Reports and Letters

Quantitative Analysis for Thorium by X-ray Fluorescence

Techniques are being developed by the U.S. Geological Survey for the quantitative analysis of rocks, minerals, and ores for thorium by fluorescent x-ray analysis. This work is part of a program that is being conducted on behalf of the Division of Raw Materials of the U.S. Atomic Energy Commission.

The method is a modification of the thorium method described by I. Adler and J. M. Axelrod [*Anal. Chemistry* **27**, 1002 (1955)]. It differs from their method in that (i) selenium is used instead of thallium as the internal standard and (ii) the samples are not briquetted.

The equipment consists of an unmodified Norelco x-ray spectrometer and plastic sample holders. These sample holders fit into the sample holder supplied by the manufacturer. In the top of the plastic blocks there is a rectangular recess 34 by 21 by 1 mm.

Standards are prepared by grinding together for ½ hr in an agate mortar 2 g of reagent-grade ThO_2 and a matrix of ground silica and Fe_2O_3, 3:1 by weight; 400 mg of silicon carbide No. 320; and 40 mg of 10-percent elemental selenium. The silicon carbide aids in the grinding process by further reducing the particle size of the other constituents. The powder is packed in the plastic sample holder. The sample is then inserted into the conventional Norelco x-ray spectrometer, and three positions are measured—$ThL\alpha_1$, $SeK\beta$, and a nearby convenient background position. This background position is determined by scanning over the spectrum region of the $ThL\alpha_1$ and $SeK\beta$ lines. The position is chosen by visual inspection of the graph. Using a LiF crystal, 29.5° was found to be a satisfactory background position in most cases.

The background is subtracted from the intensities of the $ThL\alpha_1$ and $SeK\beta$ lines. The ratio of the intensities of $ThL\alpha_1$ to $SeK\beta$, in counts per second, is plotted against a percentage of thorium (as the ordinate). The resulting curve is a straight line up to about 0.7 percent of thorium.

Above about 0.7 percent of thorium, the curve deviates from linearity because of coincidence losses in the counting circuits and poor resolution. This causes some $ThL\alpha_1$ radiation to be counted as $SeK\beta$. Coincidence losses can be overcome by the use of apertures to reduce the counting rate to about 500 counts/sec. Adler has shown that better resolution, but poorer sensitivity, can be obtained by using a quartz crystal instead of LiF.

Samples are prepared in much the same fashion. To a 2-g sample, 40 mg of 10-percent selenium in silica and 400 mg of SiC is added. This mixture is ground for ½ hr and is then measured in the same way as the standards. In each case, the background count is subtracted from the $ThL\alpha_1$ and $SeK\beta$ before computation.

The x-ray fluorescent analyses are compared with quantitative spectrographic analyses and with radiochemical analyses of the same samples in Table 1.

One sample was packed into the holder and counted ($ThL\alpha_1$) six times. This process was repeated six times, yielding 36 measurements. Variance was calculated on these replicate analyses. The actual quantity used was the time in seconds required to count 12,800 counts. The sample concentration was 0.215 percent of thorium. The standard deviation owing to packing, or more probably to sampling, was 0.72 sec. The error, or precision standard deviation, was 0.32 sec. The total time for 12,800 counts was about 40 sec. Sampling and packing doubled the error caused by recounting alone.

The error variance was calculated both on routine samples (ores) and on synthetic standards. Ores ranging from 0.4 to 0.8 percent of thorium had a standard deviation of 0.026 percent of thorium, based on 18° of freedom. Ores in the range of 0.01 to 0.05 percent of thorium had a standard deviation of 0.0026 percent of thorium based on 12° of freedom. Synthetic standards in the range of 0.1 to 0.3 percent of thorium had a standard deviation of 0.0042 percent of thorium based on 6° of freedom. In the range from 0.02 to 0.3 percent of thorium the standard deviation was 0.0026 percent thorium with 6° of freedom.

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27 April 1955

Table 1. Accuracy of fluorescent x-ray analysis for percentage of thorium

Sample	Quantitative spectrographic	Radiochemical	Synthetic spectrographic standard	Average of quantitative spectrographic and radiochemical	Fluorescent x-ray analysis
A, synthetic			0.10		0.10
B, synthetic			0.02		0.023
C, synthetic			0.032		0.037
D, synthetic			0.32		0.324
E, synthetic			0.75		0.71
F, ore	0.63	0.61		0.62	0.64
G, ore	0.49	0.47		0.48	0.48
H, ore	0.66	0.66		0.66	0.80
I, ore	0.34	0.38		0.36	0.43
J, ore	0.52	0.70		0.61	0.75
K, ore	0.43	0.51		0.47	0.57
L, ore	0.12			0.12	0.12
M, ore	0.17	0.17		0.17	0.18
N, ore	0.70	0.66		0.68	0.75
O, ore	0.48	0.49		0.485	0.48

Pathways of Glucose Metabolism in Corneal Epithelium

It has been previously shown that in the corneal epithelium the direct oxidative pathway, or the hexose monophosphate shunt, is an alternate route for glucose metabolism (*1*). It appears that in ocular tissues, such as the corneal epithelium and especially in lens (*2*), the shunt mechanism accounts for most of the CO_2 produced from glucose. To approximate the significance of the alternate pathway in the corneal epithelium, a comparison was made of the incorporation of C^{14} from 1-C^{14} and 6-C^{14} glucose into CO_2 (*3, 4*) and lactic acid (*5*).

Table 1. Incorporation of glucose-C^{14} into $C^{14}O_2$ and C^{14}-lactic acid by corneal epithelium. The values are given as the mean ± the standard error in counts per minute, per gram of wet weight, per 3 hr. The specific activity of 1-C^{14} and 6-C^{14} glucose was 480 counts/min µmole.

Determinations		Total counts/min recovered		Ratio C-1/C-6
Compound	No.	1-C^{14} glucose	6-C^{14} glucose	
CO_2	12	3370 ± 68	507 ± 15	6.7
Lactic acid	6	5330 ± 221	8105 ± 204	0.65
Sum		8700	8612	

Bovine corneal epithelium (700 to 800 mg) was incubated for 3 hr at 37.5°C with 20 µmole/ml of glucose in 6.0 ml of Krebs-Ringer bicarbonate buffer equilibrated with a gas phase of 95-percent O_2 and 5-percent CO_2. At the end of the incubation period the reaction mixture, contained in an erlenmeyer flask equipped with a side arm and center well, was acidified, and the CO_2 released was absorbed by a sodium hydroxide solution injected into the center well.

Aliquots of the CO_2 recovered were analyzed for CO_2 content (6) and radioactivity. The radioactivity of CO_2 was counted as $BaCO_3$ with an end-window Geiger tube. The specific activity of 1-C^{14} and 6-C^{14} glucose was 480 counts/min µmole and was determined by the combustion method of Van Slyke and Folch (7). The amount of lactic acid recovered after incubation was measured by the method of Barker and Summerson (8). The specific activity of the lactate was determined after oxidation to acetaldehyde and was counted as the dimedon-acetaldehyde complex (9). To employ this method it was necessary to remove glucose from the reaction mixture. This was accomplished by deproteinizing the reaction mixture with 10-percent trichloroacetic acid.

The resulting supernatant, neutralized to pH 5 to 6, was then placed on a Dowex 1 (chloride) column 30 by 1.2 cm. The column was washed with 1 lit of water to remove all traces of the isotopic glucose. Upon subsequent elution with 0.01N HCl, the lactic acid was recovered within a 50-ml fraction, and without further treatment it was oxidized according to the method of Brin (9).

The reaction mixtures from two experiments were usually pooled to give a sufficient amount of lactate (60 µmole) for this determination. As a control of this method, nonradioactive lactate was separated from a simulated reaction mixture containing isotopic glucose, and no radioactivity was detectable when the recovered lactate was converted to the dimedon-acetaldehyde complex.

The results of the experiment when corneal epithelium was incubated with either of the two isotopic forms of glucose are shown in Table 1.

As is shown in Table 1, the ratio of $C^{14}O_2$ from 1-C^{14} glucose/6-C^{14} glucose of 6.7 indicates that there does occur a preferential cleavage of the C-1 of glucose in contrast to C-6. If glucose were metabolized via the glycolytic and citric acid pathways exclusively, a ratio of 1 would have been observed. It appears that the shunt mechanism is particularly active in the production of CO_2 in this tissue. That this is the case, even though most of the glucose is metabolized via the glycolytic scheme, suggests that the citric acid cycle is relatively inactive in this tissue.

From the amount of radioactivity incorporated into lactic acid, as shown in Table 1, it is apparent that there exists an active glycolytic pathway, and an approximation of the relative extent of this route and the shunt mechanism is possible. Since the C-1 of glucose is oxidized directly to carbon dioxide in the shunt mechanism, it would appear that the 5330 counts/min recovered in the lactate from 1-C^{14} glucose is derived solely by the glycolytic route. An identical amount of radioactivity in lactate must be furnished by glycolysis when 6-C^{14} glucose is the substrate, and the additional amount observed is that derived from the direct oxidative pathway. Therefore, the ratio of C^{14}-lactate from 1-C^{14} glucose/6-C^{14} glucose of 0.65 can be taken as the approximate fraction of glucose metabolized via the glycolytic pathway. It appears that about 65 percent of the glucose is metabolized via the conventional glycolytic scheme and 35 percent by the shunt mechanism.

It is of interest to note that the sum of radioactivity incorporated into carbon dioxide and lactic acid is identical from the two forms of labeled glucose. This indicates that in this tissue, the intermediates of the direct oxidative pathway do not accumulate to any appreciable extent. Furthermore, it must mean that the triose phosphates formed in the shunt mechanism are eventually converted to lactic acid.

In the corneal epithelium, even though most of the glucose is metabolized by the glycolytic pathway, the presence of the hexose monophosphate shunt is indicated because a preferential oxidation of the C-1 of glucose occurs. Moreover, since the citric acid cycle appears sluggish, the shunt mechanism probably plays a more conspicuous role in the production of biological energy in the cornea than it does in other mammalian tissues.

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References and Notes

1. J. H. Kinoshita and T. Masurat, Arch. Biochem. and Biophys. 53, 9 (1954).
2. J. H. Kinoshita, Abstracts of 126th Meeting Am. Chem. Soc. (1954).
3. B. Bloom and D. Stetten, J. Am. Chem. Soc. 75, 5446 (1953).
4. S. Abraham, P. F. Hirsch, I. L. Chaikoff, J. Biol. Chem. 211, 31 (1954).
5. This work was supported by the U.S. Atomic Energy Commission.
6. D. D. Van Slyke and J. M. Neill, J. Biol. Chem. 61, 523 (1924).
7. D. D. Van Slyke and J. Folch, ibid. 136, 509 (1940).
8. S. B. Barker and W. H. Summerson, ibid. 138, 535 (1941).
9. M. Brin and R. E. Olson, ibid. 199, 475 (1952).

8 April 1955

Acclimation of the Critical Thermal Maximum of the Reptile Urosaurus ornatus

Experiments were designed for preliminary investigation of acclimation of temperature tolerance in a reptile, the small lizard Urosaurus ornatus linearis Baird. The relatively small sample sizes ($N = 12–15$) have proved adequate. A total of 45 individuals was used, including young and adults in the body-weight range of 1.26 to 4.85 g. Measurements were restricted to those for the upper lethal temperature range.

In such investigation it is useful to explore two different but related expressions of temperature tolerance—that is, the critical thermal maximum, or incipient upper lethal temperature, and the resistance time. General methods currently used for determining such values for fishes were not employed (1). In the present work, the critical thermal maximum was first determined ($= 43.1 ± 0.25$°C); afterward the resistance time at 44.0°C was measured. Resistance time at a given constant temperature may be given as the arithmetic mean, or the geometric mean, survival time. The animals were tested singly in air in 100-ml-capacity glass tubes, immersed in an electrically heated and stirred water bath, with air supplied through coils of immersed copper tubing. In tests for the critical thermal maximum, the animals were started at 39°C and the temperature was raised 0.5°C every 30 min. The animal's body temperature reached the new environmental