The apparent K_m calculated from the line is $0.8 \times 10^{-3} M$.

Initial studies have been made on the absorption of Se^{75}-selenomethionine in man after oral ingestion (Fig. 2). Fifteen microcuries have been employed, but larger quantities (approximately 50 μc) will probably have to be used in clinical studies in order to have a sufficiently high counting rate from blood samples. Radioactivity in the blood reached a peak at about 3 hours and was essentially unchanged 5 hours after ingestion. Whether or not Se^{75}-selenomethionine is absorbed by man in parallel with S^{35}-methionine, it may offer an approach to the quantitation of amino-acid absorption in vivo (*4*).

RICHARD P. SPENCER
Department of Biophysics, University of Buffalo School of Medicine and *Radioisotope Service, Veterans Administration Hospital, Buffalo, New York*

MONTE BLAU
Department of Nuclear Medicine, Roswell Park Memorial Institute, Buffalo

References and Notes

1. D. Nathans, D. F. Tapley, J. E. Ross, *Biochim. et Biophys. Acta* **41**, 271 (1960).
2. M. Blau, *ibid.* **49**, 389 (1961); ——— and R. F. Manske, *J. Nuclear Med.* **2**, 102 (1961).
3. T. H. Wilson and G. Wiseman, *J. Physiol. (London)* **123**, 116 (1954).
4. This research was supported by grants A-4887 and CS-9600 from the Public Health Service.

29 September 1961

Inhibition of Hypoxylon pruinatum by Pyrocatechol Isolated from Bark of Aspen

Abstract. Pyrocatechol has been identified as one of two main substances isolated from bark of aspen (*Populus tremuloides* Michx.) which inhibited *Hypoxylon pruinatum* (Klot.) Cke. Total inhibition of this fungus was obtained at a dosage of 640 parts per million. Tests with bark extract from two localities showed that inhibition was maximal in samples obtained in the dormant season and from the base of the trees.

The fungistatic action of bark extract of different poplar species was first reported by Grosjean in 1943 and 1950 (*1*). Klöpping and van der Kerk in 1951 (*2*) isolated from *Populus candicans* Ait. several fungistatic substances including pyrocatechol, salicin, saligenin, salicylic acid, and two unknown substances, which were tested against *Botrytis cinerea* Pers., *Penicillium italicum* Wehm., *Aspergillus niger* van Tiegh., and *Rhizopus nigricans* Ehr. The two unknown fractions were more active than the other substances tested. The first fraction was thought to be benzyl gentiate, but the second was not identified. French and Oshima (*3*) in 1959 showed that the outer bark of *Populus tremuloides* favored germination of spores of *Hypoxylon pruinatum*, while the green layer inhibited their germination for 24 hours. In 1959 and 1961, Jung (*4*) observed the fungistatic action of the cambium of different hardwood species but did not determine or characterize the active substances. A similar observation was made by Sussex *et al.* (*5*) in 1961. Bark extracts from different poplar species have been reported also by Butin and Loeschcke in 1960 (*6*) as fungistatic to *Dothichiza populea* Sacc. et Briard. The investigation reported here was undertaken to test bark extracts of poplars against *Hypoxylon pruinatum* and to attempt purification and identification of the inhibitory substances.

The inhibiting potency of bark extracts of different poplars against *Hypoxylon pruinatum* was investigated as follows. Bark meal was prepared by grinding freshly collected bark in a Waring blender with Dry Ice (*7*) and then stored at −20°C. Twenty milliliters of a 5-percent bark-meal agar were poured into petri dishes, and a 4-mm agar disk from an active plate culture of the fungus was placed in the center of each dish. The cultures were incubated at room temperature for 3 weeks, and the diameter of the colonies was measured every 3 days.

The fungistatic action varied in intensity with several different species of poplars tested, aspen (*Populus tremuloides*) producing the greatest inhibition. Inhibition was consistently greater in aspen bark collected during fall and winter than in bark obtained during summer from two different localities near Quebec City. There was a consistent difference in inhibitory powers between bark from the two localities. In addition, the activity was greater in bark at the base than in bark elsewhere on the trees.

The extraction of active substances was carried out as follows. Bark meal was treated with ethyl acetate at 4°C for 20 hours, and the filtrate was separated into basic, neutral, and acid fractions with sodium carbonate and acetic acid. Each fraction was evaporated under vacuum at 60°C, the residue was dissolved in 10 ml of 96-percent ethanol, and 2.5 ml of the resulting solution was mixed with 100 ml of malt agar before autoclaving and testing for activity. Because it was found that the active substances were contained in the acid phase, this fraction was further separated with a Dowex 50 ion-exchange column. Solutions of acetic acid ranging in concentration from 0.1 to 100 percent were used to elute the substances. Fifty parts of 20 ml each were collected in this manner. Tests in malt agar showed that fractions Nos. 11 to 14 and 33 completely inhibited the growth of the fungus, while fractions Nos. 10, 15 to 17, 30, 31, 34, and 41 were only partially active. The active substance sublimed from fractions Nos. 11 to 14 during evaporation under vacuum.

This compound is characterized as follows: melting point 104° to 106°C; green reaction with $FeCl_3$; R_F, 0.88 on Whatman paper No. 1, with butanol, acetic acid, and water (4:1:5); and grey spot with orange contour with diazonium sulfanilic acid. From these data, in conjunction with infrared absorption studies, it is concluded that the substance is identical to pyrocatechol. The chemical nature of fraction No. 33 is not known.

Additional tests of the fungistatic action of pyrocatechol against *Hypoxylon pruinatum* were carried out with a commercial preparation obtained from British Drug House, Toronto, Ontario. The lowest dosage to produce a visible growth reduction was 80 ppm, and total inhibition was obtained at 640 ppm (*8*).

MARTIN HUBBES
Forest Entomology and Pathology Laboratory, Sillery, Quebec, Canada

References and Notes

1. J. Grosjean, *Tijdschr. Plantenziekten* **49**, 172 (1943); *Nature* **165**, 853 (1950).
2. H. L. Klöpping and G. J. M. van der Kerk, *Nature* **167**, 996 (1951).
3. D. W. French and N. Oshima, *Forest Sci.* **5**, 255 (1959).
4. J. Jung, *Naturwissenshaften* **46**, 657 (1959); **48**, 134 (1961).
5. I. M. Sussex, J. B. Lutinski, L. J. Dilks, *Botan. Gaz.* **121**, 171 (1961).
6. H. Butin and V. Loeschcke, *Naturwissenshaften* **47**, 451 (1960).
7. P. A. Manigault and C. Stoll, *Experientia* **14**, 409 (1958); H. Kern, *Phytopath. Z.* **40**, 301 (1961).
8. Further laboratory studies on this subject are in progress. The author is grateful to René Pomerleau for advice and translation of the manuscript and to D. E. Etheridge, R. Martineau, and G. B. Ouellette for reviewing the paper. This report is contribution No. 811, Forest Entomology and Pathology Branch, Department of Forestry, Ottawa, Canada.

6 November 1961