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6. Indole was measured by the method of Smith and Yanofsky [*Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5]; the tryptophan content of the toluene-extracted broth was measured by the method of Dickman and Crockett [*J. Biol. Chem.* **220**, 957 (1956)].
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10. *Escherichia coli* mutant T3 was furnished by Dr. C. Yanofsky, and mutant 5 MTR-6 by Dr. H. S. Moyed. We appreciate stimulating talks with Dr. B. Magasanik. Work supported in part by the Nutrition Foundation.

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Cysteine Stimulation of Glucose Oxidation in Thyroid Tissue

Abstract. *Oxidation of* D-*glucose-C*^{14} *by bovine thyroid slices was stimulated by cysteine, 2-aminoethanethiol, and cystamine. Amino acids tested, other than cysteine, were not stimulatory, except tyrosine and arginine to a slight degree. The pattern of stimulation resembled that brought about by thyroid-stimulating hormone. Stimulation of labeled* CO_2 *production was greater when glucose-1-C*^{14} *rather than glucose-6-C*^{14} *was the substrate.*

Thyroid-stimulating hormone (TSH) can be inactivated by oxidation. It can then be partly reactivated by exposure to reducing substances (*1*). Some of the reducing substances used in these reactivation experiments produce goiters, but others do not, and cysteine has been reported to belong to the second category (*2*). Since such reducing substances which do not produce goiters have never been shown to exert a direct action on the thyroid in the absence of TSH, it is of interest that cysteine and several related substances exert an action resembling that of TSH on thyroid slices in vitro.

Fresh beef thyroids obtained from local slaughterhouses were brought immediately to the laboratory in ice-chilled beakers. They were stripped of adventitial tissue and sliced on a Stadie-Riggs hand slicer into pieces about 0.5- to 1.0-mm thick weighing approximately 75 mg. These slices were then placed in 25-ml erlenmeyer flasks fitted with removable glass center wells and containing 2 ml of Krebs-bicarbonate medium, 2.0 mg of D-glucose, and 0.50 μc of D-glucose-1- or -6-C^{14} (*3*). The flasks were flushed for 20 seconds with a mixture of 95 percent O_2 and 5 percent CO_2, and incubated for 45 minutes at 37°C while being shaken. At the end of the incubation, 1 ml of "Hyamine" base was added to the removable center well and 0.2 ml of 10*N* H_2SO_4 to the medium in each flask. The flasks were then shaken for another hour at room temperature. The "Hyamine" was transferred to crystallite vials and counted in a Tri-Carb liquid-scintillation counter. The results are expressed as counts per minute per gram of wet weight of thyroid tissue. The TSH used in these experiments ("Thytropar," Armour) had a specific activity of approximately 1 U.S.P. unit per mg.

Table 1. Radioactivity recovered as $C^{14}O_2$ from D-glucose-1-C^{14}. Results are given in 10^2 counts per minute per gram of tissue, wet weight. Each set of figures represents the mean ± the standard deviation for three to five flasks.

Control	TSH*	L-Cysteine, $10^{-2}M$	Cystamine-2HCl, $10^{-2}M$	2-Amino-ethanethiol-HCl, $10^{-2}M$
		Experiment 1		
350 ± 55	673 ± 225‡	621 ± 138§	803 ± 85‡	1,185 ± 189‡
		Experiment 2		
965 ± 84	1,161 ± 135†	1,248 ± 68†	1,400 ± 79‡	2,285 ± 67§
		Experiment 3		
317 ± 15	497 ± 76†	404 ± 89†	573 ± 99‡	1,012 ± 313‡

* TSH concentration: 0.25 U.S.P. units per vessel. † $p < 0.05$. ‡ $p < 0.01$. § $p < 0.001$.

The results in Table 1 indicate that $10^{-2}M$ cysteine stimulates glucose oxidation (25 experiments) in thyroid tissue as does $10^{-2}M$ 2-aminoethanethiol (7 experiments) and $10^{-2}M$ cystamine (5 experiments). Other amino acids tested as controls were arginine, tyrosine, aspartic acid, proline, methionine, lysine, leucine, tryptophane, serine, and alanine. Tyrosine and arginine sometimes gave slight but statistically significant stimulation of glucose oxidation; the others appeared to be inert or inhibitory. All amino acids were tested at a concentration of $10^{-2}M$. It has been reported that tyrosine was inert in such a system (*4*), but it was tested then only at a concentration of $10^{-4}M$. Although occasionally the combined stimulation in the presence of both TSH and cysteine was greater than that from either alone, we were unable to demonstrate an additive or synergistic effect of the two substances. The pattern of stimulation from cysteine resembled that resulting from TSH, since oxidation of glucose-1-C^{14} to $C^{14}O_2$ was accelerated more than oxidation of glucose-6-C^{14} (four experiments). When the incubation time was shorter, stimulation by cysteine was evident within 5 minutes, another resemblance to TSH (*5*).

The action of TSH on thyroid slices has been considered fairly specific, since other known hormones of adenohypophysis do not show corresponding activity (*5*). Similar stimulation is seen with epinephrine, norepinephrine, serotonin, and acetylcholine (*6*); however, a number of other biologically active substances (for example, glucagon, insulin, methimazole, triiodothyronine, histamine, thiolhistidine, and others) have been tested and do not stimulate glucose oxidation under these conditions (*7*). It has been postulated that all of the stimulatory substances act by increasing the availability of oxidized triphosphopyridine nucleotide though perhaps by several different mechanisms (*8*). While this may be the case with the thiols mentioned in this paper, the exact mechanism by which such stimulation would take place is not immediately apparent. A purely preservative action of these thiols cannot be ruled out by our data, but the several similarities to TSH stimulation suggest that the mechanisms may be alike in the two cases.

These results emphasize the importance of proper controls when reducing agents are used as stabilizing agents during the preparation or storage of TSH (*9*).

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Glauconite from the Precambrian Belt Series, Montana

Abstract. *Glauconite from the upper part of the Missoula Group of the Belt Series, Flathead County, Montana, has been dated at 1070 million years by potassium-argon and rubidium-strontium analyses. This is the first glauconite of Precambrian age reported in North America.*

The assignment of the Belt Series to the Precambrian has been generally accepted. The age of the uppermost beds of the Missoula Group of the Belt Series in Montana, however, has been questioned by Nelson and Dobell (*1*), who suggest that these beds may be Cambrian.

Glauconite from the upper part of the Missoula Group and within a few thousand feet of the Middle Cambrian Flathead Quartzite, the oldest known Paleozoic formation in the section, gives similar K-Ar and Rb-Sr ages that average 1070 million years (Table 1). This is considered a minimum age for the Precambrian Missoula Group.

The glauconite occurs as dark-green pellets in a pink and dark-green spotted, medium-grained, feldspathic quartzite that was sampled (Fig. 1) at an elevation of 6000 feet on the east flank of the ridge between Ringer and Cruiser mountains in the southeast corner of the Marias Pass quadrangle, Flathead County, Montana.

The glauconite pellets are typical aggregates of crystallites that show low order interference colors. Structurally the mineral is a partially disordered 1M-mica polymorph, as is shown by the broadness of the x-ray peaks and by the poor resolution of some principal peaks of ordered 1M-mica. Interlayering of the mica-montmorillonite type is suggested in the x-ray pattern of a sample treated with ethylene glycol. Expanded layers are estimated at 10 to 15 percent, based on Weaver's (*2*) method and data. The partially disordered 1M-mica structure is considered a good indication that the rock has not undergone severe metamorphism.

The principal minerals in the glauconite-bearing quartzite are quartz, K-feldspar, plagioclase, chlorite, and barite. Minor constituents include hematite, apatite, and a carbonate. Chlorite occurs in pellets and appears to be an iron-rich type. Barite is a secondary mineral and apparently is fairly widely distributed in the rocks of the Missoula Group in the Flathead region.

Analytical data for the K-Ar and Rb-Sr ages are given in Table 1. The Ar^{40} and Sr^{87} reported are radiogenic. The glauconite, outgassed overnight at 75°C, required a correction for atmospheric Ar^{40} of about 1 percent. The Sr^{87} represents approximately 40 percent of the total Sr^{87}. The analyzed sample contains chlorite, quartz, and K-feldspar as impurities. The K-feldspar is estimated at 1 to 2 percent, and should not affect the determined age appreciably.

Although the Belt Series occurrence is the first Precambrian glauconite reported in North America, a number of glauconite samples dated at 1000 million years or more have been reported from the U.S.S.R. and China (*3*). No mineralogical data are given for these glauconites; however, the K-Ar ages given by Polevaya, Murina, and Kazakov (*3*) range to 1260 million years. A sample from the Sinian of Hopei Province, China, was dated at 1040 million years. Three samples from the Murmansk-Kola region range from 1020 to 1060 million years. Seven samples from the southern Urals range from 570 to 1260 million years, and six samples from Siberia show a similar range of 502 to 1260 million years. The K_2O content of the glauconite samples is variable, ranging from 3.0 to 7.7 percent and averaging 5.9 percent. Argon was determined volumetrically and tested for purity with a mass spectrometer. This method has been abandoned in this country in favor of the isotope dilution technique. The results are particularly interesting in their promise of a method for correlating the Precambrian nonfossiliferous strata.

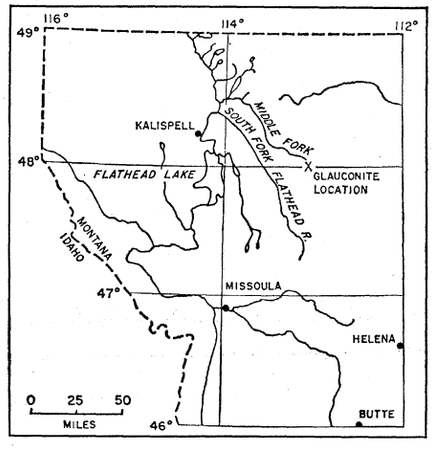

Fig. 1. Map of western Montana showing location of glauconite sample.

Although the U.S.S.R. glauconite ages appear to fit the geological time scale (*4*) rather well, such agreement is not common for glauconite samples dated in this country. Summaries of the extensive studies of glauconite in various laboratories are given by Hurley *et al.* (*5*), Folinsbee *et al.* (*6*), and Evernden *et al.* (*7*). Many of the K-Ar ages for glauconites appear to be satisfactory; however, more commonly the ages are too low. Hurley *et al.* (*5*) found that the glauconite results may be 10 to 20 percent lower than ages measured for micas from igneous rocks whose positions can be reasonably determined stratigraphically.

In view of the uncertainty of the isotopic ages for glauconites, the age of 1070 million years for the Belt Series sample should be considered a minimum age. This age, however, is considerably greater than that reported by Goldich *et al.* (*8*) for illite from the Siyeh Limestone of the Belt Series. The 2M-illite from a light-green shale bed near the top of the Siyeh Limestone, Logan Pass, Glacier Park, Montana, gave a K-Ar age of 740 million years.

Table 1. Age determinations for Belt Series glauconite, by K–Ar and Rb–Sr analyses (*13*). Ages are given in millions of years.

K–Ar analysis			Rb–Sr analysis			
K_2O (%)	Ar^{40} (ppm)	Age	Rb (ppm)	Sr (ppm)	Sr^{87} (ppm)	Age
8.36	0.733	1090	294	22.2	1.30	1050

Constants: K^{40}, $\lambda\varepsilon = 0.585 \times 10^{-10}$ per year, $\lambda\beta = 4.72 \times 10^{-10}$ per year. $K^{40} = 1.22 \times 10^{-4}$ gm/gm K. Rb^{87}, $\lambda\beta = 1.47 \times 10^{-11}$ per year. $Rb^{87} = 0.283$ gm/gm Rb.