ently attributed to surface effects rather than to the total K and Na or ^{42}K and ^{24}Na contents of the sample.

A good elution flow rate through the column is about 1 ml per minute. If we include the time for both digestion and elution, the fastest separation we have achieved thus far is approximately 1 hour, and it is possible to reduce this time further.

We are applying this procedure in our studies of trace elements in normal and diseased human tissues. We have also demonstrated its usefulness in the study of contaminants in reactor cooling water and in investigations of cyclotron-produced radionuclides, in both of which cases copious quantities of interfering ^{24}Na are produced when aluminum components or target wrapping foils are subjected to fast neutron bombardment.

CHUNG-WAI TANG
CONSTANTINE J. MALETSKOS
Cancer Research Institute,
New England Deaconess Hospital,
and *Department of Pathology,*
Harvard Medical School,
Boston, Massachusetts 02215

References and Notes

1. G. E. Gordon, K. Randall, G. G. Goles, J. B. Corliss, M. H. Beeson, S. S. Oxley, *Geochim. Cosmochim. Acta* **32**, 369 (1968).
2. J. R. DeVoe, Ed., *Modern Trends in Activation Analysis, Proceedings of the 1968 International Conference, Gaithersburg, Maryland, 7–11 October 1968* [*Nat. Bur. Stand. U.S. Spec. Publ. 312* (1969), vols. 1 and 2.]
3. See List of Investigators and Investigations, Lunar Sample Program, *Ind. Res.* **11**, 26 (July 1969).
4. N. Spronk, *Nucl. Appl.* **2**, 259 (1966).
5. C. V. Banks and J. J. Richard, *Talanta* 2, 235 (1959).
6. H. Sanke Gowda and W. I. Stephen, *Anal. Clin. Acta* **25**, 153 (1961).
7. M. P. Menon and R. E. Wainerdi, in *Proceedings of the 1965 Conference Modern Trends in Activaiton Analysis* (Texas A & M Univ., College Station, 1965), p. 152.
8. F. Girardi and E. Sabbioni, *J. Radioanal. Chem.* **1**, 169 (1968).
9. Available from Carlo Erba, Milan, Italy.
10. Preliminary experiments show that NaBr and Na_2SO_4 are effective in removing radiosodium. For obvious reasons we are presently concentrating on the NaCl system.
11. Our early experiments were conducted with columns made from glass tubes (5 mm in inner diameter, 30 to 60 cm long) drawn to a nozzle on one end and plugged with a wad of glass wool. Later we used wider-diameter glass tubes fitted with glass frits (1 cm in inner diameter); at present we use polypropylene chromatographic tubes (1.5 cm in inner diameter, 15 cm long), each sealed at one end with a perforated polypropylene disk and fitted with a removable nozzle. To prevent the fine crystals from passing through the perforations, a filter paper disk (1.4 cm in diameter) is placed at the bottom of the polypropylene tube. These commercially available plastic columns are easier to pack, give more uniform and faster flow rates, and are convenient to handle. More NaCl is required, but, as compared to passage through three or more NaCl columns 10 cm long in glass tubes 5 mm in inner diameter, one pass through a column of 230- to 325-mesh NaCl 5 to 7 cm long in the plastic tube is usually sufficient to remove ^{24}Na from 100 mg of irradiated lyophilized muscle tissue.
12. The physics and chemistry of the water-acetone-HCl system and the mechanism of exchange are not clearly understood at present. The small amount of water presumably plays an important role in recrystallization. Columns packed with NaCl equilibrated with a mixture of acetone and HCl for over 2 weeks appear to give lower decontamination factors than columns packed with fresh materials.
13. The low boiling point (56°C) of acetone allows one to rapidly concentrate the eluant and the washings by evaporation at relatively low temperature.
14. For reasons not understood, the addition of a little hexone in the eluate appears to improve the decontamination; however, it is not essential, since an acetone-H_2O mixture alone can remove ^{24}Na or ^{42}K, respectively, with NaCl or KCl. The H_2O-acetone-HCl system described here may not be optimum, but it is the most effective system found thus far.
15. Supported by AEC contract AT(30-1)-3778 with the New England Deaconess Hospital. We thank Professors C. D. Coryell and G. E. Gordon, Department of Chemistry, Massachusetts Institute of Technology and University of Maryland, respectively, for discussions and review of the manuscript.

9 July 1969; revised 10 November 1969

Maxillary Mycangium in the Mountain Pine Beetle

Abstract. *A mycangium containing blue-stain fungi and yeasts is located in the cardo of the maxilla of the mountain pine bettle,* Dendroctonus ponderosae *Hopk. Symbiosis between one or more of the microorganisms and the insect is indicated.*

We report here a mycangium in the maxilla of a scolytid beetle; we have cultured blue-stain fungi from this specialized structure. Mycangia have been reported previously. Nunberg speculated that special organs (now called mycangia) in the ambrosia beetle *Trypodendron lineatum* Oliv. = *Xyloterus* Oliv., were involved in fungus transmission (*1*). Mycangia were subsequently found in this and several other ambrosia beetles and in some bark beetles. The occurrence of mycangia, their distribution, and function have recently been reviewed (*1*). Blue-stain fungi colonize phloem and sapwood of trees successfully attacked by bark beetles. Farris (*2*) reported evidence of a mycangium in the pregular region of the mountain pine beetle. We now describe the location, structure, and contents of this mycangium.

We obtained newly emerged beetles from infested lodgepole pine *Pinus contorta* Dougl. var. *latifolia* Engelm., in the East Kootenay region of British Columbia. Insects not used immediately were stored individually up to 1 week at 4°C with pieces of lodgepole pine phloem. Sagittal and transverse sections and whole mounts of maxillae were stained (*3*) for examination for fungal material. To culture microorganisms from mycangia, we separated aseptically the cardines from the maxillae that were extracted from recently removed heads. The cardines were placed in either sterile water on microslides or in a petri plate of 2 percent water agar or potato dextrose agar with 0.02 percent yeast extract. Cultures were kept in a moist chamber on the laboratory bench and examined at ×450 at 12 hour intervals. Pure cultures originating from mycangial openings were obtained from hyphal tips or spores. For scanning electron microscopy (*4*), unfixed whole maxillae were mounted with egg albumen on a cover glass affixed to a specimen stub with high conductivity paint, coated with approximately 100 Å of carbon and 300 Å of gold paladium at an angle of 45° from the point of evaporation, and examined at a beam voltage of 3 kv.

Cultures from aseptic dissections of the pregular region consistently showed fungal growth arising toward the proximal end of the cardines. The mycangium was located during detailed examination by light and scanning electron microscopy (Fig. 1). It was present in both cardines of both sexes of this beetle and opened on the inner surface slightly above the fossa between the muscle processes (*5*). The position of the mycangium on the contorted cardo was readily seen under the scanning electron microscope (Fig. 1). This greatly facilitated examination and comprehension of whole mounts and sections of cardines viewed by light microscopy (×100). A column of hyphae and spores was seen frequently extending from the mycangial opening. One column was approximately as long as the cardo (about 175 μm). Stained sections (Fig. 2) and whole mounts of maxillae revealed a reticulate tube containing fungus material in one of the two muscle processes of the cardo. The structure of this mycangium, and the peglike protrusions on its inner surface as seen in Stereoscan preparations, resemble those of several species of *Trypodendron* (*6*).

The blue-stain fungi and yeasts that occur in lodgepole pine attacked by the mountain pine beetle (*7*) were cultured from mycangia. Isolations were made from one or the other mycangium of 32 unsexed insects. The fungi obtained and the number of mycangia that produced them were: *Ceratocystis montia* Rhumb., 5; *Europhium clavigerum* Robinson and Davidson, 16; yeasts, 18

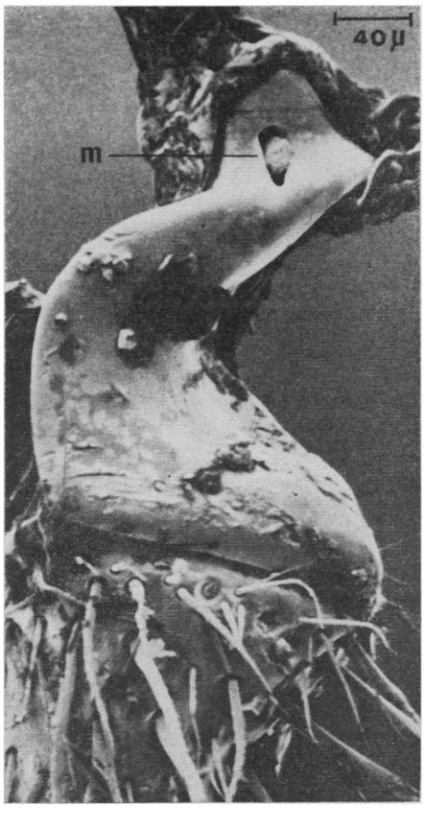

Fig. 1. Scanning electron micrograph of a cardo of *Dendroctonus ponderosae*. Mycangium (*m*) near proximal end with mycelium and spores of a blue-stain fungus or yeast, or both, in the opening.

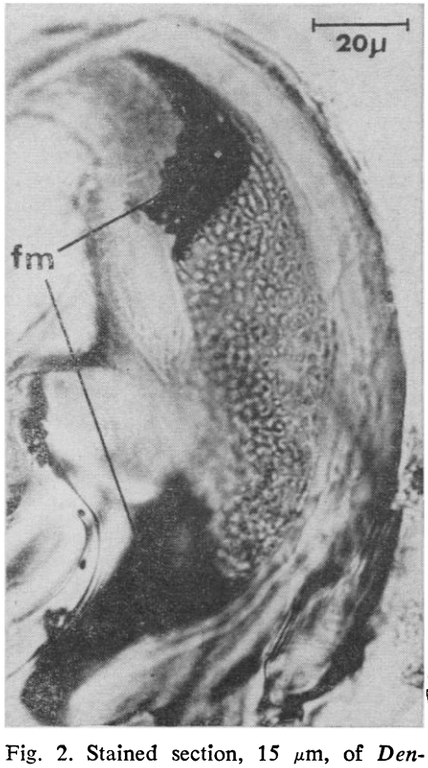

Fig. 2. Stained section, 15 μm, of *Dendroctonus ponderosae* mycangium showing reticulate structure. Fungus material (*fm*) is darkly stained.

[mostly *Pichia pini* (Holst) Phaff, *Hansenula capsulata* Wickerham, or *H. holstii* Wickerham]; *Trichoderma* spp., 4; *Penicillium* spp., 3; and *Cladosporium* spp., 2. Three mycangia yielded yellow bacterial colonies only, and two did not yield any microorganisms. Some mycangia yielding blue-stain fungi also yielded one, and sometimes two, yeasts. However, the two blue-stain fungi, *C. montia* and *E. clavigerum*, were never found together in the same mycangium, and the nonblue-stain fungi were usually solitary.

Mycelia and spores were observed but not cultured in 59 (48 females, 11 males) of 72 additional insects examined. However, when two of the 13 showing no visual evidence were cultured, both yielded *Ceratocystis montia* and a yeast. Of 29 *Dendroctonus ponderosae* adults sectioned and stained, fungi were present in the mycangia of 17 of 22 females and 5 of 7 males. The maxillae of 9 adult females examined in stained whole mounts all showed fungus material in the mycangia.

The variable length of fungal columns extending from the mycangia, the absence of microorganisms from some mycangia, and the occasional presence of fungi other than blue-stain or yeasts in other mycangia suggested that the microorganisms in the mycangium undergo change.

Symbiosis between certain fungi and wood and bark-inhabiting beetles of the family Scolytidae has been recognized for over 100 years (*8*). Discovery of a mycangium containing blue-stain fungi and yeasts in *D. ponderosae* indicates symbiosis between one or more of these microorganisms and this insect.

H. S. WHITNEY
Forest Research Laboratory, Department of Fisheries and Forestry, Calgary 2, Alberta, Canada

S. H. FARRIS
Forest Research Laboratory, Department of Fisheries and Forestry, Victoria, British Columbia, Canada

References and Notes

1. M. Nunberg, *Ann. Mus. Zool. Polon.* **14**, 261 (1951); H. Francke-Grosmann, in *Symbiosis*, S. M. Henry Ed. (Academic Press, New York, 1967), vol. → L. R. Batra, *Trans. Kans. Acad. Sci.* **66**, 213 (1963).
2. S. H. Farris, *Can. Dep. For. Bimon. Prog. Rep. 21* (1965), p. 3.
3. S. H. Farris and A. Funk, *Can. Entomol.* **97**, 527 (1965).
4. "Stereoscan"—Cambridge Instrument Company, England.
5. A. D. Hopkins, *U.S. Dep. Agr. Bur. Entomol. Tech. Ser.* **17**, part 1 (1909).
6. H. Francke-Grosmann, *Verhandl. Deut. Ges. Angew. Entomol.* **14**, 139 (1957); L. P. Abrahamson, H. Chu, D. M. Norris, *Ann. Entomol. Soc. Amer.* **60**, 1107 (1967).
7. R. C. Robinson, *Can. J. Bot.* **40**, 609 (1962).
8. K. Graham, *Annu. Rev. Entomol.* **12**, 105 (1967).
9. We thank R. D. Mills (Civil Engineering Department, University of Calgary, Atla.) for the use of the scanning electron microscope, and J. Bloor of the same department for coating and photographing our specimens. We are grateful to the Department of Food Science and Technology, University of California, Davis, for identification of yeast cultures. J. Chapman of the Victoria Research Laboratory gave valuable advice during the study.

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Potassium Ions Asymmetrically Activate Erythrocyte Membrane Phosphatase

Abstract. *In reconstituted ghosts of human red cells, the cell membrane phosphatase is activated by external, but not by internal, potassium. This finding is consistent with the possible participation of the membrane phosphatase in the enzymatic system responsible for cation transport.*

The hydrolysis of adenosine triphosphate (ATP) by the cell membrane ($Na^+ + K^+$)-adenosine triphosphatase system seems to follow a sequence of at least two steps: (i) A ($Na^+ + Mg^{2+}$)-dependent transference of the terminal phosphate of ATP to the protein of the enzyme, and (ii) a K^+-activated release of inorganic phosphate from the phosphorylated protein. This sequence of reactions can be written as follows.

$$\text{ATP} + \text{enzyme} \xrightleftharpoons[Mg^{2+}]{Na^+} \text{enzyme-P} + \text{ADP}$$

$$\text{enzyme-P} + H_2O \xrightarrow{K^+} \text{enzyme} + P_i$$

It has been suggested that the membrane-bound *p*-nitrophenyl phosphatase activity, activated by K^+ and sensitive to ouabain, is the expression of the ability of the ($Na^+ + K^+$)-adenosine triphosphatase system to hydrolyze other phosphate esters apart from the phosphorylated intermediate (enzyme-P) (*1*). If the suggested identity of the K^+-activated phosphatase activity with the last step in the adenosine triphosphatase reaction is true, both enzymes would share the same K^+ sites. One of the main predictions that this assumption leads to is that the activation of the phosphatase by K^+ would take place, as in the ($Na^+ + K^+$)-adenosine triphosphatase system, only when K^+ is at the external surface of the cell membrane (*2*). We report experiments which test this prediction on "reconstituted ghosts" of human red cells.

The red cell membrane is highly permeable to the phosphatase substrate