rinsed three times in sterile distilled water, minced, resuspended in 100 ml of NaCl-antibiotic solution and shaken vigorously by hand. After standing for 30 minutes, 50 ml of the surface fluid was centrifuged and the sediment was resuspended in 10 ml of NaCl-antibiotic solution. *Histoplasma capsulatum* was not isolated from any of the mice injected with these four preparations when the mice were killed and tissues were subcultured after 3 weeks (*1*).

The remaining 50 ml of the feather antibiotic suspension was stored at 4°C. After 4 weeks it was again shaken and allowed to settle for only 5 minutes prior to removal of an additional 10 ml for injection into mice. This specimen differed from the others in that it contained numerous tiny feather fragments. When mice inoculated with this sample were killed 3 weeks later, the spleens yielded many colonies grossly consistent with *H. capsulatum*. The colonies appeared after incubation on standard isolation media (*3*) at room temperature for 8 to 12 days. The organism also grew as a yeast which was indistinguishable morphologically from *H. capsulatum* in the yeast phase when incubated on Francis glucose cystine agar at 37°C (*6*). However, macroconidia characteristic of the organism in its filamentous phase were not produced even after transfer to a variety of media such as Sabouraud's agar, potato dextrose agar, corn meal agar, and other media which often induce sporulation in sterile mycotic strains.

Further attempts to induce sporulation were made when similar colonies, which also failed to sporulate, were isolated from mice after injection of two additional 10-ml samples of the feather-antibiotic suspension. Several colonies were selected and after serial transfer on potato dextrose agar they were again injected into mice, reisolated from the spleens on the isolation media and serially transferred several times on potato dextrose agar slants. Following the sixth passage through mice, the isolates after only one transfer to potato dextrose agar revealed many small, smooth-walled, round to pyriform spores (Fig. 1). After three additional transfers on this medium, the macroconidia typical of *H. capsulatum* were also observed (Fig. 2).

This home-made pillow was brought from Hungary more than 30 years ago. It had not been cleaned for many years and when not in use it was stored in attics and closets accessible to both birds and bats (*7*). It is, therefore, not possible to determine whether the organism was present on or in the feathers when the pillow was made or whether the organism was deposited later by other means. In any event, this situation is not unique, especially in rural areas, and it is not improbable that other infants and, indeed, adults might acquire histoplasmosis from a similar source.

CHARLOTTE C. CAMPBELL
GRACE B. HILL
BARNEY T. FALGOUT

Department of Bacteriology, Walter Reed Army Institute of Research, Washington, D.C.

References

1. C. W. Emmons, *Public Health Repts.* **64**, 892 (1949).
2. J. T. Grayston and M. L. Furcolow, *Am. J. Public Health* **43**, 665 (1953); L. Ajello and L. D. Zeidberg, *Science* **113**, 662 (1951); J. T. Grayston, C. G. Loosli, E. R. Alexander, *ibid.* **114**, 323 (1951); C. G. Loosli, J. T. Grayston, E. R. Alexander, F. Tanzi, *Am. J. Hyg.* **55**, 392 (1952); C. W. Emmons *Public Health Repts.* **76**, 591 (1961).
3. C. C. Campbell, *J. Lab. Clin. Med.* **50**, 841 (1957).
4. L. Ajello, *Mykosen* **3**, 43 (1960); H. Campins, C. Zubillaga, L. Gomez-Lopez, and M. Dorante, *Am. J. Trop. Med. Hyg.* **5**, 690 (1956); R. C. Arellano and J. B. Galvez, *Am. Fac. Med. Lima* **38**, 1092 (1955).
5. C. C. Campbell, *Ann. N.Y Acad. Sci.* **89**, 163 (1960).
6. ———, *J. Bacteriol.* **54**, 263 (1947).
7. M. H. Shacklette, F. H. Diercks, N. B. Gale, *Science* **135**, 1135 (1962).

23 January 1962

Course of Cation Absorption by Plant Tissue

Abstract. The absorption of rubidium by excised barley roots from solutions containing calcium was a strictly linear function of time for 1 hour and was temperature-sensitive throughout; there was no evidence of an initial nonmetabolic exchange phase of uptake. The rubidium absorbed reached concentrations many times the external concentration without any slackening of the rate of absorption—evidence for a high degree of irreversibility of the overall absorption process. This is discussed in terms of the "enzyme-kinetic" model of ion transport by carriers.

We have studied the course and extent of the absorption of rubidium by excised barley roots in a series of experiments. A typical experiment is presented, and its implications are discussed in regard to (i) the time course of absorption and the significance of the so-called initial phase of cation uptake and (ii) the high degree of irreversibility of the overall mechanism of cation absorption by this tissue.

In our experiments, 1-liter Erlenmeyer flasks each containing 1000 ml of the experimental solution were maintained at the temperature of the respective experimental runs. The solution contained 0.10 mmole of RbCl, the rubidium being labeled with Rb^{86}, and 0.50 mmole of $CaCl_2$. The *p*H, initially 5.6, did not vary by more than 0.2 *p*H unit during the experimental runs. The roots were excised from barley seedlings, variety Arivat, grown as described elsewhere (*1*).

Just before the absorption period began, each 1-g (fresh weight) sample of roots was rinsed for 1 minute with water of the same temperature as the experimental solution for that sample. The roots were then transferred to the aerated experimental solution. At the end of the absorption period the roots were rinsed for 1 minute with at least four 250-ml portions of distilled water; the first two portions were at the same temperature as the experimental solution, and the other two were at room temperature. Radioactive assay was as described elsewhere (*1*). The experimental technique is such that no replications are needed, and the values reported represent single samples.

The results of a typical experiment (Fig. 1) show the absorption of rubidium to be a strictly linear function of time, the line extrapolating to zero absorption at zero time. Absorption was depressed at the lower temperature and was essentially uniformly depressed with respect to temperature at all times. Amounts of rubidium absorbed at 4.5°C were 0.10 of those at 30°C, on the average, the fractions ranging from 0.08 for the 30-minute sample to 0.13 for the 3-minute run. If a 1-g (fresh weight) sample of roots is taken to be equivalent to 1 ml of water, the concentration of rubidium in the tissue reaches equality with that in the external solution (0.10 μmole/ml) within 1 minute after the beginning of the absorption period, and reaches 60 times that value in 60 minutes. Actually, 1 g of root tissue corresponds to less than 1 ml of water, and the accumulated ions are concentrated within only a fraction of this volume—probably largely in the vacuoles. The actual accumulation ratios are therefore higher than those given above.

Evidence has been presented (*2*) that various cations can displace each other on the cation-exchange spots of plant root tissue. This nonmetabolic exchange in roots of barley seedlings requires about 30 minutes for equilibration. Anions do not seem to get involved in such nonselective, reversible

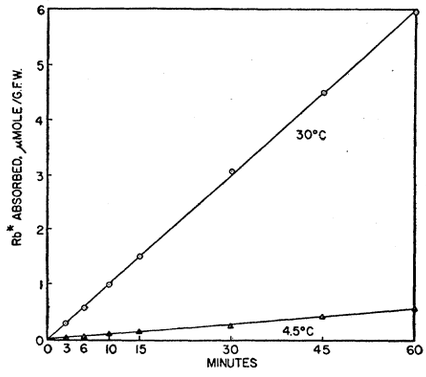

Fig. 1. Absorption of rubidium by barley roots as a function of time and temperature. Roots, 1.00 g solution: 0.10 mmole/lit. of $Rb^{86}Cl$, 0.50 mmole/lit. of $CaCl_2$, *p*H 5.6, aerated.

ion exchange, and their initial penetration into the tissue is by diffusion into the "outer" (*3*) or "water free" (*4*) space. On the basis of such findings, a generalized diagram has been presented by Briggs, Hope, and Robertson (*5*) according to which the initial uptake of the cation of a salt exceeds that of the anion and its initial equilibration is much slower than that of the anion. After this initial period, both the cation and the anion are absorbed by the active transport mechanisms, at steady rates.

Figure 1 shows that when calcium is present—the physiological condition (*1*)—there is no more evidence for an appreciable period of equilibration before the onset of steady-state, metabolically mediated absorption of rubidium than there is with anions. The first measurement was made 3 minutes after the start of the absorption period. At 30°C the rate of absorption for the first 3 minutes was 0.10 μmole per gram per minute—the same rate that was maintained for the full 60-minute absorption period. Absorption even during the first few minutes is fully temperature-sensitive; that is, it is metabolically mediated and does not represent "outer" space equilibration, which evidently occurs extremely rapidly (see *6*). It is concluded that when calcium at physiological concentrations is present, the general, nonselective cation-exchange capacity of the tissue is largely satisfied by calcium ions and the time course of the absorption of monovalent cations under these physiological conditions is precisely like that of anions. Overt, nonselective cation exchange plays no role, and the diagram given by Briggs *et al.* (*5*) is not a valid general representation of the time course of the absorption of monovalent cations.

We want to point out parenthetically that there is no evidence, under these physiological conditions (which include the presence of calcium in the solutions), of measurable instantaneous adsorption (zero-time intercepts) ostensibly reflecting the concentrations of the specific ion-carrying sites (*7*). In our experiments zero-time intercepts of the order specified would be readily apparent.

Kinetic studies (*8*) have resulted in the following model of ion transport across cellular membranes. The ion combines with a carrier which resides in and is part of a membrane not permeable with respect to free ions (ions not combined with a carrier):

$$R + M_{\text{outside}} \underset{k_2}{\overset{k_1}{\rightleftarrows}} RM$$

where R is the carrier and M is the ion. Once the carrier-ion complex RM has reached the far side of the membrane, the ion dissociates from the carrier into the "inner" (transmembrane) compartment or space, as a result of a chemical change in the carrier:

$$RM \underset{k_4}{\overset{k_3}{\rightleftarrows}} R' + M_{\text{inside}}$$

The model is analogous to the mechanism of enzyme action:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES; \quad ES \underset{k_4}{\overset{k_3}{\rightleftarrows}} E + P$$

Michaelis-Menten enzyme kinetics are based on the postulate that the rate of the reaction is proportional to the concentration of enzyme-substrate complex ES. When both k_2 and k_4 are appreciable, the net rate of the reaction depends not only on the concentration of the enzyme-substrate complex but on the concentration of product P as well, and it decreases as the concentration of product rises. In work with reversible enzyme reactions this effect is avoided by measuring the "initial rate" of the enzyme reaction, before the concentration of product reaches an appreciable value.

In carrier kinetics, the rate of transport is considered proportional to the concentration of carrier-ion complex RM, in analogy with enzyme-mediated catalysis, and "M_{inside}"—that is, absorbed ions—corresponds to "product" (*8*). However, in our experiment, the concentration of "product"—that is, ions absorbed—does not remain negligible for any length of time (Fig. 1). Within less than 1 minute the internal concentration of Rb rises to a value well above the concentration in the external solution. Despite the subsequent buildup of internal concentrations far in excess of the external concentration, the rate of absorption does not slacken; that is, the rate is independent of the rising internal concentrations of Rb ("product"). This means, in terms of the foregoing hypothesis, that the overall process is to a marked degree irreversible. It is recognized that the internal concentration will eventually reach a level at which the rate of absorption will slow down. The experiment shows, however, that the equilibrium is far to the right—that is, either k_1 *or* k_3 (or both) is greatly in excess of the corresponding reverse rate. Other, independent evidence (*9*) bears out this conclusion concerning the high degree of irreversibility of the absorption process (*10*).

EMANUEL EPSTEIN
D. W. RAINS
WALTER E. SCHMID
Department of Soils and Plant Nutrition, University of California, Davis

References and Notes

1. E. Epstein, *Plant Physiol.* **36**, 437 (1961).
2. ——— and J. E. Leggett, *Am. J. Botany* **41**, 785 (1954).
3. E. Epstein, *Plant Physiol.* **30**, 529 (1955).
4. G. E. Briggs, A. B. Hope, M. G. Pitman, *J. Exptl. Botany* **9**, 128 (1958).
5. G. E. Briggs, A. B. Hope, R. N. Robertson, *Electrolytes and Plant Cells* (Blackwell, Oxford, England, 1961), Fig. 32.
6. S. Pettersson, *Physiol. Plantarum* **14**, 124 (1961).
7. M. Fried, J. C. Noggle, C. E. Hagen, *Soil Sci. Soc. Am. Proc.* **22**, 495 (1958).
8. E. Epstein and C. E. Hagen, *Plant Physiol.* **27**, 457 (1952); E. Epstein, *Nature* **171**, 83 (1953).
9. G. G. Laties, *Ann. Rev. Plant Physiol.* **10**, 87 (1959).
10. This research was supported by a grant from the National Science Foundation. We thank L. L. Ingraham and G. W. Butler for helpful discussion of the experiments and conclusions.

19 January 1962

Formation of Double Hydroxides and the Titration of Clays

When increasing amounts of MgO are added to a known quantity of Al^{3+} saturated Wyoming bentonite in a dilute salt solution, the resulting titration curve shows two buffering regions, one below and the other above *p*H 7.0. The latter occurs at a *p*H considerably below that at which the precipitation of $Mg(OH)_2$ would be expected, and our report deals exclusively with it. Similar buffering