Table 1. Lead isotopic composition of galena specimens from South African pre-Cambrian rocks

Locality	Geologic occurrence	Lead isotopic composition 206/204	207/204	208/204	Apparent common lead age (10^9 yr)
Southern Rhodesia					
1) S. T. Mine near Bindura*	Veins of galena in arkosic metasediments	14.16	15.06	34.22	2.2 ± 0.1
2) Kingsley Hoard Mine, Bindura, Mazoe Dist.	Replacement ore in arkosic and semipelitic metasediments	13.99	14.89	33.97	2.3 ± 0.2
3) Hardy (gold) Mine, Darwin Dist.	Gold vein in basement complex	14.02	14.74	33.85	2.4 ± 0.2
4) St. Ives Mine, near Turli, Bubi Dist.	Quartz vein in volcanic greenstone country	13.74	14.57	33.69	2.5 ± 0.2
5) Elba (gold) Mine, Wankie Dist.	Gold vein in basement complex	16.79	15.46	37.97	1.0 (?)
6) Cobra (gold) Mine, Gwanda	In greenstone country near granite contact	14.00	14.89	34.05	2.3 ± 0.2
7) C. S. C. Mine, Wedza, Marandellas Dist.	W, Pb deposit in marbles, pelitic metasediments, and Fe quartzites	14.15	15.17	33.86	2.3 ± 0.2
Southwest Africa					
8) Ombonna 89, Otjiwarongo Dist.†	Vein in reddish phase of Salem (?) granite	18.71	15.52	38.95	< 0.8
Union of South Africa					
9) Leeuwenkoof 97, Pretoria Dist.	Replaces dolomite of Transvaal system	14.92	15.06	34.21	2.1 ± 0.2
10) Uitloop 291, Potgietersrust Dist.	Quartz vein in granite older than Bushveld igneous complex	14.97	15.07	34.71	2.0 ± 0.2
11) Stavoren (tin) Mine, Potgietersrust Dist.	Cassiterite pipes in granophyres of Bushveld igneous complex	18.14	15.76	38.47	< 0.8
12) Appelfontein 71, Zoutpansberg Dist.	Quartz vein in "Old Granite"	15.94	15.40	36.76	1.4 ± 0.3
13) Rosetta Mine, Barberton Dist.	Quartz vein in Jamestown complex intrusive into Figtree schists	12.58	14.11	32.77	2.9 ± 0.1
14) Dyasonsklip, Gordonia, about 15 mi SW of Uppington	Vein in Namaqualand granite-gneiss	18.09	15.57	37.80	< 0.8
15) Keimos, Orange River Valley, Cape Province‡	Associated with either Namaqualand pegmatites or older Archean intrusive rocks that are cut by the pegmatites	20.41	15.66	41.49	Anomalous
16) East Geduld (gold) Mine, Witwatersrand		18.03	15.98	34.48	Anomalous, probably pre-Cambrian

* Samples 1–7 and their descriptions were supplied by R. M. Tyndale-Biscoe, acting director, and A. M. Macgregor of the Geological Survey of Southern Rhodesia.
† Samples 8–14 and 16 and their descriptions were provided by L. T. Nel, director, and B. Wasserstein of the Geological Survey of South Africa. Survey museum numbers corresponding to table numbers are as follows: No. 8, 4523a; No. 9, 4415; No. 10, 4480; No. 11, 3487; No. 13, 758; and No. 14, 4512. Samples 12 and 16 are from Wasserstein's personal collection.
‡ Sample and description supplied by A. Poldervaart, geology department staff, Columbia University, New York, N.Y.

The Rosetta galena (No. 13) was identical to that submitted by Nel to the Toronto group, and the isotopic composition here reported agrees well with the earlier measurement (*1*). Our age of 2.9×10^9 years is essentially identical to the 2860×10^6 years reported in the Canadian work (*1*).

A more complete history of this important area may be reconstructed with the aid of additional isotopic analyses of common leads and of uranium-bearing minerals. The tentative nature of the age determinations in this paper must be emphasized in view of the uncertainties in the values of the constants used in the lead growth Eqs. 1–3 and of possible inhomogeneities in the Pb/(U + Th) ratios in the crust.

GEORGE L. BATE*
J. L. KULP
Lamont Geological Observatory, Columbia University, Palisades, New York

References and Notes

1. P. E. F. Gretener, R. M. Farquhar, J. T. Wilson, *Trans. Roy. Soc. Can.* 48, 17 (1954). See also, J. T. Wilson *et al.*, *Nature* 174, 1006 (1954).
2. Lamont Geological Observatory contribution No. 164. Work supported in part by Research Division of U.S. Atomic Energy Commission under contract AT(30-1)-1114.
3. G. L. Bate, "Variations in the isotopic composition of common lead and the history of the crust of the earth," Ph.D. thesis, Department of Geology, Columbia University, New York (1955).
4. E. McCrady, *Trans. Am. Geophys. Union* 33, 156 (1952); R. D. Russell *et al.*, *ibid.* 35, 301 (1954).
5. R. M. Farquhar and G. L. Cumming, *Trans. Roy. Soc. Can.* 48, 9 (1954).

* Postdoctoral fellow of National Science Foundation.

20 July 1955

Tetrazolium-Reduction Test for Milk

Tetrazolium salts are redox indicators; their oxidized state forms colorless or pale yellow solutions in water. When reduced, they become insoluble in water and form highly colored crystals of formazan. The formazan of triphenyltetrazolium chloride (TTC) is red, that of neotetrazolium chloride (NT) is purple, and that of blue tetrazolium (BT) is dark blue.

In 1951 Schönberg (*1*) introduced the use of TTC in the indirect determination of the bacterial content and the keeping quality of milk. He concluded that the TTC-reduction test is best suited as a rapid screening test for milk samples with a high bacterial content. Later (*2*) he pointed out that the reliability of the results obtained with TTC in diffuse daylight of changing intensity may be questioned because light has a

marked accelerating effect on the reduction of TTC.

This paper (*3*) reports the applicability of TTC, NT, and BT for the testing of milk under different conditions. The tetrazolium tests were performed simultaneously with a conventional methylene blue test in unstoppered glass tubes. The final concentrations of the tetrazolium salts were 10^{-3} and 10^{-4} (9 ml of milk and 1 ml of 1.1-percent weight per volume stock solutions of TTC, NT, and BT).

The incubation temperatures were 37°C and room temperature was 19° to 21°C. At room temperature, the tests were performed simultaneously in darkness and under the influence of scattered light of blue sky or of direct sunlight. The bacterial contents of the milk samples were estimated customarily by counting the number of the colonies on agar and gelatin plates.

Tetrazolium salts in a concentration of 10^{-3} were inhibitory to the milk bacteria since the reduction times were definitely prolonged. The concentration 10^{-4} was, therefore, more suitable for the testing of milk. The results obtained using a 10^{-4} concentration of the tetrazolium salts and a dark thermostat of 37°C are summarized in Table 1.

The table shows that the reduction of the tetrazolium salts in the milks with 4×10^6 bacteria per milliliter or less becomes visible earlier than the reduction of methylene blue. In good milks with long reduction times the colors of formazans are first seen at the bottoms of the tubes because of the sedimentation of bacterial clumps. In milks highly contaminated with bacteria, TTC and NT seem to be equal with methylene blue in the rapidity of reduction, while the use of BT is hampered by its slower reduction.

As is known, room temperature (19° to 21°C) is inapplicable to the methylene blue test because reduction occurs in a reasonable time only with milks highly contaminated with bacteria. In contrast, it is a definite advantage of tetrazolium salts that they may be employed at room temperature when milk samples with high bacterial contents are screened under field conditions. The formation of formazan is relatively little delayed in such milks by room temperature.

When the effect of light on the reduction of the tetrazolium salts was studied, it was found that TTC was the most photosensitive. Even plain water solutions of TTC became red under the influence of light, whereas the water solutions of NT and BT were stable. In milk the reduction of tetrazolium salts is accelerated by the light more than the reduction of methylene blue. The reduction in light was partly nonenzymatic, for boiled samples of milk also exhibited formazan formation on the side of the tube directed toward the light source. The higher the concentration of tetrazolium salts in the milk, the more photosensitive the reaction and the poorer the correlation between the reduction time and the bacterial content. We can agree with Schönberg (*2*) that the tetrazolium-reduction test performed in a diffuse daylight of unknown intensity using TTC in a final concentration of 10^{-3} yields inconsistent results concerning the bacterial content. Therefore, the tests should be conducted in complete darkness.

Thirty-eight percent formaldehyde up to a final concentration of 5×10^{-3} accelerated the reduction of methylene blue, but this concentration was definitely inhibitory to the formation of the formazans. Aldehydeoxidase of Schardinger seems, therefore, to play a smaller role in the reduction of tetrazolium salts than it does in the reduction of methylene blue. Salicylic acid was found to inhibit equally the reduction of the tetrazolium salts and that of methylene blue.

When a continuous stream of air was conducted simultaneously into identical tests with methylene blue and with the tetrazolium salts, it was found that methylene blue was not completely reduced to the leuco form, whereas the reduction of the tetrazolium salts was only slightly delayed. The stability of formazans against oxygen seems to be a great advantage of the tetrazolium salts over methylene blue, which is readily reoxidized by the atmospheric oxygen (*4*). Moreover, the number of bacteria that are capable of reducing tetrazolium salts can be estimated by microscope since the intensely colored microcrystals of formazan are intracellularly deposited in the bacteria.

Our attempts to develop a quantitative colorimetric method by extracting the formazans from milk with lipid solvents (*5*) were unsuccessful. Quantitative yields were not recovered with the use of ether, chloroform, benzene, toluene, xylene, methanol, ethanol, propanol, butanol, amyl alcohol, and their combinations with glacial acetic acid.

Table 1. Reduction times of the tetrazolium salts and methylene blue (MB) as influenced by the bacterial content of milk samples

Bacteria (No./ml)	Reduction time (min)			
	TTC	NT	BT	MB
$< 10^5$	$>$ 420	$>$ 420	$>$ 420	$>$ 660
10^5 to 5×10^5	420 to 270	420 to 270	420 to 270	660 to 360
5×10^5 to 4×10^6	270 to 90	270 to 90	270 to 90	360 to 120
4×10^6 to 20×10^6	90 to 25	90 to 15	90 to 60	120 to 20
$> 20 \times 10^6$	$<$ 25	$<$ 15	$<$ 60	$<$ 20

KIMMO K. MUSTAKALLIO
E. O. AHOS
E. O. AUTIO
Department of Hygiene, University of Helsinki, Helsinki, Finland

References and Notes

1. F. Schönberg, *Lebensmitteltierarzt Deut. tierärzt. Wochschr.* **2**, 52 (1951).
2. ———, *ibid.* **4**, 137 (1953).
3. We are indebted to W. Lojander, head of the department of hygiene, for his unfailing interest during our study.
4. W. Lojander, thesis, *Acta Soc. Med. Fennicae.* Duodecim 6, fasc. 2 (1925).
5. A. R. Fahmy and E. O'F. Walsh, *Biochem. J. London* 51, 55 (1952).

5 July 1955

Fluorescence Characteristics of 5-Hydroxytryptamine (Serotonin)

Although no definite function has been ascertained for 5-hydroxytryptamine (5-HT), it is already evident from the reports of numerous investigators that it may be an important physiological agent. The most interesting development is the possible connection between 5-HT and brain function as first suggested by Gaddum (*1*), and indicated by recent studies of Shore, Silver, and Brodie (*2*).

To obtain more information about this compound, it is necessary to develop methods for its analysis in tissue extracts. A number of bioassays for 5-HT have been reported (*3, 4*) and these have contributed important information concerning the distribution of this compound. However, standard chemical procedures are not sufficiently sensitive to detect the small amounts that are present in tissues such as brain and blood.

In a previous communication, it was reported that 5-HT in dilute acid or at neutral *p*H fluoresces at 330 mμ when activated at 295 mμ (*5*). The fluorescence in the ultraviolet was detected with a specially designed spectrophotofluorometer. The sensitivity of the fluorescence in the ultraviolet is such that less than 0.1 μg can be readily measured. However, extracts of certain tissues such as brain contain other materials that fluoresce near 330 mμ and therefore make analysis difficult.

It has now been found that in stronger acid (3*N* HCl), 5-HT and other 5-hydroxyindoles fluoresce at 550 mμ and