is m, and whose intercept is log k. This graphical method gives results identical with those presented in Table 1. However, once the M' values have been calculated, it is a simple matter to get the M'' values, then m and k.

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References

1. A. A. Frost and R. G. Pearson, *Kinetics and Mechanism* (Wiley, New York, 1953), p. 40.
2. See also H. Pessen, *Science* **134**, 676 (1961).
3. H. Margenau and G. M. Murphy, *The Mathematics of Physics and Chemistry* (Van Nostrand, New York, 1943).
4. A. G. Worthing and J. Geffner, *Treatment of Experimental Data* (Wiley, New York, 1943).
5. N. V. Sidgwick, *The Chemical Elements and Their Compounds* (Oxford Univ. Press, Oxford, England, 1950), vol. 1, p. 615.
6. A. A. Blumberg and S. C. Stavrinou, *J. Phys. Chem.* **64**, 1438 (1960).

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Intestinal Transport of Selenium-75 Selenomethionine

Abstract. When measured simultaneously, selenium-75 selenomethionine accumulated identically with sulfur-35 methionine across everted hamster intestinal sacs in the presence of carrier methionine. The apparent K_m for L-methionine transport was 0.8×10^{-3} M, as calculated from the beta emissions of S^{35}-methionine or the gamma emissions of Se^{75}-selenomethionine. Absorption of Se^{75}-selenomethionine from the gastrointestinal tract of man also occurred, with peak blood levels being reached in about 3 hours. Because of the 122-day half-life of selenium-75 and its multiple gamma emissions, Se^{75}-selenomethionine may serve as a tool in evaluating amino-acid absorption.

Availability of an amino acid labeled with a gamma-emitting radioisotope would be an aid to studies of intestinal absorption, since the emission could be detected without extensive preparation and fragmentation of the sample. Although I^{131}-tyrosine (*1*) and its derivatives have been employed during in vitro studies of absorption, the short half-life of iodine-131 (and consequently its short shelf-life) and the cleavage of iodine from the molecule in biologic systems have limited its applicability for in vivo studies. The report of biosynthesis of Se^{75}-selenomethionine (CH_3-Se^{75}-CH_2•CH_2•$CHNH_2$•$COOH$) with the description of some of its biological properties has provided another amino acid labeled with a gamma-emitting isotope (*2*).

In the experiment reported here, in vitro transport of Se^{75}-selenomethionine against a concentration gradient was compared with that of S^{35}-methionine; everted hamster intestinal sacs, as described by Wilson and Wiseman (*3*), were utilized. Hamsters were sacrificed, and the small gut of each was made into three everted sacs containing 1 ml inside (serosal fluid) and 5 ml outside (mucosal fluid). Initially, serosal and mucosal fluids contained solutions of identical composition. A stock solution contained L-methionine ($8 \times 10^{-3}M$), S^{35}-methionine (0.05 μc/ml), and Se^{75}-selenomethionine (0.02 μc/ml) in Krebs-bicarbonate buffer at *p*H 7.4 (without calcium, magnesium, or glucose). From the stock solution successive dilutions were made with Krebs-bicarbonate buffer to provide L-methionine concentrations of 4, 2, and $1 \times 10^{-3}M$. Two animals (six intestinal sacs) were studied at each concentration. An incubation period of 1 hour in an oscillating water bath at 37°C was employed after preliminary gassing with 95 percent oxygen and 5 percent carbon dioxide.

Sacs were removed from the bathing fluid, drained, and weighed. Mucosal and serosal solutions were centrifuged to remove sloughed tissue and were then assayed. Mucosal and serosal solutions (0.5 ml each) were counted in a Tracerlab gamma ray spectrometer and corrected for background. The S^{35} beta emissions did not interfere with assaying the Se^{75} gamma emissions. However, some of the weaker gamma rays of Se^{75} were recorded by a Packard Tri-Carb scintillation counter as though they were beta emissions [0.05 ml of solution to be analyzed, 10 ml of toluene containing 0.5 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene, 40 mg of 2,5-diphenyloxazole, and 3 ml absolute ethanol]. Accordingly, the S^{35}-methionine beta counts were in each case corrected for the Se^{75}-selenomethionine present on the basis of the counts recorded from a known amount of Se^{75}-selenomethionine and the gamma counts of each sample. The correction was small (about 3 percent) but was made in each case.

Shown in Table 1 are the ratios of serosal concentration to mucosal concentration calculated in each case by the beta counts of S^{35}-methionine and the gamma counts of Se^{75}-selenomethionine. The values are practically identical, indicating that S^{35}-methionine and Se^{75}-selenomethionine are handled identically by the everted hamster intestinal sac under these conditions. Initial chromatographic studies of the mucosal and serosal solutions have revealed but one gamma-emitting peak, suggesting that Se^{75}-selenomethionine is not appreciably degraded during its transmural transport. A Lineweaver-Burk type plot of the transport data is shown in Fig. 1.

Table 1. In vitro intestinal concentration of L-methionine measured by S^{35}-methionine and Se^{75}-selenomethionine. Each value for the ratio of serosal concentration to mucosal concentration is the mean of six determinations. The standard deviations were about 0.5. The original concentrations of L-methionine are given in italic type.

Serosal concn./mucosal concn. (at 1 hour)		Concn. ratio by β / Concn. ratio by γ
By β activity	By γ activity	
8×10^{-3}M L-methionine		
2.11	2.20	0.96
4×10^{-3}M L-methionine		
3.86	3.70	1.04
2×10^{-3}M L-methionine		
4.51	4.33	1.04
1×10^{-3}M L-methionine		
7.60	8.20	0.93
	Mean	0.99

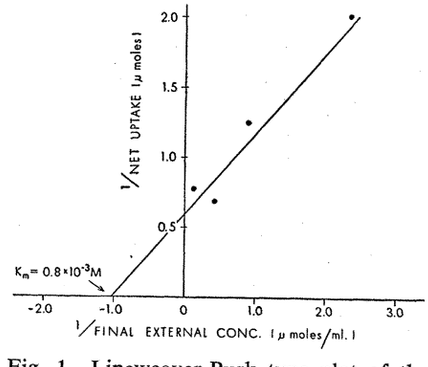

Fig. 1. Lineweaver-Burk type plot of the net serosal uptake of L-methionine as a function of the final mucosal concentration.

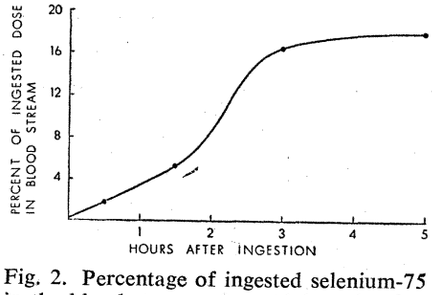

Fig. 2. Percentage of ingested selenium-75 in the blood stream of an adult male after ingestion of 15 μc of Se^{75}-selenomethionine in 0.53 g of L-methionine (0.1 g of L-methionine per 15 kg body weight).

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*).

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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*).

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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.

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