Determination of Rubidium in Biological Materials Using Atomic Absorption Spectroscopy

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In order to establish a reliable and rapid method for the determination of rubidium in biological materials using atomic absorption spectroscopy, chemical interference as well as ionization interference due to co-existing elements and some organic substances was studied. In the presence of potassium of sufficient concentration, most of the interference was found to be negligible. The temperature of the sample solutions proved to be a possible source of error. The process of sample preparation was examined, including a determination of the loss of rubidium in dry ashing. The accuracy and precision of the method was tested by analyzing NBS SRM 1577 Bovine Liver. The rubidium contents of typical biological materials—foodstuffs, human tissues and urine—were determined by the present method and a possible relationship between the rubidium and potassium concentrations is indicated.

Rubidium is an element the behavior of which in biological systems is similar to that of potassium.[1] However, its data on the transfer of this element from the environment to man and on its distribution in the human body are relatively few.[2,3] Rubidium has a natural radioactive isotope, ^{87}Rb, the half-life of which is 4.8×10^{10} years and the abundance of which is 27.85 %.[4] The radioactivity of ^{87}Rb is calculated to be 52.9 disintegrations per min per milligram of rubidium, which cannot be neglected from the standpoint of radiological health problems. The development of rapid and precise methods for the determination of natural rubidium in biological and environmental materials has recently become urgent.

Rubidium has usually been determined using emission spectrography, X-ray fluorescence analysis, or neutron activation techniques,[5] which require rather complicated equipment and are not considered to be very convenient methods in most laboratories. Flame emission spectrometry, which is claimed to be simple and of satisfactory sensitivity, is subject to unavoidable interference due to the neighboring strong potassium lines even when spectrometers of high spectral resolution are used.[6,7] This problem necessitates wavelength scanning which is time consuming.

Only a few papers have been reported on the determination of rubidium in biological materials by atomic absorption spectroscopy. In this report, in order to establish a rapid method of atomic absorption spectroscopic determination of rubidium, the chemical interference due to the co-existing elements and acids, as well as the ionization interference effect, was studied. It was confirmed that rubidium in typical biological materials can be rapidly determined, free from the effect of interference, using the proposed method, which was proven using a biological standard material.

Experimental

Instrument. Perkin-Elmer Model 303 and 403 atomic absorption spectrophotometers were used with 10-cm slot burner heads for air-acetylene flames. An Osram rubidium discharge lamp covered with filter paper having an 8 mm × 8 mm square aperture was used. Thus, about a two-fold increase in intensity was obtained supposedly due to the integration of the emitted light by reflection. A UV-cut filter (Hoya Glass Works, Tokyo, O-56) was placed just in front of the entrance slit of the Model 303 instrument. A Hitachi 056 strip chart recorder was employed. A Medical Spectrometer (Tokyo Shibaura Electric Co.) with a well-type NaI(Tl) detector was used for gamma scintillation counting.

Reagents. Rubidium chloride and cesium chloride were purchased from E. Merck AG. and used in most of the experiments. A 1000 μg cm^{-3} rubidium stock solution for quantitative determination was prepared by dissolving 1.415 g of Johnson Matthey "Specpure" rubidium chloride in 1000 ml of distilled water. The rubidium-86(tracer with a half-life of 18.66 d, supplied as a chloride, a specific activity of 0.833 mCi/mg-Rb) was obtained from the Radiochemical Centre, U. K., and diluted before use. Most of the other reagents used were of JIS Special Grade or Analytical Grade.

Results and Discussion

Optimum Operating Conditions. Typical operating conditions adopted are summarized in Table 1. The light beam, 2, 6, and 15 mm in height above the burner, approximately sampled the primary, interconal and outer zones, respectively. A comparatively small variation in absorbance was observed over the range of acetylene flow rate examined in the presence of potassium at a height of 7 mm (Fig 1). Some enhancement of the rubidium absorbance was observed when the acetylene cylinder pressure was reduced below 5 kg/cm^2. This effect, which occurred up to 30% of the relative absorbance, was suspected to be due to acetone vapor liber-

TABLE 1. OPERATING CONDITIONS

Wavelength	780.0 nm
Discharge lamp current	350 mA
Slit setting	4 divisions
Spectral band width	1.4 nm
Acetylene flow,	
meter setting	9.0 divisions
flow rate	3.4 dm^3/min
Air flow, meter setting	7.5 divisions
flow rate	17.0 dm^3/min
Beam height above burner (with potassium added)	7 mm

Perkin-Elmer Model 303. Similar conditions were adopted for Model 403.

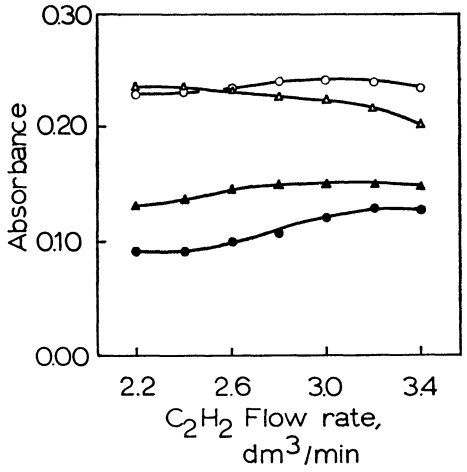

Fig. 1. Effect of acetylene flow rate on rubidium absorbance. Air flow rate: 17.0 dm^3/min (const). 5 μg cm^{-3} Rb, 3 mm (▲) and 7 mm (●) above burner top 5 μg cm^{-3} Rb+1000 μg cm^{-3} K, 3 mm (△) and 7 mm (○) above burner top.

ated from absorbing beds.

Comparison between Atomic Absorption and Emission Spectroscopy. The effect of the spectral band width on the signal-to-noise ratio of the rubidium absorbance signal in the presence of potassium was negligible as expected from theoretical considerations,[8] as is shown in Table 2. The peak-to-background ratio of the emission spectrum seriously deteriorated when wider slit widths were used, revealing the need for time-consuming scanning employing a narrow slit width (Fig. 2). Furthermore, it should be born in mind that many molecular emission bands such as the CrO 777.8- and 781.2-nm bands, the LaO 775.9-nm band, the FeO 777.5-nm band and the VO 785.1-nm band can interfere with the rubidium 780.0-nm line.[8,9]

The Effect of Other Alkali and Alkaline-earth Metals. Lithium, sodium, potassium, and cesium, as chlorides at concentrations greater than 100 μg cm^{-3} caused the rubidium absorbance to increase. In the interconal zone, the enhancement reached saturation at metal concentrations of greater than 1000 μg cm^{-3} while the saturation point shifted to the range from 2500 to 5000 μg cm^{-3} in the outer zone (Fig. 3). In the case of cesium, the rubidium absorbance apparently increases linearly with the concentration of cesium at concentrations greater than 1000 μg cm^{-3}. This was proven to be due to the rubidium impurity in the cesium chloride used (0.40 μg/mg of Cs as determined by atomic absorption) and the absorbance corrected for the reagent blank disclosed saturation at cesium concentrations greater than 500 μg cm^{-3}. The degree of enhancement was compared for these elements on a molar basis. The order was found to be Li<Na<K<Cs, which agrees with the inverse order of their ionization energies: 5.39 eV for Li, 5.14 eV for Na, 4.34 eV for K, and 3.89 eV for Cs.[8] The effect was more marked in the outer zone (Fig. 3). Flame temperatures in the outer zone were found to be higher than in the lower part for an air-acetylene flame closely matched to the flame conditions used here.[10] From these results, it is concluded

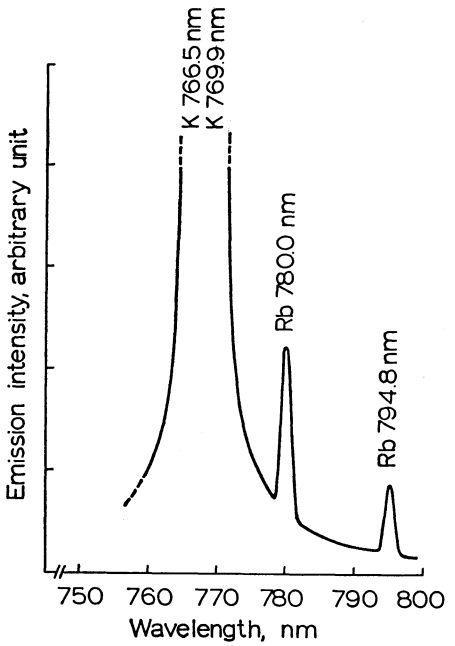

Fig. 2. A typical emission spectra of rubidium in the air-acetylene flame in the presence of potassium obtained by scanning. 2 μg cm^{-3} Rb+2000 μg cm^{-3} K (0.1 mol dm^{-3} HCl). Spectral band width: 1.4 nm; Scanning speed: 10 nm/min.

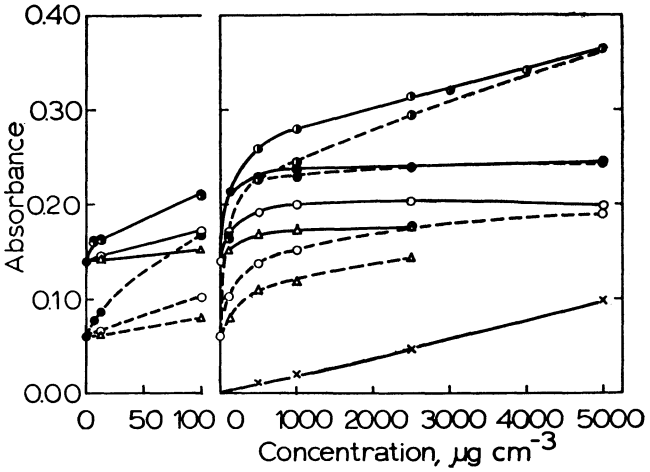

Fig. 3. Effect of other alkali metals. 5 μg cm^{-3} Rb+ Li (△), Na (○), K (●), Cs (◑); Cs only (×). ——: interconal zone; ---: outer zone.

Table 2. The effect of the slit width or spectral band width on rubidium determination by atomic absorption and emission in the presence of potassium

Slit		Spectral band width (nm)	Atomic absorption S/N ratio	Atomic emission Peak/background ratio
Setting (scale)	Width (mm)			
3	0.3	0.4	72	11
4	1	1.4	133	3.2
5	3	4	123	0.9
6	10	14	107	—

2 μg cm^{-3} Rb+2000 μg cm^{-3} K (0.1 mol dm^{-3} HCl).

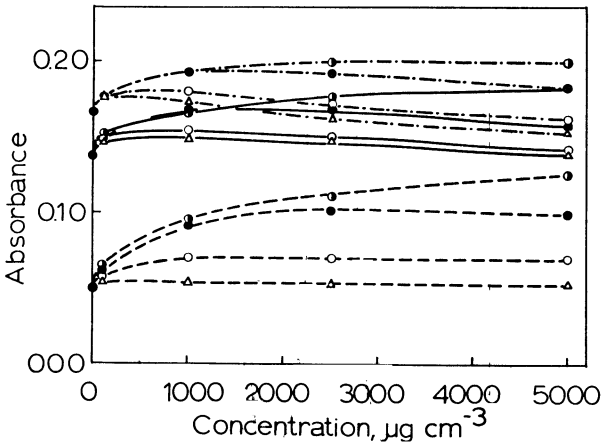

Fig. 4. Effect of alkaline earth metals. 5 μg cm^{-3} Rb+ Mg (△), Ca (○), Sr (●), Ba (◑). —·—·: primary zone; ——: interconal zone; ---: outer zone.

that the suppression of rubidium ionization by other easily ionized alkali metals[11,12] is the essential mechanism of this effect.

Alkaline-earth metals show a less pronounced but similar effect and the enhancement was found to be Mg<Ca<Sr<Ba, which is the inverse of their ionization energies: 7.65 eV for Mg, 6.11 eV for Ca, 5.69 eV for Sr, and 5.21 eV for Ba.[8] Magnesium, calcium, and strontium at greater than 1000 μg cm^{-3}, however, exhibited a slight suppression of the rubidium absorbance which had been enhanced at lower concentration levels. This negative interference was not observed in the outer zone (Fig. 4). It appears that the alkaline-earth metals were ionized to some extent in air-acetylene flames exhibiting ionization interference and that, at higher concentrations (2500 to 5000 μg cm^{-3}), they caused chemical interference by retarding the atomization of rubidium, possibly through occlusion in and/or adsorption to clotlets in the primary and interconal zones.

The enhancements of alkali and alkaline-earth metals at 10—20 mmol dm^{-3} were compared, with the resulting order in the interconal zone: Li, Mg<Ca< Sr<Ba<Na<K<Cs. This is in good agreement with the inverse order of the ionization energies for all these elements except lithium.

The Effect of Several Transition Metals and Aluminium. Iron, chromium, zinc, and copper at concentrations of 10 to 1000 μg cm^{-3} exhibited small positive interference while the effect of manganese was negligible at concentrations below 100 μg cm^{-3} (Fig. 5). Aluminium, as a nitrate at concentrations in the range from 100 to 2500 μg cm^{-3}, exhibited a small enhancement although it noticeably lowered the rubidium absorbance at 5000 μg cm^{-3} as either a nitrate or a chloride. For systems containing 1000 μg cm^{-3} of potassium, sodium showed no influence on the rubidium absorbance up to 1000 μg cm^{-3} and virtually no effect was observed for calcium, iron, copper, zinc, manganese, and aluminium up to 1000 μg cm^{-3} (Fig. 6). A small negative interference due to magnesium was observed in the presence of potassium. The use of nitric acid proved favorable when aluminium is present in sample solutions.

The Effect of Mineral Acids. No appreciable effect due to nitric acid was observed. Hydrochloric and perchloric acid exhibited slight enhancment at 0.1 mol dm^{-3} but a negative interference of about −30% at

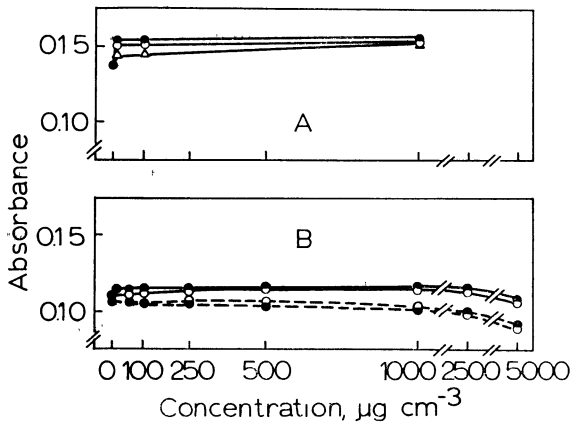

Fig. 5. Effect of transition metals and aluminium.
(A) 5 μg cm^{-3} Rb+Fe, Cr, Zn (●), Cu (○), Mn (△).
(B) 5 μg cm^{-3} Rb+Al as nitrate (●), as chloride (○).
—— interconal zone; ---: primary zone.

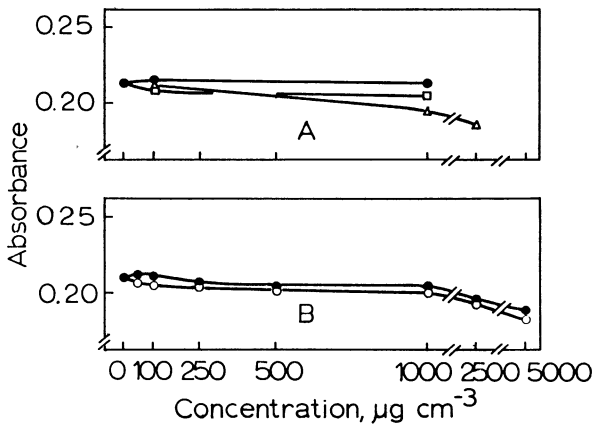

Fig. 6. Effect of transition metals and aluminium in the presence of potassium.
(A) 5 μg cm^{-3} Rb+1000 μg cm^{-3} K. ●: Na, △: Mg, □: Ca, Fe, Cu, Zn, Mn.
(B) 5 μg cm^{-3} Rb+1000 μg cm^{-3} K, Al as nitrate (●) as chloride (○).

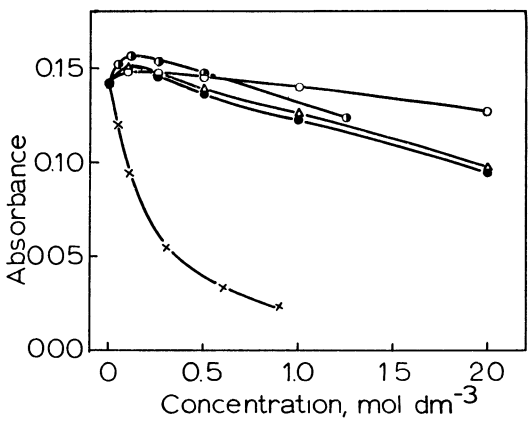

Fig. 7. Effect of mineral acids.
5 μg cm^{-3} Rb. ○: HNO_3, ●: HCl, △: $HClO_4$, ◑: H_2SO_4, ×: H_3PO_4.

2 mol dm^{-3} was observed (Fig. 7). This interference was inferred to be due to the formation of the diatomic molecule, RbCl, in the flame.[13] Phosphoric acid severely interfered causing a −50% decrease in signal even at 0.2 mol dm^{-3}. With sulfuric acid, the absorbance decreased and the aspiration rate of the sample solution was found to be reduced. In the presence of 1000 μg cm^{-3} of potassium, the negative interference for these acids at a concentration of 0.1 mol dm^{-3} was negligible, except for phosphoric acid in which case the interference was reversed to only −22% at 0.2 mol dm^{-3}. Precipitation occurred in solutions containing potassium and of perchloric acid concentrations greater than 0.5 mol dm^{-3}, which perturbed the rubidium absorbance measurements (Fig. 8).

The Effect of Phosphates. Phosphates at concentrations in the form of P below 2500 μg cm^{-3}, including free orthophosphoric acid, exhibited interference, except when the counter cation was potassium (Fig. 9). For an added potassium concentration of 1000 μg cm^{-3}, the interference of phosphoric acid, ammonium phosphate and calcium phosphate was reversed to from −3 to −5% at a P concentration of 1000 μg cm^{-3}. For the purpose of suppressing the phosphate interference, potassium should be added so as to render the K/P ratio greater than two.

The Effect of Silicates. No systematic study on the effect of silicates was carried out but some interference was observed. Since silicates exist in considerable amounts only in plant materials, this interference is expected to be negligible for most biological materials.

The Effect of Organic Acids and Their Ammonium Salts. Several ammonium salts of organic acids are used for separating alkali elements from matrices by ion-exchange column chromatography. In this connection, the effects of formic, acetic, lactic and tartaric acids and their ammonium salts were examined and the results are shown in Fig. 10. Trichloroacetic acid was selected since it is used for precipitating proteins from biological fluids. Acetic acid enhanced the rubidium absorbance while its trichloro derivative caused a severe reduction especially in the presence of 1000-μg cm^{-3} potassium concentrations. The effect of trichloroacetic acid is assumed to be due to liberated chlorine which tends to form RbCl. Ammonium formate, ammonium acetate and ammonium lactate in concentrations up to 0.5 mol dm^{-3} in the presence of 1000 μg cm^{-3} of potassium caused only a negligible effect and these are the most probable cases for practical application. These organic

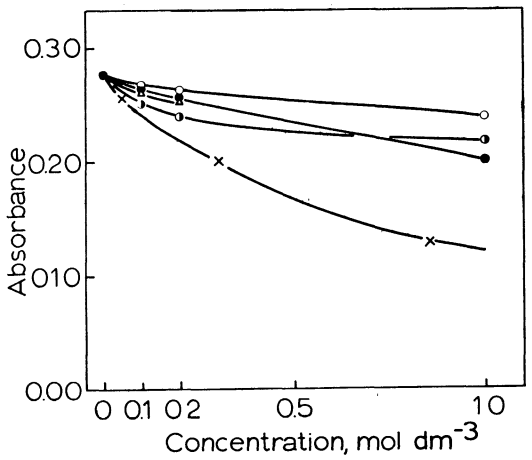

Fig. 8. Effect of mineral acids in the presence of potassium.
5 μg cm^{-3} Rb+1000 μg cm^{-3} K. ○: HNO_3, ●: HCl, △: $HClO_4$, ◑: H_2SO_4, ×: H_3PO_4.

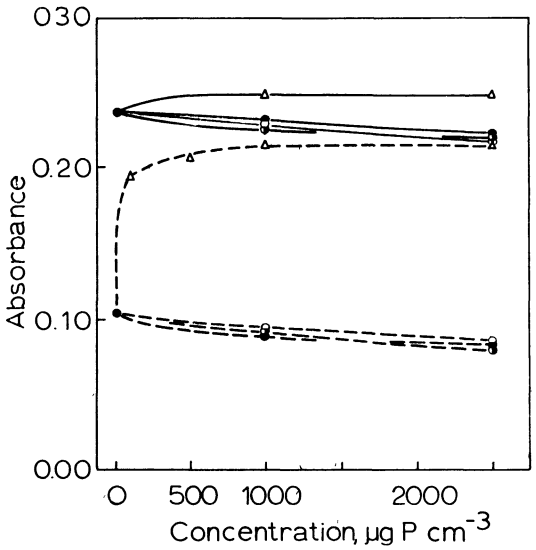

Fig. 9. Effect of phosphates.
---: 5 μg cm^{-3} Rb; ——: 5 μg cm^{-3} Rb+1000 μg cm^{-3} K.
●: H_3PO_4, ○: $(NH_4)_2HPO_4$, ◑: $Ca(H_2PO_4)_2$, △: KH_2PO_4.

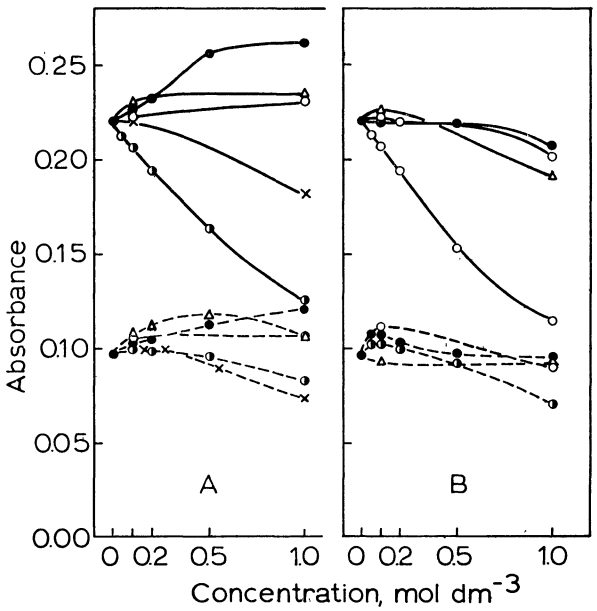

Fig. 10. Effect of organic acids and their ammonium salts.
---: 5 μg cm^{-3} Rb. ——: 5 μg cm^{-3} Rb+1000 μg cm^{-3} K.
(A) ○: HCOOH, ●: CH_3COOH, ◑: CCl_3COOH, △: $CH_3CH(OH)COOH$, ×: $(CH_2(OH)COOH)_2$.
(B) ○: $HCOONH_4$, ●: CH_3COONH_4, ◑: CCl_3COOH/NH_4OH equimolar mixture, △: $CH_3CH(OH)COONH_4$.

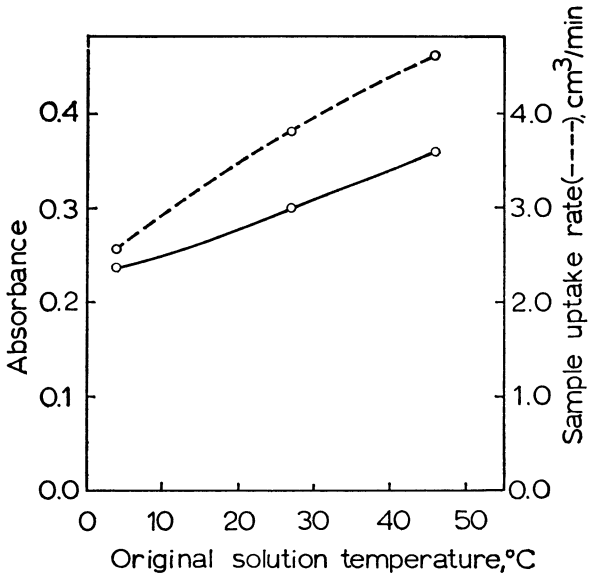

Fig. 11. Effect of sample solution temperature on rubidium absorbance and sample uptake rate.
5 μg cm^{-3} Rb+1000 μg cm^{-3} K.

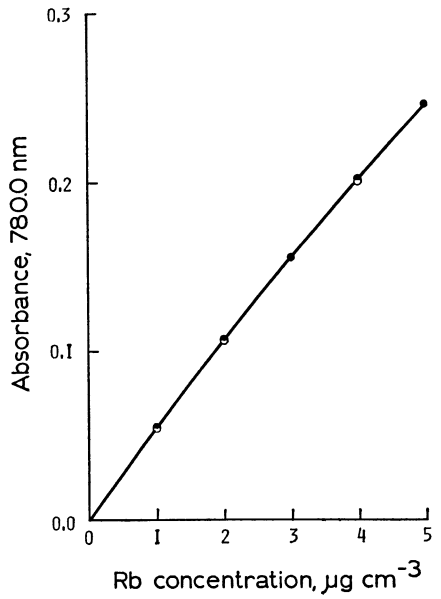

Fig. 12. Working curves for rubidium.
●: 1000 μg cm^{-3} K. ○: 1000 μg cm^{-3} K+500 μg cm^{-3} Na+500 μg cm^{-3} Ca+500 μg cm^{-3} P.

substances, however, should be decomposed prior to nebulization, because they can clog the burner slot.

The Effect of the Solution Temperature. At higher sample solution temperatures, a considerable increase in absorbance was observed (Fig. 11). This phenomenon was accompanied by an increase in the rate of sample aspiration through a capillary. If sample and standard solutions of different temperatures are measured, considerable error will result.

Sensitivity and Detection Limit. Typical analytical curves for rubidium with potassium added are shown in Fig. 12. The slight non-linearity can be attributed to self-absorption in the light source. It is not expected that this can be avoided by replacing the discharge lamp with a hollow cathode, because no essential difference was observed between Na and K analytical curves obtained using metal discharge lamps and those obtained using hollow-cathode lamps. This non-linearity excludes the use of the standard addition method in precision analysis.

Only less than a 1% reduction in sensitivity was observed when further addition of sodium, calcium and phosphorus, each at 500 μg cm^{-3}, were made. The sensitivity for rubidium under these conditions was calculated to be 0.08 μg cm^{-3} for 1% absorption (0.0044 absorbance). The detection limit obtained by digital signal averaging over about 10 s was 6.5 ng cm^{-3}.

Decomposition of Biological Materials. The loss of rubidium during dry ashing and the conventional procedure for removing silica using concentrated hydrochloric acid (Table 3) was compared with the loss during

TABLE 3. LOSS OF RUBIDIUM DURING DRY ASHING AND PREPARATION OF SILICA-FREE SAMPLE SOLUTIONS DETERMINED BY Rb-86

Material	Wet weight (g)	Ashing temperature (°C)	Ashing time (h)	Recovery (%)
Cabbage	100	450	24	99.6
		550	3	94.5
Beef	100	450	24	99.8
		500	8	97.1

Cabbage was homogenized using a Waring blender, transferred to a porcelain dish, mixed thoroughly with a quantity of Rb-86 tracer and dried at 100 °C prior to ashing in a muffle furnace. Beef was minced, mixed with a quantity of Rb-86 tracer, and treated in a similar manner.

TABLE 4. DETERMINATION OF RUBIDIUM IN NBS SRM 1577 BOVINE LIVER

Analysis number	Sample weight[a] (g)	Absorbance[b] (780.0 nm)	Concentration (μg Rb/g)	Content (μg Rb/g)	Certified value (μg Rb/g)
1	0.9221	0.093_8	1.71	18.6	
2	0.9659	0.098_9	1.81	18.7	
3	0.9146	0.092_8	1.69	18.5	18.3±1.0
4	0.9220	0.095_4	1.75	19.0	
5	0.9334	0.094_3	1.72	18.4	
				Mean ± s. d.	
				18.6 ± 0.20	

a) Dried at 90°C for 24 h. The ash was taken up in 10.00 cm^3 of 0.1 mol dm^{-3} HNO_3. b) Mean of five measurements with relative standard deviation of 0.6% for an approximately 10 s digital integration ("100 AVR").

acid digestion (the recovery found, 99.7%). No appreciable rubidium loss was found for dry ashing. When extremely accurate determinations are required, a temperature of 450 °C should be used.

Preparation of Samples. Fifty to two hundred grams of fresh vegetables and cerials were dried at 90 °C for 24 h and ashed. Silica was removed as usual. The edible parts of about 50 g of fresh fish and shellfish were selected and processed as stated above without the removal of silica. About 50 g of meat was processed in the same manner. Two hundred milliliters of milk was evaporated in a porcelain evaporating dish on a water bath and then ashed. Urine sampled for 24 h, about 1.5 dm^3, was digested by repeated treatment with concentrated nitric acid and 30% hydrogen peroxide with heating, then evaporated to dryness and dissolved with distilled water. Regarding human tissue, about 10 g of fresh material was used. Finally, sample solutions were adjusted to 0.1 mol dm^{-3} using nitric or hydrochloric acid and the content of potassium was determined by atomic absorption or by emission in order to confirm that the concentration was in the range of 1000 to 5000 μg cm^{-3}. As necessary, potassium was added to sample solutions.

Accuracy and Precision. A biological reference material, NBS SRM 1577 Bovine Liver, was analyzed and the results are summarized in Table 4. The sample solution contained roughly 1300 μg cm^{-3} of potassium. The analytical results were in good agreement with the certified value.

Rubidium Content of Biological Materials. Some analytical results for typical biological materials made using the present method are listed in Tables 5 and 6. The values of the rubidium content found for these samples were in the range from 0.4 to 7 μg/g fresh weight. Higher figures were found for meat and milk than those for vegetables and fish. This relationship was again found when the results were compared on a potassium weight basis: 2—3 μg-Rb/mg-K for meat and milk as compared to 0.2—0.6 μg-Rb/mg-K for vegetables and fish. Human lung, liver, and spleen were found to contain slightly higher concentrations than the other tissues examined. The Rb/K ratios fall in the range 1.5—3.5 μg-Rb/mg-K for human tissues. From these results it may be inferred that mammals including man concentrate rubidium more than potassium during the incorporation of these alkali metals from precursors.

Table 5. Analytical results for rubidium in food stuffs

Material	Rb concentration		
	mg/g ash	μg/g wet wt	μg/mg-K
Vegetable			
Spinach, leaf	0.187	2.61	0.603
Japanese radish, root	0.149	0.412	0.436
Japanese radish, leaf	0.101	1.65	0.404
Fish			
Carp	0.082	0.747	0.314
Saurel	0.050	0.968	0.280
Mackerel	0.076	0.968	0.263
Bonito	0.083	0.680	0.289
Tuna	0.083	0.643	0.278
Shellfish			
Tapes sp.	0.560		0.196
Pork	0.753	7.11	2.38
Milk, processed	0.555	4.02[a)]	2.68

a) Equivalent to 1030 μg-Rb/dm^3 of milk.

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Table 6. Analytical results for rubidium in human organs and urine

Material	Subject		Rb concentration		
	Age	Sex	mg/g ash	μg/g wet wt	μg/mg-K
Lung	76	Male	0.472	5.05	3.49
Liver	76	Male	0.513	4.36	3.73
	73	Male	0.330	3.44	2.63
	24—81	F, M[a)]			1.96±0.75
Kidney	38	Female	0.283		1.87
	79	Male	0.255		1.72
Spleen	38	Female	0.316		1.92
	79	Male	0.217		1.61
Pancreas	60	Male	0.555		3.01
Thyroid	24	Male	0.319		1.91
Diaphragm	55	Female	0.312		1.65
	[b)]	Female	0.473		2.14
	24	Male	0.334		1.83
	79	Male	0.196		1.54
Small intestine	73	Male	0.559	1.61	3.38
	24—79	F, M[c)]			1.94±0.55
Urine, 24-h	26	Male		1.40	1.80

a) Sample number: 8. b) Age not identified. c) Sample number: 8.

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