Determination of rubidium in human serum

H.-R. Schulten[1], P. B. Monkhouse, C. Achenbach and R. Ziskoven

Institut für Physikalische Chemie der Universität Bonn, Wegelerstr. 12, D-5300 Bonn 1 (Federal Republic of Germany), and Physiologisches Institut II der Universität Bonn, Wilhelmstr. 31, D-5300 Bonn 1 (Federal Republic of Germany), October 19, 1982

Summary. Field desorption mass spectrometry (FD-MS) combined with stable isotope dilution has been used to determine rubidium concentrations from human serum. Samples obtained from 110 healthy volunteers (50 males, 60 females) were examined. The rubidium concentrations found varied from 0.96 to 3.56 µmoles/l, the average value being 1.96 µmoles/l. The precision of the measurements within a batch was 0.8%. The time for 1 analysis, including sample preparation is about 30 min, the total sample consumption is 100–200 µl. The corresponding potassium concentrations were also determined from all these serum samples; a weak trend towards higher potassium levels with increasing rubidium concentration is found.

Up to now, the alkali metal rubidium has been considered to be a 'nonessential' trace element in mammalian metabolism[2,3], its precise function being unknown. However, due to its chemical and physical properties, the physiological behavior of the rubidium cation can be expected to bear some relation to that of the potassium cation and to a lesser extent to the thallium cation[4]. In a recent study of pregnant rabbits and their offspring[5,6], correlations were found between the rubidium concentrations in maternal serum, the bone growth of the fetus and the formation of milk. An investigation on multiple sclerosis patients[7] has shown distinct similarities in the 24-h excretion profiles between Li^+ and Na^+ and between K^+ and Rb^+. Furthermore, the multiple sclerosis patients in this study had rubidium levels averaging more than 25% below the average concentration of a group of healthy individuals, after correcting for dietary conditions. The concentration of rubidium in serum has been determined by other analytical methods such as atomic absorption[8,9], emission spectroscopy[10–12], neutron activation analysis[13–16] and X-ray spectroscopy[17,18]. The reported values vary between 0.47 and 6.78 µmoles/l. However, before continuing our studies, we consider it essential to establish a reliable and more accurate baseline for healthy persons. In the present investigation, therefore, we have collected and examined serum samples from a series of healthy volunteers.

The FD method, which we employed in this study, has been used increasingly in recent years for qualitative and quantitative investigations of metals in biological samples. It has proved to be a highly sensitive, specific and fast technique for the detection of metal cations[19–22]. In combination with the stable isotope dilution technique, quantitative assays of a variety of metals, including rubidium, have been performed with high precision from physiological fluids and tissues[23–26].

Material and methods. Serum samples were obtained from 110 apparently normal individuals, 50 men and 60 women, attending the University Polyclinic, Bonn for routine tests. Blood was sampled i.v. after an overnight fast and centrifuged within 30 min after sampling. Sodium and potassium were determined by flame photometry in the analytical laboratories of this clinic, whilst rubidium was determined by FD-MS and isotope dilution in our laboratory (Institute of Physiology II). For the rubidium assay, the serum sample (100–200 µl) was mixed with a known amount of enriched rubidium chloride (Rohstoff-Einfuhr GmbH, Düsseldorf, FRG) as internal standard. Ethanol (1 ml) was added, the mixture shaken and than centrifuged for at least 20 min at 10,000 rpm before applying several µl to the FD emitter. The isotopic abundances of the standard were checked by FD-MS prior to quantification and were found to be correct within a few tenths of a percent[21]. For each serum sample 3 or 4 independent measurements were performed, the average value and SD calculated.

FD measurements were performed using a double focussing mass spectrometer (Varian MAT 212, Bremen, FRG)

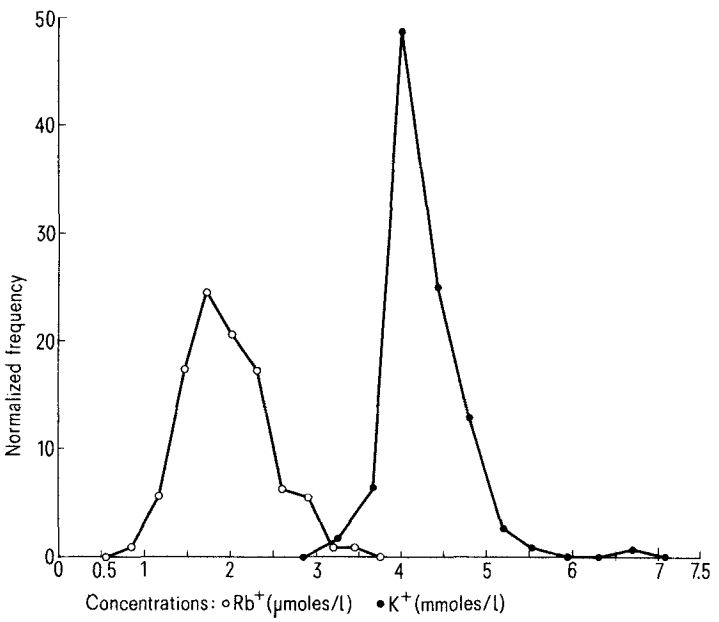

Figure 1. Normalized frequency distributions of rubidium (○) and potassium (●) concentrations found in human serum.

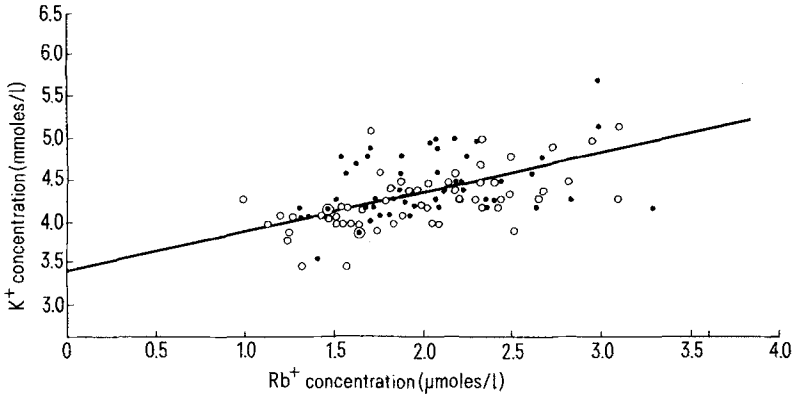

Figure 2. Plot of potassium concentrations against corresponding rubidium concentrations. Linear regression is shown by the solid line.

equipped with a combined EI/FD-ion source. The ion source potentials were +3 kV for the field anode and −4 kV for the counter electrode. For integrating accumulation of FD ions, a multichannel analyzer (Varian CAT 1024) was used and the peaks plotted on a recorder (Kipp and Zonen, BD 4004-06). Signal amplification was performed with an SEV at −2 kV. For each measurement about 50 scans were accumulated. For all measurements, standard high temperature activated carbon FD emitters with a 10 μm tungsten core were employed[27]. The emitter wire was heated directly with a manually operated heating current up to 70 mA.

Results and discussion. The serum samples from 110 adult volunteers were examined. Rubidium concentrations found ranged from 3.2 to 1.27 μmoles/l for males and 3.56 to 0.96 μmoles/l for females. The average value for males was 1.97 μmoles/l, that for females 1.94 μmoles/l, with a combined average of 1.96 μmoles/l. The variation coefficients for the measurements within a batch varied from 0.1 to 3.0%, averaging 0.8%.

The results were devided into classes of fixed width 0.3 μmoles/l and the normalized frequency distributions calculated (fig. 1). The slight asymmetry in the distribution obtained is not thought to be significant.

For comparison, a compilation of quantitative data on rubidium in serum is available[28]. These data are taken from 10 independent investigations and are based on a total of 361 measurements using the techniques atomic absorption spectroscopy, emission spectroscopy, X-ray fluorescence spectroscopy and neutron activation analysis. The range of values given extends from 0.47 to 6.78 μmoles/l. Comparing the results of our method with atomic absorption and emission spectroscopy we observe with one exception[8] that the Rb^+ concentrations reported are generally higher. One possible explanation is matrix effects. For instance, in serum the effects of potassium and sodium ions on rubidium absorbance and an enhanced absorbance due to interference of these cations has been described[9]. It is noteworthy that our values using stable isotopes as internal standards and FD-MS are in good agreement with those obtained with neutron activation analysis and X-ray spectroscopy[13-17]. However, one should consider that for neutron activation[16] 25 ml are reported as the amount of sample (higher by the factor of 250) and a total analysis time of approximately 1000 h, which is more than 3 orders of magnitude longer than with FD-MS.

The potassium concentration in the serum of our volunteers ranged from 3.4 to 6.59 mmoles/l over the whole series, with 95% of the values falling between 3.58 and 5.11 mmoles/l. The normalized frequency distribution is also shown in figure 1. The variance found is much smaller than that for rubidium, and is similar to that given by Iyengar et al.[28] for potassium in serum, 4.09–5.39 mmoles/l, although their weighted average value (4.88 mmoles/l) is higher than our average value of 4.29 mmoles/l.

The variation of sodium concentration in our experiments was found to be only a few percent, from 0.14 to 0.15 moles/l, with an average value of 0.14 moles/l, the same as is found in the literature[28].

The data were also examined for a potassium correlation between corresponding potassium and rubidium serum concentrations. Linear regression ($[K]^+$ against $[Rb]^+$, see fig. 2) gives the relation $[K]^+$ (mg/l) $= 2.55[Rb]^+$ (μg/l) + 3.34, with a correlation coefficient of 0.55. Thus a weak trend towards higher potassium levels with increasing rubidium concentration is found.

We believe that further investigations along these lines could prove useful in the study of certain diseases, such as multiple sclerosis. However, factors such as dietary intake should certainly be taken into consideration. In our previous studies on multiple sclerosis patients[7,20] a group of patients from the same hospital but not suffering from multiple sclerosis gave rubidium concentrations in urine averaging well below the average of the healthy volunteers, although significantly above that for the multiple sclerosis patients. Also, any study on multiple sclerosis patients should be carried out at known stages of the illness and under strict medical supervision.

In general, FD-MS using stable isotope dilution can be regarded as a fast, reliable and definite method for trace and ultratrace analysis of metals. It is superior to the established analytical methods when the specimens available are less than 10 mg and for metal concentrations at the ppb level and below.

1 Present address and to whom reprint requests should be addressed: Fachhochschule Fresenius, Department of Trace Analysis, Dambachtal 20, D-6200 Wiesbaden (Federal Republic of Germany). This work was generously supported by the Umweltbundesamt, Berlin (F+E Vorhaben Nr. 10660013/04) and the Deutsche Forschungsgemeinschaft, Bonn. The authors are grateful to P. Lemke, Institute of Physiology II, for technical assistance and A. Schneider, University Polyclinic, for preparation of serum samples.

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Altered hyperemic response of the coronary arterial bed in alloxan-diabetes

M. Zs. Koltai, M. Wagner and G. Pogátsa[1]

Research Unit, National Institute of Cardiology, P.O.B. 9-88, H-1450 Budapest (Hungary), May 3, 1982

Summary. Reactive hyperemic responses of the coronary arterial bed, provoked by asphyxia or clamping of the coronary artery, were compared in alloxan-diabetic and metabolically healthy dogs. In alloxan-diabetic dogs the response of the coronary arterial bed lasted longer, and its reactivity to hypoxia was lower. Treatment with adenosine caused less vasodilation in diabetic animals than in controls. These changes may be due to the altered reactivity of diabetic vascular smooth muscle.

Ischemic heart diseases[2,3] and cerebrovascular alterations[4-6] are more frequent and severe in cases where diabetes mellitus is present. Biochemical and morphological changes and altered innervation of the coronary arteries may all play an important role in ischemia of the myocardium in diabetes. It has already been demonstrated that the reactivity of the coronary arterial bed to norepinephrine and to electrical stimulation of the cardiac plexus is considerably diminished in animals with diabetes as compared to metabolically healthy animals. These alterations have been detected even in early stages of diabetes when macro- or microangiopathy are not observed[7]. In the present study the reactivity of the coronary arterial bed to hypoxia was investigated during hyperemia provoked in different ways in alloxan-diabetic dogs.

Methods. 26 healthy young mongrel dogs of both sexes, each weighing 14–35 kg, were selected for the study. All received the same diet, consisting of 25% protein, 60% carbohydrate, 15% fat, vitamins and mineral salts ad libitum. 12 dogs were made diabetic using alloxan (560 mmoles/kg i.v., alloxan tetrahydrate, Merck). 14 dogs served as controls. Plasma disappearance rates of glucose[8], plasma glucose[9] and urea nitrogen[10] from venous blood were determined at the beginning of the study and at least once monthly during the observation period, as well as on the day before the hemodynamic investigation, in the fasting state. The glucose[9] and aceton[11] contents of urine collected over 24 h were determined at least once weekly during the observation period.

The hemodynamic investigation was carried out 3 months after the induction of diabetes. Blood flow in the left anterior descending coronary artery was measured continuously by an electromagnetic flowmeter (Godard-Statham, SP 2202) in dogs ventilated with positive pressure under

Metabolic and hemodynamic variables of control and alloxan-diabetic dogs

	Plasma disappearance rate of glucose (μmoles/min)	Plasma glucose (mmoles/l)	Glucose excretion (mmoles/day)	Body weight (kg)	Mean arterial blood pressure (kPa)	Coronary blood flow (ml/min)
Control (n = 14)	18 ± 1	4.96 ± 0.18	0 ± 0	22.1 ± 1.7	13.7 ± 0.7	38.2 ± 11.5
Diabetic animals before alloxan	18 ± 1	5.19 ± 0.23	0 ± 0	21.8 ± 1.0	-	-
After alloxan (n = 12)	7 ± 2[b]	14.35 ± 0.85[b]	496 ± 120[b]	18.8 ± 1.1[b]	17.5 ± 1.7[a]	52.2 ± 9.7

Data are expressed as mean ± SEM. [a]$p < 0.05$; [b]$p < 0.01$.