No evidence of postprecipitation could be found in 22 cultures subsampled four times over a 48-hour period, although the initial subsample was not taken until 3 hours after the iron was added. One would also suspect, although the mechanisms of postprecipitation are not fully understood (8), that the carbon so precipitated would be localized peripherally on the iron colloids and hence be vulnerable to acid rinses. One might therefore conclude that the carbon is occluded within the iron oxide colloid where it cannot be removed by conventional washing procedures.

These results indicate the importance of preventing inorganic precipitation in experiments in which a radioactive carbonate tracer is used. It should be emphasized that precipitation may result not only from additions of nutrients, but also from pH shifts accompanying photosynthesis in closed bottles, from temperature and redox potential changes which can develop during prolonged incubation, from changes in the solubility of iron under the influence of light (9), and from particulate matter serving as precipitation nuclei. The addition of the basic solution containing carbon-14 itself, in some circumstances, could alter the pH enough to cause inorganic precipitation in natural waters. Dark bottles alone may not serve as adequate controls. We therefore recommend the inclusion of both light and dark prefiltered controls for all culture and productivity studies in which carbon-14 is used where inorganic precipitation may occur (10). CHARLES R. GOLDMAN

# DAVID T. MASON

Department of Zoology, University of California, Davis

#### **References** and Notes

- 1. Methods. unless otherwise stated, are as in C. R. Goldman, Ecol. Monodescribed in C. R. Goldman, *Ecol. Mono-*graphs 30, 207 (1960); C. R. Goldman, *Ecol.*
- graphs 30, 207 (1960); C. R. Goldman, Ecology 42, 282 (1961).
  2. W. Stumm and G. F. Lee, Schweiz. Z. Hydrol. 22, 295 (1960).
  3. W. Rodhe, Symbolae Botan. Upsalienses 10, 149 (1948); G. C. Gerloff and F. Skoog, Ecology 38, 551 (1957).
  4. C. L. Schelske, Science 136, 45 (1962).
  5. D. W. Menzel and J. H. Ryther, Deep-Sea Research 7, 276 (1961).
  6. H. H. Willard and N. H. Eurman Elementary.

- Research 7, 276 (1961).
  6. H. H. Willard and N. H. Furman, Elementary Quantitative Analysis: Theory and Practice (Van Nostrand, New York, ed. 3, 1940).
  7. C. R. Goldman, "Symposia on marine pro-ductivity in the Pacific, 10th Pacific Science Congress, Honolulu, 1962," in press.
  8. G. L. Clark, L. K. Nash, R. B Fischer, Quantitative Chemical Analysis (Saunders, Philadelphia, 1949).
  9. S. I. Peltz and E. V. Lynn, J. Am. Pharm. Assoc. Sci. Ed. 27, 774 (1938).

- Assoc. Sci. Ed. 27, 774 (1938).
- 10. This work was supported by grants G-8901 and G-18020 from the National Science Foundation.
- 27 December 1961

### Histoplasma capsulatum Isolated from Feather Pillow Associated with Histoplasmosis in an Infant

Abstract. Feathers from an old baby pillow yielded Histoplasma capsulatum in an epidemiologic study of histoplasmosis in an infant. Efforts to exclude other possible sources of infection, including blood donor and placental transmission, were exhaustive. Although histoplasmosis has long been associated with soils in avian habitats, the feather itself has not been implicated heretofore.

The best documented epidemiologic studies of histoplasmosis, whether in outbreaks or in sporadic cases, reveal the source of infection in nature to be soils in and around the habitats of domesticated and undomesticated avian species (1-4). The presence of Histoplasma capsulatum in such foci has been generally attributed to the high nitrogenous content of the soils. which presumably results from prolonged fertilization with bird or fowl excreta. This report describes the isolation of H. capsulatum from chicken feathers in a baby pillow more than 30 years old, which in an extensive epidemiologic investigation of a single case of clinical disease in an infant,  $3\frac{1}{2}$  months old, was the only source in the environment from which the organism could be recovered. The feather pillow has not been recognized before as a possible source of this infection.

The diagnosis of histoplasmosis in this infant was confirmed by isolation of H. capsulatum from his spleen after its removal, because of continuing enlargement, when he was  $3\frac{1}{2}$  months old. Because the baby was erythroblastotic at birth, it had to be presumed that the baby could have acquired the disease by direct transmission either from one of three blood donors or by the placental route, as well as by exposure to soils contaminated with the agent. The most usual means, exposure to contaminated soil, seemed the most unlikely, for the earth was blanketed with snow from the time the infant was discharged from the hospital at the age of 2 weeks until he was readmitted 3 months later. Nevertheless, when all members of the baby's family, including his mother, the three blood donors, and the family's pet dog proved by skin test and serology to be negative for histoplasmosis (5), soil samples and other types of specimens including several from potted house plants, were collected from the child's home environment. Histoplasma capsulatum was not isolated from any of these samples after injection into mice as described by Emmons (1). When it appeared that the baby had not acquired his infection in any of these three most probable ways, the baby's feather pillow was also investigated.

The small pillow, approximately 8 by 12 by 2 inches and covered with heavy muslin, was immersed and tumbled for a few minutes in sterile distilled water. Four specimens were prepared for intraperitoneal injection of 0.5 ml quantities into white albino mice weighing 14 to 16 g. Three specimens were sediments from surface rinse water collected after 30 minutes, and after 48 and 96 hours at room temperature. Each sample of 100 ml of rinse water was centrifuged to 5000 rev/min for 20 minutes and the sediment was resuspended in 10 ml of 0.85 percent sodium chloride solution containing 100 units of penicillin and 200 units of streptomycin per milliliter. The fourth specimen comprised washings from feathers removed from the case after 96 hours, by which time the case and feathers had begun to decompose. The feathers were



Fig. 1. Smooth-walled, round-to-pyriform conidia found in isolate from feathers after repeated mouse passage and one transfer on potato dextrose agar (about imes530).



Fig. 2. "Macroconidium" characteristic of those observed in isolates of feathers after repeated mouse passage and several transfers on potato dextrose agar (about  $\times$ 680).

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 featherantibiotic 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, smoothwalled, 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 22 JUNE 1962

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
- C. W. Emmons, Public Health Repts. 64, 892 (1949).
   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).
   C. C. Campbell, J. Lab. Clin Med. 50, 841
- 3. C. C. Campbell, J. Lab. Clin. Med. 50, 841 (1957)
- (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)
- C. C. Campbell, Ann. N.Y Acad. Sci. 89, 163 (1960).
   ..., J. Bacteriol. 54, 263 (1947).
   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<sup>86</sup>, and 0.50 mmole of CaCl<sub>2</sub>. The pH, initially 5.6, did not vary by more than 0.2 pH 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  $4.5\,^{\rm o}{\rm C}$  were 0.10 of those at 30  $^{\rm o}{\rm C},$  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  $\mu$ mole/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