SCI. REP. KYOTO PREF. UNIV. (NAT. SCI. & LIV. SCI.) No. 31, Ser. B, p. 13~21 (NOV. 1980)

Selenium Levels in Human Erythrocyte, Saliva, Milk, Urine and Nail

YASUJI HOJO

(Received July 28, 1980)

Selenium, protein (or related substances) and lipid concentrations were measured for human erythrocyte, saliva, colostral and mature milk, urine and nail samples. Low negative correlation coefficients were produced between selenium and protein concentrations in saliva and mature milk and between selenium and lipid concentrations in mature milk. In erythrocyte, colostral milk and urine, high positive correlations were found between selenium and protein (or related substances). In all individual tissues examined, high (low) and positive (negative) correlation coefficients between selenium and protein (or related substances, lipid) concentrations corresponded thoroughly to lower (higher) variation coefficients of average selenium levels expressed as content per content of tissue protein (or related substances, lipid) than that per tissue volume. When selenium levels were expressed in content per protein (or related substances) content, urine selenium produced high negative correlations with erythrocyte and saliva selenium, and high positive correlation coefficient with colostral milk selenium. High positive correlation coefficient was also found between erythrocyte and saliva selenium. Average selenium levels decreased in the order, nail > erythrocyte > urine > colostral milk > mature milk > saliva when expressed in content per tissue volume (or weight), and, in the order, urine > saliva > mature milk > colostral milk > erythrocyte when expressed in content per tissue protein (or related substances) content. Selenium levels for individual tissues examined were considerably similar to values from literatures. These results give a support to essential function of selenium element for human beings.

Introduction

Toxic effects from excess selenium have been recognized even longer, and the essentiality of selenium for life is well established for mammals. Although it is difficult to assess whether selenium functions as a micronutrient for man, several lines of reasoning from existent data suggest it [1]. This element is known to modify profoundly the toxicity of both organic and inorganic mercury compounds [2]. The protective action of selenium salts against various reactions initiated by AFB_1 is also important [3]. Selenium has been linked to the human diseases of cancer [4], muscular distrophy [5], infant death [6], dental caries [7] and teratogenicity [8].

In assessing the role of selenium in human health and disease, it is fundamental to investigate the selenium status in various tissues of human body and to examine the relations of selenium levels among different tissues.

This paper is a report for (1) relation between selenium and the tissue protein (or related substances) concentrations, (2) relation of selenium concentrations between different combinations of tissues, and (3) comparison of the selenium levels expressed as content per tissue

Laboratory of Food Hygiene, Department of Food Science, Faculty of Living Science, Kyoto Prefectural University, Shimogamo, Kyoto 606, Japan

protein (or related substances) content and per tissue volume, in human erythrocyte, saliva, milk, urine and nail samples.

Materials and Methods

Materials: All samples for selenium determination came from normal humans. Four volumes of a venous sample was collected into one volume of acid citrate dextrose (ACD) solution. Hemoglobin concentration was measured spectrophotometrically as cyanmethemoglobin [9]. The blood solution prepared as mentioned above was mixed with 10 volumes of 0.154 M KCl and centrifuged at $1000 \times g$ for 10 minutes. The cell was resuspended in the same volume of KCl and hemolyzed by diluting with 6 volumes of distilled deionized water [10]. Hemolysate from 1 ml of blood sample was used for selenium determination. From six of ten persons who offered blood samples, saliva and single urine samples were obtained. Mature milk from nine mothers at three months postpartum was analyzed for selenium and lipid. First secreted colostral milk and urine samples were taken from five mothers, and one of them offered milk and urine samples between 35 and 85 days postpartum. Milk samples from the five mothers were measured for selenium and protein. All the chemicals used were of reagent grade.

Methods: The spectrofluorimetric method with 2,3-diaminonaphthalene was used for selenium determination [11]. Sample volumes used were 4, 2, and 2 ml for saliva, milk and urine samples, respectively. The protein was determined by Lowry method [12], when milk and saliva samples were diluted to 500 and 20 volumes, respectively. The creatinine in urine and the lipid in milk were determined by Folin-Wu method [13] and Roese-Gottliebe method, respectively. Fluorimetric and absorbance measurements were made with a model 204 Hitachi spectrofluorimeter and a model 139 Hitachi spectrophotometer, respectively.

Results

Selenium and hemoglobin concentrations in erythrocyte produced positive and statistically significant correlations ($r=0.70$, $p<0.05$), as found in Fig. 1. Unlike it, Fig. 2 shows that selenium concentrations in saliva are not found to have significant relationship with protein concentrations. Selenium levels in erythrocyte expressed in content per content of hemoglobin have a tendency to increase with increasing saliva selenium levels per protein content (Fig. 3). As the methods to estimate selenium level in both single and 24-hr urines, the expression in content per creatinine content was shown to be much better than that per volume by the author [14]. Fig. 4 represents good correlations ($r=0.82$, $p<0.05$) between selenium and creatinine contents per urine volume, using single urine samples taken from six persons who offered saliva and erythrocyte samples. This observation gives again a validity to the expression of urine selenium levels in the form of content per creatinine content. In Fig. 5, selenium levels in erythrocyte and saliva expressed in content per content of hemoglobin and protein, respectively, have a tendency to decrease with increasing selenium levels in urines per creatinine content. Saliva had always higher selenium levels than erythrocyte at every urine selenium level.

In mature milk, selenium concentrations produced low negative correlations with lipid (Fig. 6) and protein (Fig. 7) concentrations. Contrary to these observations, selenium concentrations increase with protein concentrations in the first secreted colostral milk (Fig. 7). For urines collected from the mothers who offered milk samples, Fig. 8 represented correlation

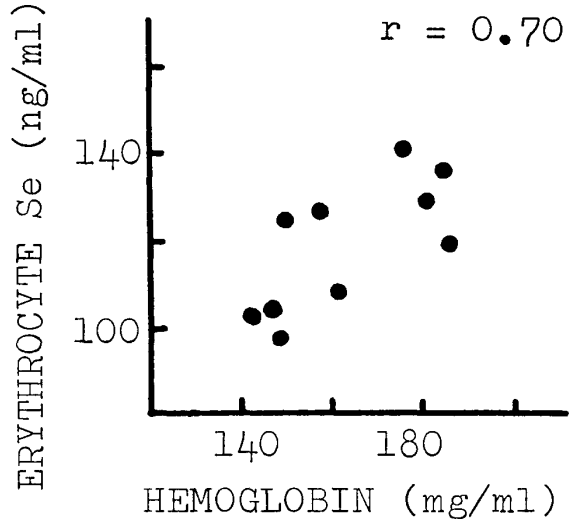

Fig. 1. Individual selenium concentration plotted against hemoglobin concentration in erythrocyte. Both variables are expressed as values for erythrocyte contained in 1 ml of blood sample.

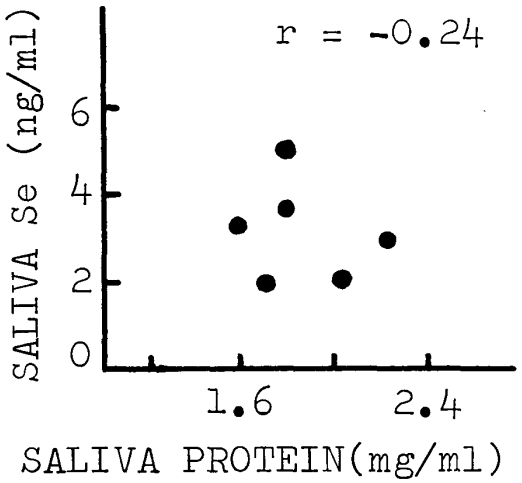

Fig. 2. Individual selenium concentration plotted against protein concentration in saliva.

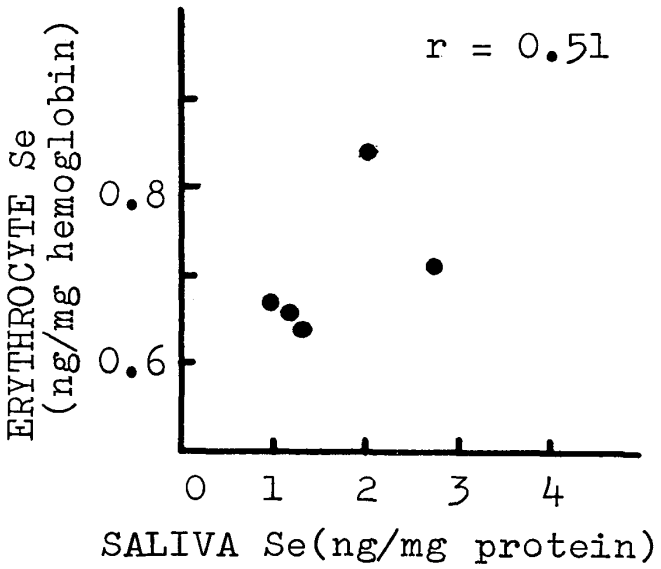

Fig. 3. Individual selenium level in erythrocyte plotted against that in saliva.

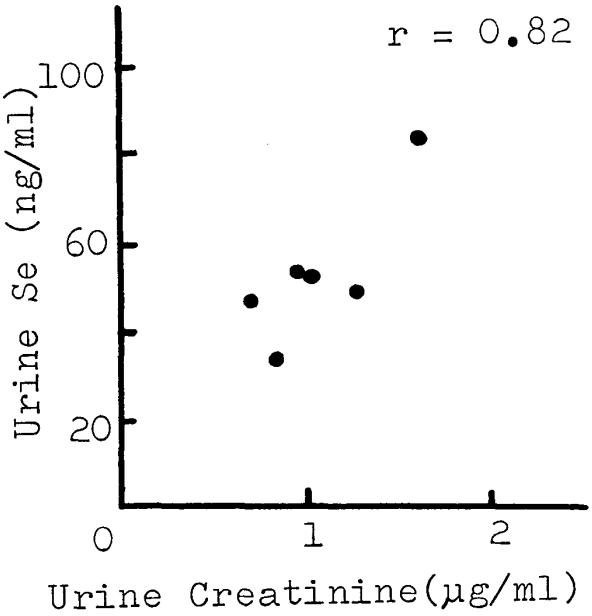

Fig. 4. Individual selenium concentration plotted against creatinine concentration in urine collected from six persons who offered saliva and erythrocyte samples.

between selenium and creatinine concentrations. Fig. 9 shows that the urine selenium levels expressed per creatinine contents increase with the selenium levels expressed per protein contents in the first colostral milk and have no correlation with that in the mature milk collected from one mother between 35 and 85 days postpartum.

The average, range and variation coefficient of selenium levels in human erythrocyte, saliva, milk, urine and nail samples are summarized in Table 1. The average selenium levels expressed in the form of content per tissue volume or tissue weight decreased in the order, nail > erythrocyte > urine > first colostral milk > mature milk > saliva, even if considering that the selenium levels in erythrocyte were expressed as that in the erythrocyte contained in 1 ml of blood samples. When the selenium levels were expressed as content per tissue protein (or related substances) content, they were in the order, urine > saliva > mature

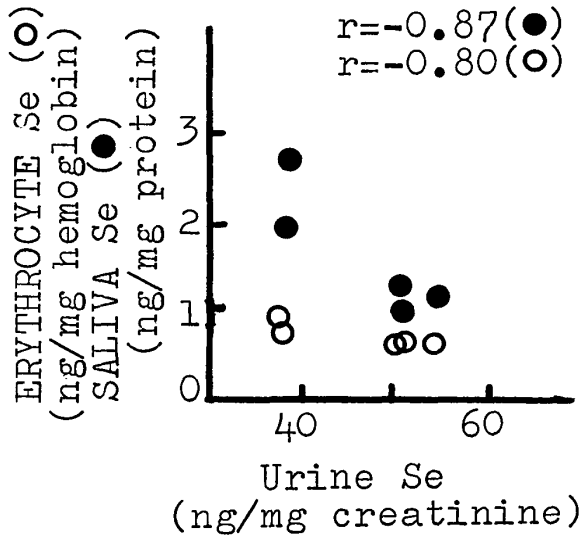

Fig. 5. Individual selenium level in erythrocyte and saliva plotted against that in urine.

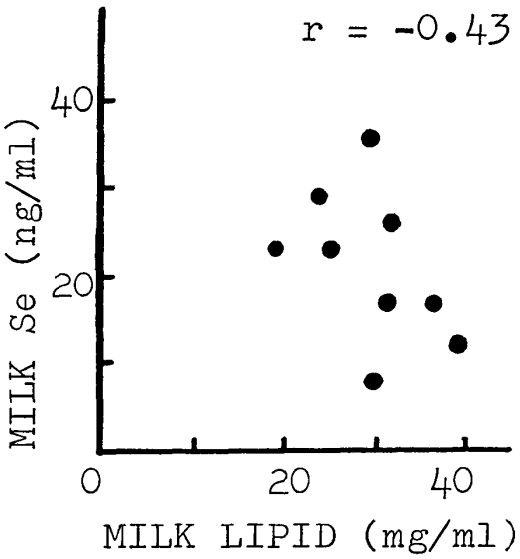

Fig. 6. Individual selenium concentration plotted against lipid concentration in mature milk.

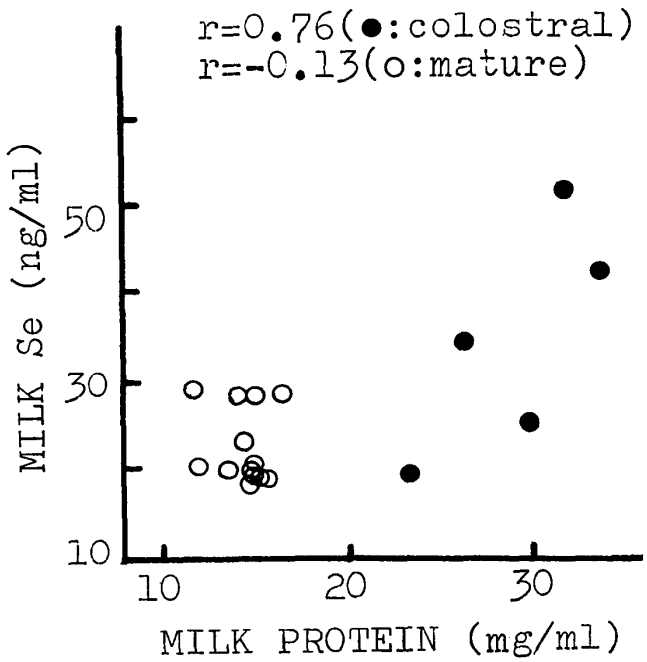

Fig. 7. Individual selenium concentration plotted against protein concentration in first colostral milk taken from five mothers and in mature milk taken from one mother between 35 and 85 days postpartum.

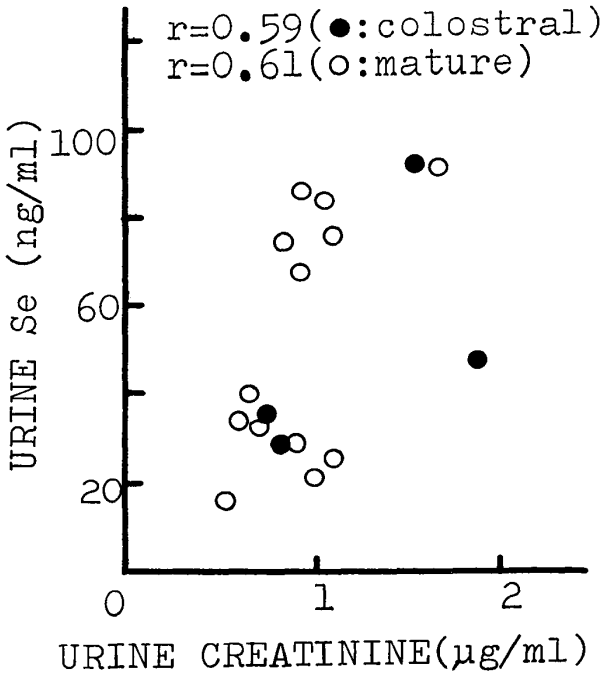

Fig. 8. Individual selenium concentration plotted against creatinine concentration in urine collected from four mothers who offered first colostral milk and from one mother between 35 and 85 days postpartum.

milk > first colostral milk > erythrocyte. These two orders for the tissue selenium levels were opposite in direction except for urine.

In the individual tissues, the high (low) and positive (negative) correlation coefficients between selenium and protein (or related substances, lipid) concentrations were always accompanied by the lower (higher) variation coefficients of the average selenium levels expressed in content per content of tissue protein (or related substances, lipid) than that per tissue volume (Figs. 1, 2, 4, 6, 7 and 8, Table 1).

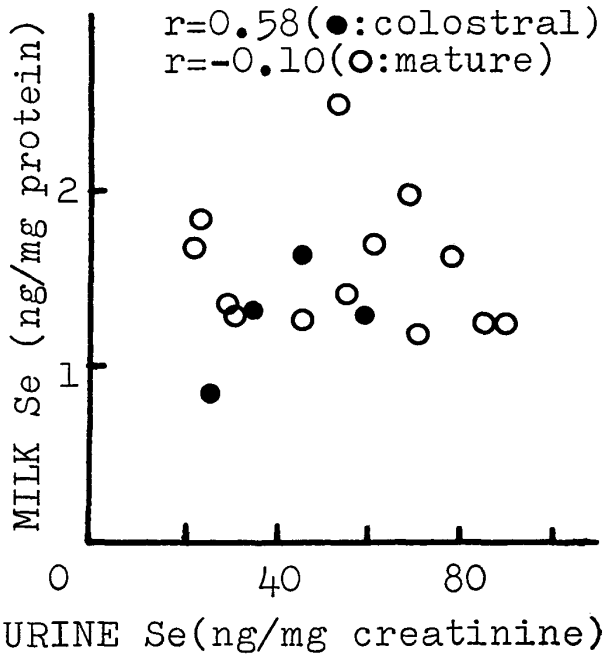

Fig. 9. Individual selenium level in first colostral milk taken from four mothers and in mature milk taken from one mother, plotted against urine selenium level.

Table 1. Selenium Levels in Human Erythrocyte, Saliva, Milk, Urine and Nail Samples

Sample	Mean±SD	Range	Coefficient of variation*	No. of samples
Erythrocyte (ng/ml)	118.8±14.7	98-140	12.4	10
Erythrocyte (ng/mg hemoglobin)	0.7280±0.0656	0.640-0.839	9.0	10
Saliva (ng/ml)	3.62±1.10	2.0-4.9	30.4	10
Saliva (ng/mg protein)	1.720±0.679	0.99-2.75	39.5	6
First secreted colostral milk (ng/ml)	34.2±12.8	19-51	37.4	5
First secreted colostral milk (ng/mg protein)	1.154±0.329	0.82-1.59	28.5	5
Mature milk (ng/ml)	21.2±8.7 22.5±4.2**	8-36 18-29	41.0 18.7	9 13
Mature milk(ng/mg lipid)	0.772±0.392	0.27-1.22	50.8	9
Mature milk (ng/mg protein)	1.573±0.371**	1.22-2.48	23.6	13
Single urine (ng/ml)	51.7±20.8*** 52.1±28.1**	28-92 16-92	40.2 53.9	10 13
Single urine (ng/mg creatinine)	46.6±12.5*** 55.6±23.6**	25-67 21-91	26.8 42.4	10 13
Nail (ng/g)	1005.0±288.0	656-1545	28.7	7

* Values defined as (SD/Mean)×100

** Samples taken from one mother between 35 and 85 days postpartum

*** Samples taken from four mothers who offered colostral milk samples and from six persons who offered saliva and erythrocyte samples

Discussion

In most biological material, selenium is found largely in the protein fraction [1]. Consequently, it seems probable that selenium status in animal tissues can be assessed more exactly in its content expressed per tissue protein (or related substances) content than that per tissue volume (or weight). In the present study, the correlation coefficients between selenium and protein (or related substances) concentrations were high positive for erythrocyte (Fig. 1), colostral milk (Fig. 7) and urine (Figs. 4 and 8), and were low negative for saliva (Fig. 2) and mature milk (Fig. 7). These results do not always support the above inference. The cause of low correlation for saliva and mature milk is not clear. For mature milk, moreover, selenium concentrations were also not correlated with lipid concentrations (Fig. 6). Anything has never been known about the selenium status in lipid tissues. For all individual tissues examined when the correlation coefficients between the selenium and protein (or related substances, lipid) concentrations were high (low) and positive (negative), then the variation coefficient of the average selenium levels expressed as content per content of tissue protein (or related substances, lipid) were lower (higher) than that per tissue volume. This result seems statistically reasonable, for the variation coefficient is a index representing the variation of a variate in a population and can be used effectively to compare the degrees of variations between different kinds of variates.

The relations of selenium status among different tissues have been hardly investigated, particularly for human beings. Human urinary selenium excretion should, in most cases, be the best measure of dietary intake of the element [1]. In New Zealand where widespread areas of selenium deficiency are well known to exist [15], subjects who ingest selenium supplements show an initial rapid rise in plasma selenium up to plateau followed by a slower increase in erythrocyte selenium [16]. Thus plasma selenium is a sensitive index of short-term selenium status, while erythrocyte selenium gives a long-term index of selenium status because of the long life span of these cells. In Venezuela, both blood selenium and urinary selenium in a children group living in a seleniferous area were much higher than that in a control group [17]. A linear correlation was also found between dietary selenium intake and the levels of selenium in the whole blood of ten American subjects [18]. These observations are quite different from the result in the present study that erythrocyte selenium levels vary inversely as urinary selenium levels as found in Fig. 5. This is partly because in this report the tissue selenium levels are expressed in content per the tissue protein (or related substances) content but not per the tissue volume used by the above authors. Animal experiments have demonstrated that retention of a dose of ^{75}Se is inversely related to the dietary selenium level [19]. This result is in agreement with that from the present study (Fig. 5), for retention of selenium is reflected in the whole blood, plasma and erythrocyte selenium levels. The relations of selenium levels between blood and urine remain to be investigated. Nothing has been known about how saliva selenium level varies with the dietary selenium intake, the exposure to selenium and the erythrocyte selenium level. Like erythrocyte, saliva selenium was correlated inversely with urine selenium (Fig. 5). Therefore, as seen from Fig. 3, saliva selenium level increased with increasing erythrocyte selenium level. The function of selenium in saliva remains to be investigated. Contrary to saliva and erythrocyte, colostral milk increased its selenium levels with increasing urine selenium level (Fig. 9). In this case, urine selenium may reflect retention level of selenium in the body. On the other hand, selenium level in mature milk was not influenced by urine selenium. For mature milk, moreover,,

selenium represented low negative correlation with lipid (Fig. 6) and protein (Fig. 7). These results for mature milk may mean that the milk selenium status is not affected by the dietary selenium intake and the milk protein and lipid, and is controlled by some specific mode. Dickson and Tomlinson measured liver, skin and muscle selenium in tissues from ten autopsies, and found no consistent relationships among different tissues from an individual [20]. Animal studies have yield more consistent values for tissue selenium.

The order of average selenium levels expressed in content per tissue volume or weight among the different tissues examined (Table 1) reflects directly the order of protein (or related substances) levels. Keratin, which is a major constituent of nail tissue, has high level of the sulfur-containing amino acid cystine (*ca.* 15%) [21]. The ability of selenium to replace sulfur in cystine and methionine explains the high selenium levels in nail [22]. The results of a recent study indicate for the first time that selenium level of human nail clippings, instead of urine, may serve as a reliable and convenient indicator of the intake of dietary selenium by man [23]. Samples of nail clipping collected from 16 individuals had the selenium level of 1.14 ppm $\pm$0.06 ppm (Mean $\pm$ SD). This is the only one value so far reported for nail selenium levels. This value is roughly in agreement with the result in the present study. For 253 subjects in Canada, selenium concentrations in whole blood, erythrocyte and plasma were 182 ng, 236 ng and 144 ng per milliliter, respectively, using a neutron activation method [20]. This erythrocyte value is somewhat lower than the value, 264 ng/ml, obtained from Table 1 by assuming hematocrit value as 45% [24]. One of the Tahitians gave plasma selenium of 0.13 μg/ml and erythrocyte selenium of 0.82 μg/ml [25]. Griffiths and Thomson found a very low mean value of 68$\pm$13 ng selenium per blood milliliter for 170 New Zealand adults [26] and Watkinson reported an almost identical value of 69$\pm$10 ng/ml for 24 North Island residents in New Zealand [27]. The value in the present study is between these extremely high [25] and low [26, 27] selenium levels and seems to show the normal level of erythrocyte selenium. The variation coefficient of selenium for erythrocyte tissue is the lowest of that for all individual tissues examined and this may mean homeostatic regulation for selenium levels in erythrocyte. Sterner and Lidfeld reported urine selenium to range between 10 and 150 ng/ml, with a mean of 42 ng/ml in normal Rochester inhabitants [28]. Glover has found British adults to excrete 34 ng selenium per milliliter, a value that approaches the Rochester level when corrections are made for differences in specific gravity [29]. Oregon school children in a low- and two high-dental caries areas had urine selenium levels of 37 ng/ml and 74 and 76 ng/ml, respectively [30]. Forty-three residents from a small community in Milan, New Mexico, where the home wells were contaminated with selenium due to a uranium mill tailing pond nearby, excreted (Mean $\pm$ SD) 79.3$\pm$38.7 ng/ml [31]. In another seleniferous region of the United States, urine selenium values ranged from 100 to 2000 ng/ml [32]. On the other hand, in two children groups in New Zealand, selenium concentrations in urine were found to be 21 and 30 ng/ml [33]. The values in Table 1 seems to be in normal range. Any report has never been published concerning selenium levels in colostral milk. The mean selenium concentration for mature milk collected from 241 subjects in 17 cities in the United States, was 18.5 ng/ml both for individual subjects (range of 7 to 60 ng/ml) and cities (ramge of 13 to 28 ng/ml) [34]. The low variation of mature milk selenium levels among individual cities indicates that a homeostatic mechanism may be responsible for regulating the selenium level of human milk. The samples collected from New Zealand were reported to range between 12 and 15 ppm in human milk selenium concentrations [35]. The average and range in Table 1 are not different from the result of the United States, and so give a

support concerning the existence of homeostatic regulation for human milk selenium levels. As in the cases of most nutrients and essential elements, selenium also showed its higher levels for colostral milk than that for mature milk, if the levels were expressed as content per tissue volume. These two observations for milk from Table 1 means that selenium element is essential for man. The selenium in human breast milk is an important source of selenium for infants, and its biological function should be investigated more and more. The presence of selenium in human saliva was reported for the first time among 26 school children, and the selenium levels ranged from 1.1 to 5.2 ng/ml with a mean concentration of 3.1 ng/ml [36]. This is the only report so far published for selenium in saliva. The value from Table 1 is close to the reported value. The low selenium levels in saliva tissue may be because the tissue consists of about 99.5% water [37]. The expression of selenium levels as content per content of protein (or related substances) has never been reported. Therefore the values expressed by this method in the present study can not be compared with others' values. The above-mentioned two orders for selenium levels among different tissues is very interesting on considering biological function of selenium and in addition means that the tissue with the lower (higher) selenium level per tissue volume has the lower (higher) protein content relative to the selenium content.

The following three observations in the present investigation—(1) the considerable similarity of the selenium concentrations obtained from all kinds of normal human tissues examined to the values from other literatures, and therefore the tendency of constancy of selenium concentrations in the individual human tissues among different areas, (2) correlations between selenium and protein (or related substances) concentrations in the individual tissues and (3) correlations of selenium levels between different combinations of tissues—establish clearly the essential role of selenium element in human biological function. In order to know more profoundly the function of selenium, the selenium in the biologically active form should be investigated.

Acknowledgment

The author is particularly indebted to Professor T. Mizutani for his continuing interest, encouragement and generous supply of human milk samples. The author is grateful for fluorimetric and absorbance measurements to the following: Mrs. Y. Akaba, *née* Wakimoto, and Misses H. Kawano, M. Matsumura and Y. Ochi.

References

[1] R. F. Burk, *Trace Elements in Human Health and Disease,* A. S. Prasad (Ed.), Academic Press, New York, 1976, Vol. II, p. 105.
[2] L. Kosta, A. R. Byrne and V. Zelenko, *Nature* **254**, 238 (1975).
[3] J. H. Halor, T. D. Kimbrough and G. C. Llewellyn, *Food. Cosmet. Toxicol.* **16**, 611 (1978).
[4] W. L. Broghamer, K. P. McConnell and A. L. Blotcky, Cancer **37**, 1384 (1976).
[5] J. N. Walton, *Proc. Nutr. Soc.* **21**, 202 (1962).
[6] U. M. Cowgill, *Lancet* **1**, 816 (1976).
[7] D. M. Hadjimarkos, *Arch. Environ. Health* **10**, 893 (1965).
[8] D. S. F. Robertson, *Lancet* **1**, 518 (1970).
[9] E. J. Van Kampen and W. G. Zijlstra, *Clin. Chim. Acta* **6**, 538 (1961).
[10] T. Noguchi, A. H. Cantor and M. L. Scott, *J. Nutr.* **103**, 1502 (1973).
[11] J. H. Watkinson, *Anal. Chem.* **38**, 92 (1966).
[12] O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).

[13] R. W. Bosnes and H. H. Taussky, *ibid.* **158**, 581 (1945).
[14] Y. Hojo, unpublished results.
[15] C. D. Thomson and M. F. Robinson, *Am. J. Clin. Nutr.* **33**, 303 (1980).
[16] M. F. Robinson, H. M. Rea and R. D. H. Stewart, *Proc. Nutr. Soc.* **35**, 34A (1976).
[17] W. G. Jaffé, M. C. Ruphael-D. and M. A. Cuevas, *Arch. Latinoamer. Nutr.* **22**, 595 (1972).
[18] G. N. Schrauzer and D. A. White, *Bioinorg. Chem.* **8**, 303 (1978).
[19] D. G. Brown and R. F. Burk, *J. Nutr.* **103**, 102 (1973).
[20] R. C. Dickson and R. H. Tomlinson, *Clin. Chim. Acta* **16**, 311 (1967).
[21] A. White, P. Handler and E. L. Smith, *Principles of Biochemistry,* 3rd ed., McGraw-Hill, New York, 1964, p. 822.
[22] D. M. Hadjimarkos, *Food. Cosmet. Toxicol.* **11**, 1083 (1973).
[23] D. M. Hadjimarkos and T. R. Shearer, *J. Dent. Res.* **52**, 389 (1973).
[24] M. M. Wintrobe, *Clinical Hematology,* 4th ed., Lea & Febiger, Philadelphia, Pa., 1956.
[25] H. M. Rea, C. D. Thomson, D. R. Campbell and M. F. Robinson, *Brit. J. Nutr.* **42**, 201 (1979).
[26] N. M. Griffiths and C. D. Cotton, *New Zealand Med. J.* **80**, 199 (1974).
[27] J. H. Watkinson, *ibid.* **80**, 202 (1974).
[28] J. H. Sterner and V. Lidfeldt, *J. Pharmacol. Exptl. Ther.* **73**, 205 (1941).
[29] J. Glover, *Bull. Selenium-Tellurium Develop. Assoc.* **8**, 5 (1969).
[30] D. M. Hadjimarkos and C. W. Bonhorst, *J. Pediat.* **59**, 256 (1961).
[31] J. L. Valentine, H. K. Kang and G. H. Spivey, *Environ. Res.* **17**, 347 (1978).
[32] M. I. Smith, K. W. Franke and B. B. Westfall, *U. S. Public Health Rep.* **51**, 1496 (1936).
[33] P. B. Cadell and F. B. Cousins, *Nature* **185**, 863 (1960).
[34] T. R. Shearer and D. M. Hadjimarkos, *Arch. Environ. Health* **30**, 230 (1975).
[35] K. R. Millar and A. D. Sheppard, *New Zealand J. Sci.* **15**, 3 (1972).
[36] D. M. Hadjimarkos and T. R. Shearer, *Am. J. Clin. Nutr.* **24**, 1210 (1971).
[37] H. A. Harper, V. W. Rodwell and P. A. Mayes, *Review of Physiological Chemistry,* 17th ed., Lange, Maruzen, Tokyo, 1979, p. 245.

要　　旨

ヒトの赤血球，唾液，初乳，成乳，尿，爪のセレン (Se)，蛋白質（関連物費を含む．以後は，この説明文は省略する．），脂肪濃度を測定した．唾液，成乳の Se と蛋白質の濃度の間，及び成乳の Se と脂肪の濃度の間に低い負の相関係数が見出された．赤血球，初乳，尿の Se と蛋白質の濃度間にも高い正の相関が示された．すべての組織において，Se と蛋白質の濃度間の高い（低い）正の（負の）相関係数は，容量当りの含量で表わされた平均 Se レベルの変動係数よりも低い（高い）蛋白質（脂肪を含む）含量当りの平均 Se レベルの変動係数と完全に対応した．Se レベルを蛋白質含量当りの含量で表わすと，尿中 Se は赤血球 Se，唾液 Se と高い負の相関を示し，初乳 Se と高い正の相関係数をもたらした．赤血球 Se と唾液 Se の間にも高い正の相関係数が見出された．組織間の平均 Se レベルは，組織容量（又は重量）当りの含量で表わせば，爪＞赤血球＞尿＞初乳＞成乳＞唾液の順に減少し，又組織蛋白質含量当りの含量で表わせば，尿＞唾液＞成乳＞初乳＞赤血球の順序で減少した．各組織の Se レベルは文献値とかなり類似した．これらの結果は，ヒトに対する Se の必須機能についての支持を与えるものである．