6}M$), activity of 90 count/sec ml. Radioautographs of exudate were not produced for these first two groups. Plants in group 3 ($10^{-6}M$) gave exudate with activity of 2240 count/sec ml. The radioautograph produced after electrophoresis (Fig. 1, path 7) shows a small amount of ferric malate. These plants were green, as were the plants in groups 1 and 2, but they were undoubtedly on the borderline of iron deficiency. The plants in group 4 were just beginning to show chlorosis. Assay of the exudate gave a result of nearly 17,000 count/sec ml, and most of the iron was held as the chelate of malic acid (path 8).

These results demonstrate a connection between the nutritional status of the plants, especially the roots, and the amount of iron absorbed and held as chelate in the exudate. However, there is no evidence that all malate that moves out of the roots is combined with iron, or that a shortage of this agent limits the flow of iron. It is now known that exudate from green plants which have absorbed very little iron through the roots (such as the plants discussed here) will bind additional iron in vitro as ferric malate.

Experiments with exudates from sunflower, cotton, and tomato (*6*) have also revealed iron compounds which migrate electrophoretically as anions and have mobilities similar to those of the compounds in soybean exudate.

Iron-binding agents more elaborate than the simple plant acids have been found in animals (*10*) and in certain fungi (*11*). Thus, it is not assumed that the common acids are the principal carriers in all biological systems.

Concerning iron transport in plant systems, however, this study shows that iron absorbed from a ferric chelate moves up the stem and is held in the exudate in other chelated forms, the principal one being iron malate. The presence of iron chelates in the exudate of plants would account for the solubility of iron at the relatively high *p*H's of these systems and at the same time suggests that chelated iron is the form being translocated in intact plants.

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25 September 1961

Electrochemical Diffusion

Abstract. The methods of irreversible thermodynamics, applied to the problem of steady-state linear diffusion, lead to the conclusion that the flux across any system of parallel membranes or phase boundaries can be expressed as a linear function of the differences in electrochemical potential across the system. The presence of fixed charges, polarizable molecules, or electric fields does not alter the flux-force relation.

The classical approach to diffusion problems is that of Fick (*1*). His treatment has been extended to the problem of diffusion of electrolytes by Nernst (*2*), Planck (*3*), and Teorell (*4*), but only the relatively simple Nernst formulation has found wide use in chemistry or biology. Goldman (*5*) and Hodgkin and Katz (*6*) introduced concepts from electrostatics in the effort to obtain equations applicable to bioelectric potentials, but their differential equations, like those of Teorell, can be integrated only for special cases and with the aid of a number of assumptions of uncertain validity.

Prigogine, Mazur and Defay (*7*) have provided the basis for a new approach to the problem by demonstrating theoretically that, in any microscopic portion of a system at constant uniform temperature and pressure the diffusion affinity A_i of any molecular species i is

$$A_i = -\text{grad}\ \mu^*_i \qquad (1)$$

The electrochemical potential μ^*_i is

$$\mu^*_i = \mu'_i - \frac{\mathbf{E}^2}{8\pi}\delta_i + z_iF\phi = \mu_i + z_iF\phi \qquad (2)$$

where μ_i is the chemical potential, μ'_i is the chemical potential in the absence of an electric field (hence, $RT\ ln\ f_iC_i$), $\mathbf{E}$ is the electric field strength, δ_i is the dielectric increment dD/dC_i, z_i is the electrochemical valence, F is the Faraday, and ϕ is the electric potential.

Suppose that two parallel planes, located in a rectangular coordinate system at $x = \alpha$ and $x = \eta$, and normal to the x-axis, bound an aqueous system, made up of molecular species a,b,...i,j,...n, which is homogeneous with respect to the y- and z-axes. The flux of any constituent $\mathbf{J}_i$ across unit area of any plane parallel to the bounding planes is

$$\mathbf{J}_i = \sum_j L_{ij}\mathbf{A}_j \qquad (3)$$

In this and subsequent equations, symbols in bold-face indicate vectorial components along the x-axis only, and the L_{ij} are linear phenomenological coefficients (*8–10*). In the steady state,

$$\mathbf{J}_i = \frac{1}{\lambda}\int_{x=\alpha}^{x=\eta}\left(\sum_{j=a}^{j=n} L_{ij}\mathbf{A}_j\,dx\right) \qquad (4)$$

where λ is the distance between α and η. The diffusion affinity $\mathbf{A}_j$ will be a continuous function of x, hence the diffusion affinity $\mathbf{A}_j{}^d$ across the system will be

$$\mathbf{A}_j{}^d = \int_{x=\alpha}^{x=\eta}\mathbf{A}_j\,dx \qquad (5)$$

If we define a set of mean linear coefficients by

$$L'_{ij} = \frac{\frac{1}{\lambda}\int_\alpha^\eta \sum_j L_{ij}\mathbf{A}_j\,dx}{\int_\alpha^\eta \mathbf{A}_j\,dx} \qquad (6)$$

we have

$$\mathbf{J}_i = \sum_j L'_{ij}\mathbf{A}_j{}^d \qquad (7)$$

The expanded differential equation for $\mathbf{A}_j$ in any plane is

$$\mathbf{A}_j = -\frac{d\mu'_j}{dx} + \frac{\mathbf{E}^2}{8\pi}\frac{d\delta_j}{dx} + \frac{\rho\delta_j}{D}\frac{d\phi}{dx} - z_iF\frac{d\phi}{dx} \qquad (8)$$

The equation therefore includes explicitly the electric field strength, the dielectric increment and dielectric constant, which are related to the presence in the system of polarizable molecules, and implicitly from Poisson's relation

$$\nabla^2\phi = -\frac{4\pi\rho}{D} \qquad (9)$$

the charge density ρ, which will include any fixed charges in the structure of the system. With the above and the electrostatic equation for field strength

$$\mathbf{E} = -\text{grad}\ \phi \qquad (10)$$

we can readily integrate Eq. 5 to

$$\mathbf{A}_j{}^d = (\mu^*_j)^\alpha - (\mu^*_j)^\eta = \Delta\mu^*_j \qquad (11)$$

and the flux Eq. 7 becomes

$$\mathbf{J}_i = \sum_j L'_{ij}\,(\Delta\mu_j + z_jF\Psi) \qquad (12)$$

where Ψ is the electrical potential difference between α and η. This relation will be valid for linear diffusion in any continuous aqueous system in which the linear phenomenological relations hold.

Equation 12 may readily be extended to include discontinuities such as phase boundaries or membranes of infinitesimal thickness; finite membranes can be treated as aqueous systems. Assume that the planes at $x = \alpha$ and $x = \eta$ are phase boundaries separating an internal phase from two infinite external phases I and II. Let $\mathbf{J}_i{}^\alpha$, $L^\alpha{}_{ij}$, and $\mathbf{A}_i{}^\alpha$ represent the flux, linear coefficients, and diffusion affinities across α, and $\mathbf{J}_i{}^\eta$ and so on those for η. Define another set of coefficients

$$L''_{ij} = \frac{\frac{1}{3}\left(\sum_j L_{ij}{}^\alpha\mathbf{A}_j{}^\alpha + \sum_j L'_{ij}\mathbf{A}_j{}^d + \sum_j L\eta_{ij}\mathbf{A}_j{}^\eta\right)}{\sum_j (\mathbf{A}_j{}^\alpha + \mathbf{A}_j{}^d + \mathbf{A}_j{}^\eta)} \qquad (13)$$

Then the flux from I to II becomes

$$\mathbf{J}_i = \sum_j (L''_{ij}\Delta\mu_j + Z_jFE) \qquad (14)$$

where $\Delta\mu_j$ and E are now the differences respectively in chemical and electrical potential between I and II, and these quantities can in general be measured experimentally.

In a more detailed paper, I have