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# The Study of Elements in Organisms by Neutron Activation Analysis. III.[1] The Distribution of Trace Elements in Various Organs of Normal Rat and in Cell Fractions of Rat Liver

Masaki Shinogi, Kayoko Fukuda, Miyuki Nakazawa, and Itsuhiko Mori

*Kobe Women's College of Pharmacy*[2]

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The use of standard reference materials (SRM's) *i.e.* NBS orchard leaves and bovine liver, as multielemental irradiation standards in NAA is discussed. This technique was applied to the analysis of trace elements in various organs of the rat and in cell fractions of the rat liver. Nondestructive neutron activation analysis was carried out to determine 13 elements (As, Br, Co, Cr, Cs, Fe, K, Mn, Na, Rb, Sc, Se and Zn) in each sample.

High concentrations of several elements were found in some organs of the rat. In particular, the testes contained many elements at high concentrations, while the contents of all elements were highest in the liver. The mean concentrations of elements in various organs of the rat were classified roughly, and the elemental concentrations in cell fractions of the rat liver are discussed. The cytosol contained the greatest quantities of the elements, with the exception of Mn.

**Keywords**——neutron activation analysis; trace element; rat organ; cell fraction; Ge(Li) detector; standard reference material

## Introduction

During the past few years, there has been an increasing realization of the importance of trace elements in biological systems. The essential trace elements in animals have been analyzed and considered by many authors.[3–9] Based on these studies, the roles of trace elements in normal body function have been well elucidated, and the disfunctions due to deficiency or excess of these elements are also well known. It has been suggested that essential trace elements in different organ systems often show normal functions only within narrow limits of their concentrations, and that deviations from the normal concentrations are often signals of disease. Analysis of the distribution patterns of trace elements should aid in elucidating the physiological and pathological roles of those elements.

To expedite these studies on trace elements, highly sensitive analytical methods are necessary. Neutron activation analysis (NAA) with high resolution gamma spectrometry has contributed more to an understanding of the role of trace elements in biological materials than any other technique.

In this work, the distributions of trace elements in various organs of the rat and in cell fractions of the rat liver were determined using NAA.

1) Part II: M. Shinogi and I. Mori, *Yakugaku Zasshi*, **98**, 596 (1978).
2) Location: *Motoyama-kitamachi, Higashinada-ku, Kobe.*
3) M.P. Siegers, K. Kasperek, H.J. Heiniger, and L.E. Feinendegen, *J. Radioanal. Chem.*, **37**, 421 (1977).
4) K. Kostič, R.J. Draškovič, M. Ratkovič, D. Kostič, and R.S. Draškovič, *J. Radioanal. Chem.*, **37**, 405 (1977).
5) B. Maziere, C. Loc'h, O. Stulzaft, A. Gaudry, and D. Comar, *J. Radioanal. Chem.*, **37**, 617 (1977).
6) M. Persigehl, H. Schicha, K. Kasperek, and L.E. Feinendegen, *J. Radioanal. Chem.*, **37**, 611 (1977).
7) R. Schelenz, *J. Radioanal. Chem.*, **37**, 539 (1977).
8) E.J. Underwood, "Trace Elements in Human and Animal Nutrition," 3rd ed., Academic Press, Inc., New York, 1971.
9) H.J.M. Bowen, "Trace Elements in Biochemistry," Academic Press, Inc., New York, 1966.

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Nadkarni and Morrison[10] proposed that standard reference materials (SRM's), usually applied to check the accuracy and precision of analytical methods or for interlaboratory comparisons, are convenient for use as irradiation standards in NAA, simplifying the procedure and eliminating errors inherent in the preparation of a large number of synthetic standards at the trace element level.

In the present study, well characterized SRM's, *i. e.* NBS orchard leaves and bovine liver, were used as multielemental irradiation standards for the various elements.

## Experimental

**Preparation of the Samples**——In the study of the distribution of trace elements in various organs of the rat, three male albino rats of the Wistar strain, each weighing approximately 250 g, were used. After decapitation, their brain, heart, lungs, thymus, spleen, liver, kidneys, adrenals, testes and muscle were removed and immediately weighed. These tissue samples were washed with distilled water. Blood samples were also collected at the same time. The samples were lyophilized and subjected to NAA.

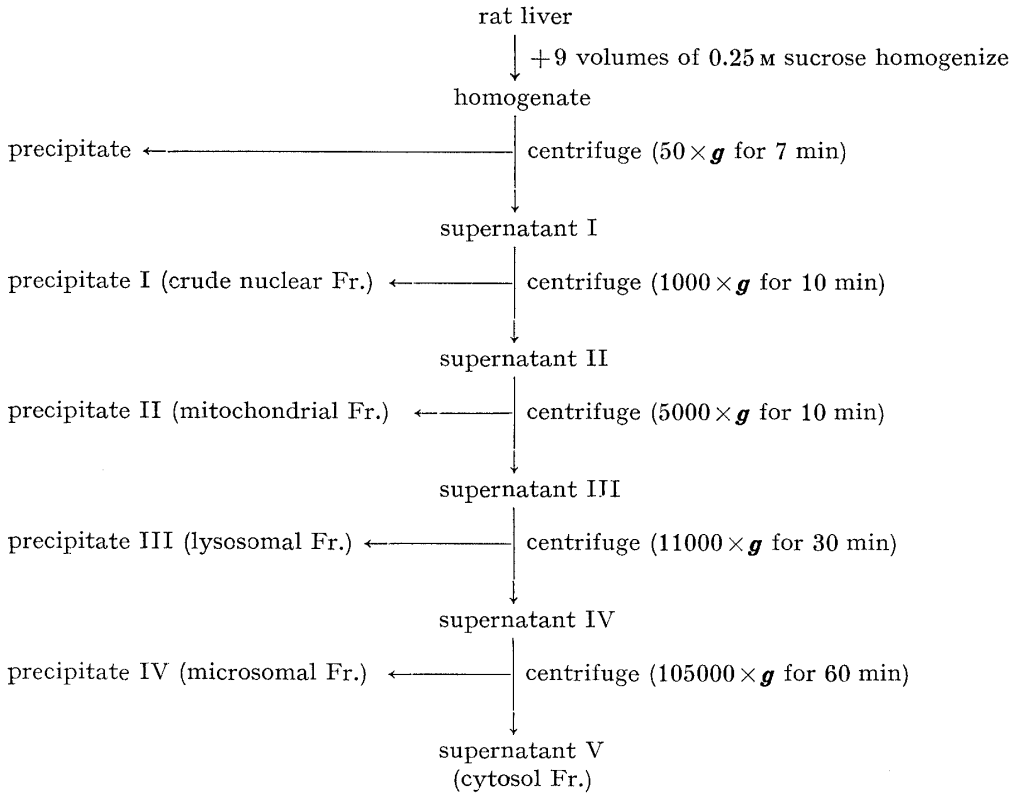


Fig. 1. Cell Fraction Separation Scheme

On the other hand, other liver samples (approximately 20 g), were washed and cut into small strips. Using a teflon homogenizer, the liver strips were homogenized in 9 volumes of 0.25M sucrose solution under cooled conditions. The homogenate was fractionated into nuclear, mitochondrial, lysosomal, microsomal and cytosol fractions by the method of de Duve *et al.*[11] (Fig. 1). To remove heavy particles, the homogenate was centrifuged at $50\times g$ for 7 min to yield supernatant I. The crude nuclear fraction, residue I, was separated from supernatant I by centrifugation at $1000\times g$ for 10 min. Supernatant II was centrifuged at $5000\times g$ for 10 min. After the removal of residue II (mitochondrial fraction), supernatant III was centrifuged at $11000\times g$ for 30 min to yield the lysosomal fraction, residue III. Further, supernatant IV was ultracentrifuged at $105000\times g$ for 60 min, and the microsomal fraction was separated as residue IV from supernatant V (the cytosol fraction). All these centrifugations were done at 4°. The five fraction samples were lyophilized and subjected to NAA together with the organ samples.

10) R.A. Nadkarni and G.H. Morrison, *J. Radioanal. Chem.*, **43**, 347 (1978).
11) C. de Duve, R. Wattiaux, and P. Buadhuim, *Adv. Enzymol.*, **24**, 291 (1962).

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**Preparation of the Standards**——NBS bovine liver (SRM-1577) and orchard leaves (SRM-1571) were used as multielemental irradiation standards. A synthetic multielemental standard was also prepared by soaking a clear filter paper (2×2 cm) in a mixed standard solution. This synthetic standard was also used for cross-checking the SRM's. Aluminum foil containing (0.1%) cobalt was used to correct for neutron flux fluctuation.

The samples and standards were dried in air in an oven at 85° for 4 hours prior to irradiation, and 100 mg of each dried sample was packed in double clean polyethylen bags for irradiation.

**Neutron Irradiation and Gamma-ray Spectrometry**——Neutron irradiation of samples and standards contained in polyethylene capsules was carried out for one hour using the pneumatic tube facility in the Kyoto University reactor with a thermal neutron flux of $1.93\times10^{13}$ n·cm$^{-2}$·sec$^{-1}$. The irradiated samples and standards were counted without any chemical separation at fixed geometry with a coaxial Ge(Li) detector (Harshaw) coupled to a 4096 channel pulse-height analyzer (Northern Scientific Inc. NS-720). The system resolution was 2.0 keV (FWHM) and the peak-to-compton ratio was 40: 1, both for the 1.332 MeV peak of $^{60}$Co.

For the measurement of $^{56}$Mn, the samples were counted for 300 sec after a decay of 3 hours. The medium-lived nuclides, such as $^{24}$Na, $^{42}$K, $^{76}$As and $^{82}$Br were measured by counting for 1000 sec with a 2- to 3-day cooling period after irradiation. For measurements of long-lived nuclides, such as $^{46}$Sc, $^{51}$Cr, $^{59}$Fe, $^{60}$Co, $^{65}$Zn, $^{75}$Se and $^{86}$Rb, counting was performed for 10000 sec after cooling for 30 days. Typical gamma-ray spectra of the liver sample obtained from these measurements are shown in Fig. 2.

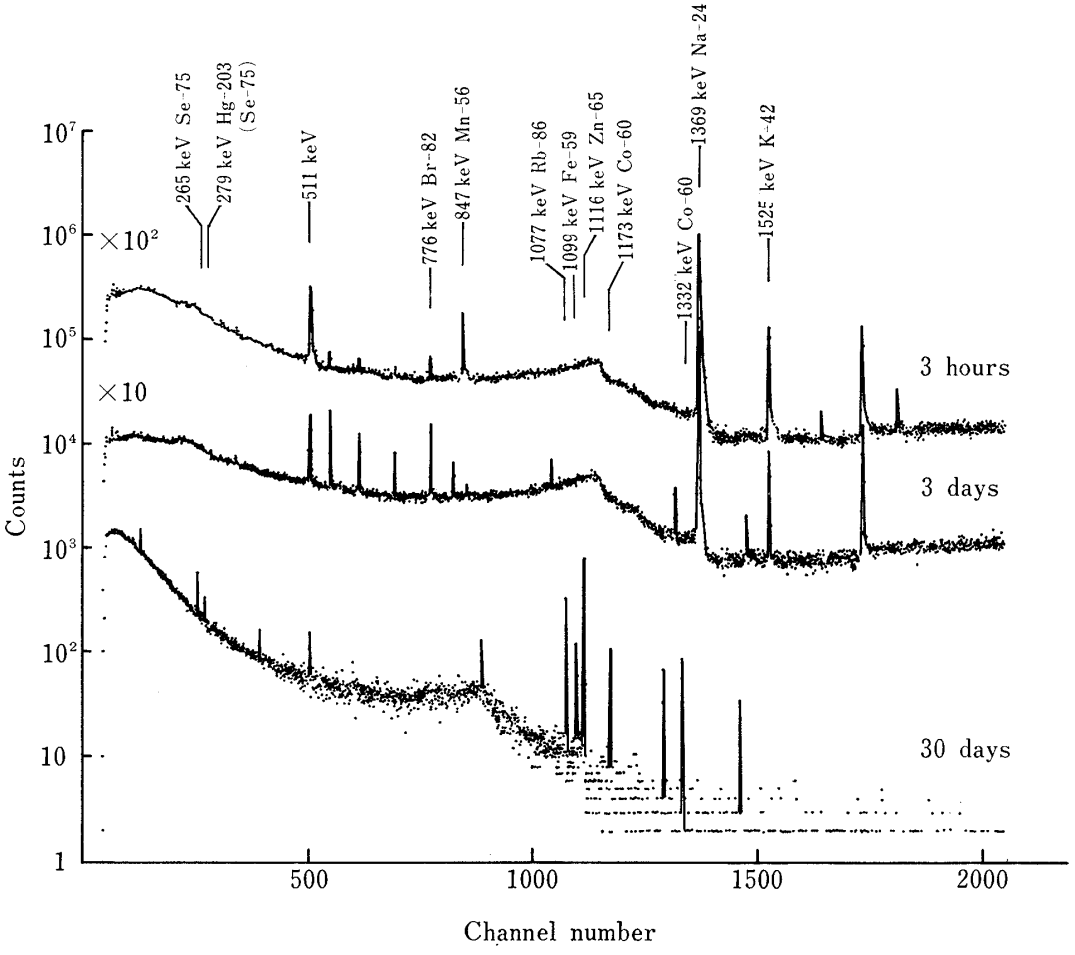


Fig. 2. Typical Gamma-ray Spectra of Rat Liver irradiated for One Hour and counted for 300, 1000 and 10000 Seconds after Decays of 3 Hours, 3 Days and 30 Days, respectively

## Results and Discussion

### Use of SRM's as Irradiation Standards

Thirteen elements in the NBS SRM's, orchard leaves and bovine liver, were analyzed by the NAA procedure using synthetic standards. The results obtained were compared

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Table I. Analysis of NBS Standard Reference Materials

| Nuclide | Half-life | $\gamma$-Ray energy (keV) | SRM[a] | Number of determinations | This study[b] (ppm) | Literature value[c] (ppm) |
|---|---|---|---|---|---|---|
| $^{76}$As | 26.5 hr | 559 | O.L. | 6 | 10.4±0.9 | 10.0±2.0 |
| $^{82}$Br | 35.3 hr | 776 | B.L. | 6 | 8.4±1.5[d] | 8.9±2.1** |
| $^{60}$Co | 5.26 y. | 1173 | B.L. | 6 | 0.27±0.03 | 0.24±0.04** |
| $^{51}$Cr | 27.8 day | 320 | O.L. | 6 | 2.6±0.6 | 2.6±0.3 |
| $^{134}$Cs | 2.05 y. | 796 | O.L. | 6 | 0.030±0.009 | 0.04 |
| $^{59}$Fe | 45.0 day | 1099 | B.L. | 6 | 290±20 | 268.0±8.0 |
| $^{42}$K | 12.4 hr | 1525 | B.L. | 6 | 11700±800 | 9700±600 |
| $^{56}$Mn | 2.58 hr | 847 | B.L. | 6 | 7.3±1.0 | 10.3±1.0 |
| $^{24}$Na | 15.1 hr | 1369 | B.L. | 6 | 2400±100 | 2430±130 |
| $^{86}$Rb | 18.7 day | 1077 | B.L. | 6 | 19.3±2.6 | 18.3±1.0 |
| $^{46}$Sc | 83.9 day | 889 | O.L. | 6 | 0.077±0.005 | 0.057±0.0063** |
| $^{76}$Se | 120.4 day | 265 | B.L. | 6 | 1.17±0.28 | 1.1±0.1 |
| $^{65}$Zn | 245 day | 1115 | B.L. | 6 | 147±8 | 130±13 |

*a*) SRM's are NBS-1577 bovine liver (B.L.) and NBS-1571 orchard leaves (O.L.).
*b*) Determination value, average and standard deviation, by the synthetic standards method.
*c*) Data with standard deviation certified by NBS. Other values, marked **, are taken from Hamaguchi *et al.* (*Bunseki Kagaku*, **26**, (1977) T23).
*d*) Analyzed using NBS orchard leaves as a standard.

with the NBS-certified and literature[12] values in Table I. Excellent agreement between our values and the literature values can be seen for most elements. These results validated the present experimental techniques, such as irradiation time, cooling time, counting time, *etc.*

On the other hand, discrepancies may be seen for some elements. In particular, for bromine in bovine liver, we obtained a value of 80±14 ppm with KBr as a standard. This value is about 9 times the literature value. The reason for this discrepancy may be the volatilization of bromine from the standard during neutron irradiation. The same phenomenon occurs in the case of mercury.[13] The value of bromine in bovine liver obtained by using orchard leaves as an irradiation standard is shown in Table I. The value was in good agreement with the literature value.[12] Therefore, it may be concluded that it is convenient to use the SRM's as irradiation standards.

In this study, bovine liver was generally used as a standard, and orchard leaves were used only for As, Cr, Cs and Sc, because the concentrations of these elements in bovine liver are lower than in orchard leaves.

## Distribution of Trace Elements in Various Organs of the Rat

Thirteen elements (shown in Table II) were determined in various organs of the rat and in cell fractions of the rat liver. Means and standard deviations for each element were calculated from 3 determinations.

As shown in Table II, the present study indicates increased levels of the following elements in some organs of the rat: Na in testes and blood, Zn in testes, Se in kidney, adrenals and testes, K in thymus, spleen and testes, Fe in spleen and blood, Br in lung, testes and blood, Rb in thymus, spleen and testes, Cs in kidney, adrenals, testes, and muscle, Co in liver, kidney and adrenals, etc. In particular, many elements such as Br, Cs, K, Na, Rb, Se and Zn are present in the testes at high concentrations.

12) H. Hamaguchi, Y. Numata, S. Iwata, M. Koyama, K. Sasajima, Y. Katayama, T. Takeuchi, M. Shinogi, T. Mamuro, Y. Kusaka, H. Tsuji, Y. Tamari, T. Sagawa, S. Ohmori, S. Nagatsuka, Y. Tanizaki, T. Suzuki, K. Tomura, Y. Hashimoto, S. Bando and T. Imahashi, *Bunseki Kagaku*, **26**, T23 (1977); T. Takeuchi and M. Shinogi, *Radioisotopes*, **28**, 729 (1979).
13) T. Takeuchi, M. Shinogi, and I. Mori, *J. Radioanal. Chem.*, **53**, 81 (1979).

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TABLE II. Elemental Concentrations in Rat Organs in ppm (Dried Tissue)

| Elment | Brain (341) | Heart (164) | Lung (202) | Thymus (107) | Liver (2970) | Spleen (114) | Kidney (429) | Adrenals (9.7) | Testes (341) | Muscle (—) | Blood (—) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| As | ND | ND | 1.39±0.21 | ND | 1.58±0.36 | 3.1±0.3 | ND | ND | ND | ND | 19.4±2.8 |
| Br | 32±3 | 44±6 | 100±6 | 5.6±1.7 | 41±4 | 72±3 | 67±4 | 35±1 | 162±22 | 25±2 | 165±16 |
| Co | 0.042 ±0.018 | 0.172 ±0.028 | 0.081 ±0.020 | ND | 0.38±0.12 | 0.109 ±0.033 | 0.84±0.12 | 0.54±0.39 | 0.037 ±0.007 | 0.036 ±0.026 | 0.032 ±0.024 |
| Cr | ND | ND | ND | ND | ND | 0.42±0.18 | ND | ND | ND | 0.27±0.10 | ND |
| Cs | 0.0159 ±0.0051 | 0.037 ±0.005 | 0.028 ±0.010 | 0.051 ±0.031 | 0.036 ±0.018 | 0.046 ±0.007 | 0.066 ±0.014 | 0.069 ±0.020 | 0.086 ±0.020 | 0.077 ±0.013 | ND |
| Fe | 63±9 | 240±30 | 310±30 | 65±30 | 260±40 | 770±20 | 192±23 | 240±60 | 116±9 | 58±4 | 2100±100 |
| K* | 1.43±0.09 | 1.09±0.20 | 1.10±0.18 | 2.1±0.2 | 1.08±0.03 | 1.86±0.14 | 0.93±0.09 | 1.11±0.33 | 2.3±0.4 | 1.52±0.10 | 0.86±0.23 |
| Mn | ND | 0.117 ±0.025 | ND | 1.04±0.20 | 6.3±1.5 | 0.097 ±0.033 | 2.32±0.03 | 13.3±3.9 | ND | 0.37±0.15 | ND |
| Na | 4500±200 | 3500±200 | 4300±300 | 2700±400 | 1780±170 | 2300±300 | 4100±200 | 2000±100 | 7400±500 | 1640±40 | 8800±500 |
| Rb | 17.0±3.3 | 27±1 | 30±1 | 44±3 | 31±13 | 43±1 | 32±3 | 21±7 | 67±9 | 33±2 | 15.0±2.4 |
| Sc | ND | 0.00128 ±0.00064 | ND | ND | ND | ND | 0.00140 ±0.00094 | 0.0096 ±0.0035 | ND | 0.0057 ±0.0039 | ND |
| Se | 0.63±0.27 | 1.73±0.32 | 1.65±0.08 | 1.35±0.21 | 2.6±0.6 | 2.2±0.9 | 4.4±0.3 | 3.58±0.04 | 5.5±0.1 | 0.66±0.13 | 2.3±0.1 |
| Zn | 64±13 | 72±2 | 97±9 | 88±15 | 88±15 | 96±12 | 83±4 | 61±10 | 157±3 | 49±3 | 22±3 |

*; %, ND; not detected, ( ); mean tissue weights of three rats (mg).

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TABLE III. Elemental Concentrations in Cell Fractions of Rat Liver in ppm

| Element | Nuclear Fr. | Mitochondrial Fr. | Lysosomal Fr. | Microsomal Fr. | Cytosol Fr. |
|---|---|---|---|---|---|
| As | 1.26±0.38 (0.43) | 0.46±0.06 (0.066) | 0.21±0.12 (0.0169) | 0.48±0.28 (0.26) | 0.129 (1.81) |
| Br | 0.62±0.09 (0.21) | 0.79±0.47 (0.113) | 0.47±0.25 (0.037) | 1.47±0.32 (0.79) | 3.9±1.2 (55) |
| Co | 0.131±0.019 (0.045) | 0.40±0.03 (0.057) | 0.189±0.046 (0.0150) | 0.068±0.022 (0.037) | 0.029 (0.41) |
| Cr | ND | 0.32 (0.45) | 0.50±0.32 (0.039) | ND | ND |
| Fe | 128±44 (44) | 93±16 (13.3) | 140±20 (11.1) | 100±12 (54) | 22±9 (310) |
| K | 1430±510 (490) | 2100±400 (290) | 530±160 (42) | 1350±100 (730) | 1710±160 (24000) |
| Mn | 9.8±3.2 (3.3) | 26±2 (3.7) | 2.6±1.6 (0.20) | 3.2±0.9 (1.74) | 0.20±0.05 (2.8) |
| Na | 77±23 (26) | 42±20 (6.0) | 88±19 (7.0) | 210±30 (111) | 280±60 (3900) |
| Rb | 3.6±1.6 (1.22) | 10.2±2.8 (1.46) | 4.1 (0.32) | 6.1±2.8 (3.3) | 10.0±2.9 (139) |
| Se | 1.25±0.07 (0.43) | 1.95±0.89 (0.28) | 0.32±0.12 (0.026) | 1.33±0.38 (0.72) | 0.23±0.04 (3.2) |
| Zn | 41±12 (14.0) | 17.7±7.7 (2.5) | 17.6±12.9 (1.40) | 41±9 (22) | 12.3±2.4 (172) |

ND; not detected, ( ); total content ($\mu$g).

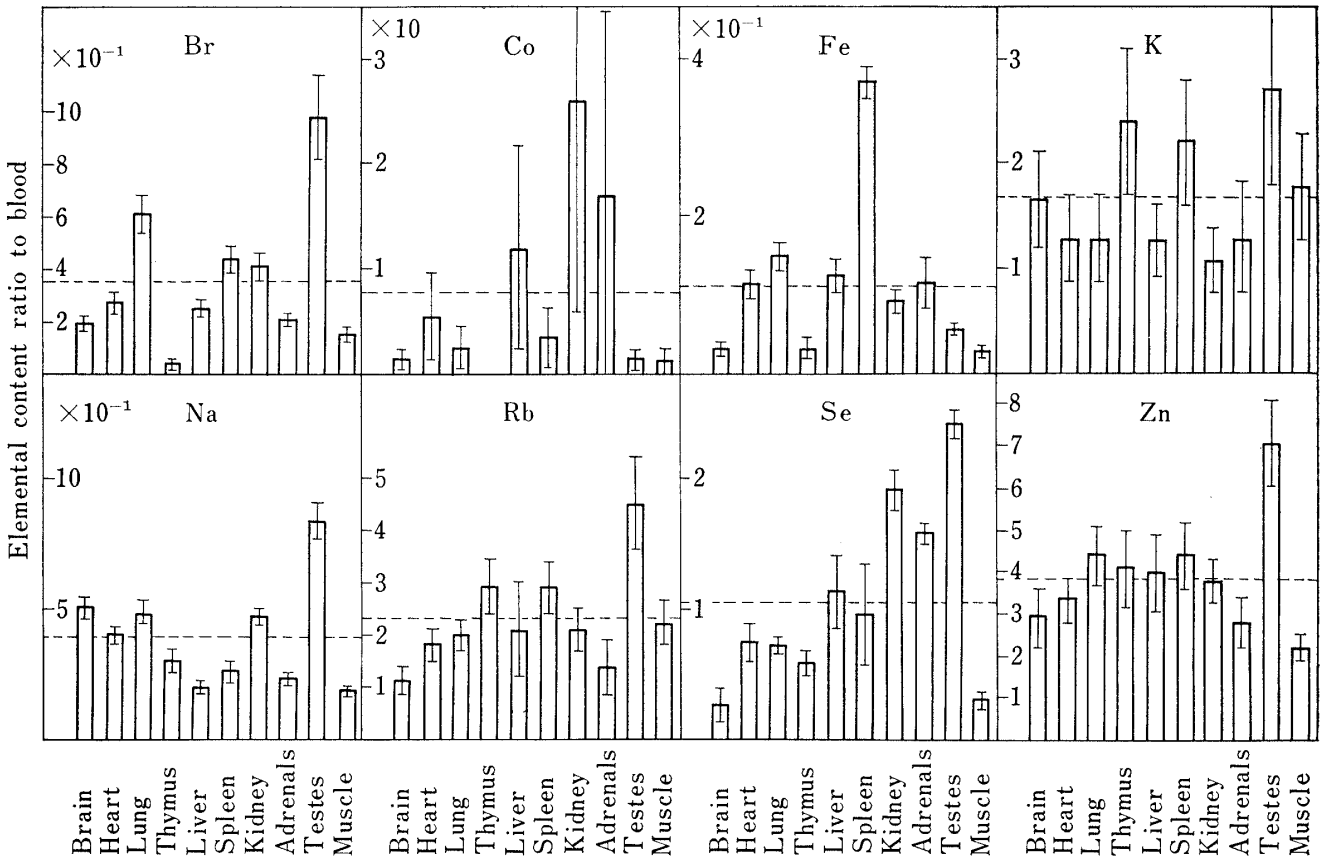


Fig. 3. Elemental Content Ratio of Some Organs of Rat relative to Blood

The heights of the columns and the vertical lines represent mean values and standard deviations, respectively. Broken lines indicate the mean content ratio in all organs.

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The orders of magnitude of mean concentrations of elements in various organs of the rat may be classified roughly as follows: K $10^4$ ppm, Na $10^3$ ppm, Fe $10^2$ ppm, Br, Rb and Zn 10 ppm, As, Mn and Se 1 ppm, Co and Cr $10^{-1}$ ppm, Cs $10^{-2}$ ppm, Sc $10^{-3}$ ppm.

The elemental concentration ratios of organs to blood are shown in Fig. 3. The values for Br, Fe and Na are less than 1.0, but those for the other elements are greater than 1.0. The concentration ratios for Co and Fe exhibit remarkably high values in the kidney, adrenals, and spleen. In the testes, high concentration ratios were found in most elements, except for Co and Fe.

The liver, the largest organ, contained the greatest quantities of the all the elements observed.

The elemental concentrations in cell fractions of the rat liver are given in Table III. The concentrations of alkaline metals such as K, Na and Rb, and Br are highest in the cytosol fraction. The cytosol fraction contained the greatest quantities of the elements observed, with the exception of Mn. The Mn content in the cytosol relative to the whole liver fraction is 24%, while those of the other elements are about 70% (80% for Zn).

In this study, the separation method for cell fractions of the rat liver employed only sucrose. If other separation methods are used, the elemental concentrations in cell fractions of the rat liver may be changed.

Thus, NAA was applid to various organs of the rat to determine 13 elements in each sample, and the distribution patterns of these elements in various organs of normal rat were defined. Deviations of trace elements from the normal distribution patterns may be signals of diseases, so a knowlege of the distribution patterns of trace elements should aid in elucidating the physiological and pathological roles of these elements.

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