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# EFFECTS OF SELENIUM INTAKE IN MAN AT HIGH DIETARY LEVELS OF SELENIFEROUS AREAS OF VENEZUELA

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## Introduction

Selenium takes a special place among the trace elements. Toxic action is known as well as deficiency syndroms and the optimal range of beneficial action seems to be smaller than for the other trace elements. Supported by epidemiological studies (1,2) there is an increasing interest in the deficiency situation of selenium in human nutrition and health. However, the treatment of a lowered selenium status of the organism is still a problem due to the small margin between the toxic and physiological dose response. In order to define the levels of a safe and adequate selenium dosage in prophylaxis and therapy information is needed regarding the influence of high selenium intakes and the physiological activity of other essential trace elements or their uptake rate. However, few data are presented in the literature on the consequences of longterm human exposure to elevated dietary selenium levels.

In order to study effects of high selenium ingestion in humans and the relationships between this element and other trace elements a research program was started in 1982 on a collaborative basis between the Hahn-Meitner-Institut Berlin and the national Venezuelan foundation for the investigation of the health status of the population FUNDACREDESA.

A field investigation was carried out in the seleniferous area of Villa Bruzual in Venezuela. In this natural laboratory Jaffê (3) started 14 years ago a clinical and biochemical investigation of school children in order to study possible health risks. In the present study the examination was extended to adults, elderly persons and lactating mothers. A total of 134 persons was examined and samples of blood, hair, breast milk and aliquots of the families meal were collected.

Sample collection and preparation

Hair

Hair was cutted from every person at the left occipital region and sealed in plastic foils. Only the first two centimeters of the hair above the roots were used for the analysis. The samples were cleaned according to the recommendation of the IAEA (4) applying a 3 step procedure using ether, bidistilled water and acetone as rinsing fluids.

Breast milk

Breast milk was obtained by manual pressure of the breast and directly collected into precleaned plastic containers avoiding any contact with the breast. A good aid for the stimmulation of the milk output was the simultaneous feeding of the child with the other breast.

Blood, serum

The blood was taken after the person rests for some minutes in a lying position. All persons were advised to be empty till the blood collection in the morning. Monovettes (Fa. Sarstedt, Germany) were used as syringes, which have been checked to be free of contamination with the elements of interest.
After coagulation (about 4o minutes waiting time) the serum was separated by centrifugation and transfered into pre-cleaned plastic tubes.

From all families food samples from meal taken before the clinical investigation were collected and sealed in plastic bugs. All samples collected in the field study were stored at dry ice temperature in a Dewar container and transported to Berlin, where the trace element analysis and the determination of some serum components has been carried out at the Hahn-Meitner-Institute.

Sample analysis

Instrumental neutron activation analysis

All fluid samples were dryed at 50 °C and carefully sealed in vials of high purity quarz, each vial containing about 100 - 300 mg dry material. Food samples were homogenized and freeze dryed before sealing. The samples were irradiated for 10 d at a neutron flux density of about $5 \cdot 10^{13}$ n $cm^{-2}$ $s^{-1}$ in the Berlin research reactor (BER II). Bovine Liver and Orchard Leaves powder (National Bureau of Standards, Washington) were used as a standard reference material.

The trace elements Co, Cs, Fe, Rb, Se and Zn were determined via the decay of their long lived radio nuclides by means of high resolution $\gamma$- spectrometry. In hair mercury could be determined after the calculation of the Se-75 interference at $E_\gamma$ = 279 KeV.

Atomic Absorption Spectrometry

The element copper was measured in serum by means of graphite furnance AAS, and to determine mercury in breast milk samples the hydrid-amalgame technique was, applied.

Serum constituents

Special serum proteins were determined as follows: Albumine, $\alpha_2$-macroglobuline and ceruloplasmine by Laser-nephelometry, C -reactive protein (CRP) and prealbumine by radial immunodiffusion, and ferritin via radioimmunoassay.

# Results and Discussion

## Serum-proteins - Health status

In general useful preliminary information on the nutritional and health status of the population group can be obtained from the levels of serum constituents. Albumine and prealbumine may serve as nutritional indicators of the long and short term protein supply, respectively. Further, C-reactive protein, $\alpha_2$-macroglobuline and ceroluplasmine the so-called acute phase proteins are very often elevated in the course of infections. The measured ferritin level gives some information about the iron storage capacity.

| | | ALL | | ADULTS | | CHILDREN | |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % |
| ALBUMIN <3500 MG/DL | ↓ | 46 | 33 | 18 | 23 | 28 | 65 |
| FERRITIN <30 NG/ML | ↓ | 34 | 24 | 16 | 20 | 18 | 42 |
| CRP >0.5 MG/DL | ↑ | 25 | 18 | 22 | 28 | 3 | 7 |
| $\alpha_2$-MACROGLOBULIN <200 MG/DL<br>>400 MG/DL | ↓<br>↑ | 14<br>6 | 14 | 11<br>4 | 19 | 3<br>2 | 11 |
| CEROLUPLASMIN <25 MG/DL<br>>45 MG/DL | ↓<br>↑ | 12<br>14 | 18 | 6<br>13 | 24 | 6<br>1 | 16 |
| PREALBUMIN <10 MG/DL | ↓ | 4 | 3 | | | | |

Tab 1: Deviation of Serum Protein Values from the Normal Range

In Table 1 the deviation of the measured values from the normal range are summarized. From the investigation of a total number of 134 persons including 46 children it is remarkable that about 65 % of the children have a low albumine serum level, indicating a certain protein deficit. Furthermore in about 42 % of the children the ferritin level is low, which might be due to an anaemic situation (latentor pre-latent).

Nearly a quarter of the adults showed a deviation in at least one of the characteristic serum proteins.

During the field investigation special attention was paid to the incidence of clinical signs which might be related to high selenium intake. Symptoms of dermatitis, pathological nails or loose hair were found only in few cases.

Analysis of food samples

From the analysis of samples of the meals of 45 families quantitative information of the mean selenium ingestion in Villa Bruzual was obtained (Fig. 1). A mean of 0.54 ppm Se was calculated on the dry weight basis. The two cases of Se-values greater than 2.2 ppm in Fig. 1 were found to be reflected also in corresponding higher Se blood levels of this families. From the mean Se-content of the meals rough estimation of the selenium intake of the population group yields a range of 300 - 400 $\mu$g Se per day without taking into account the intake of beverages. That is higher than the estimated human dietary requirement of 50 - 200 $\mu$g Se per day (5).

The measured frequency distribution of Fig. 1 corresponds to an occurance of different foods in the meals as given in Fig. 2A. From the determination of the selenium content of some of the locally produced foods (Fig. 2B ) the highest value of 28 ppm dry matter was found in the egg white. Egg accumulates obviously selenium and it might be a good monitor to localize the occurence of this element in animal feed and the environment.

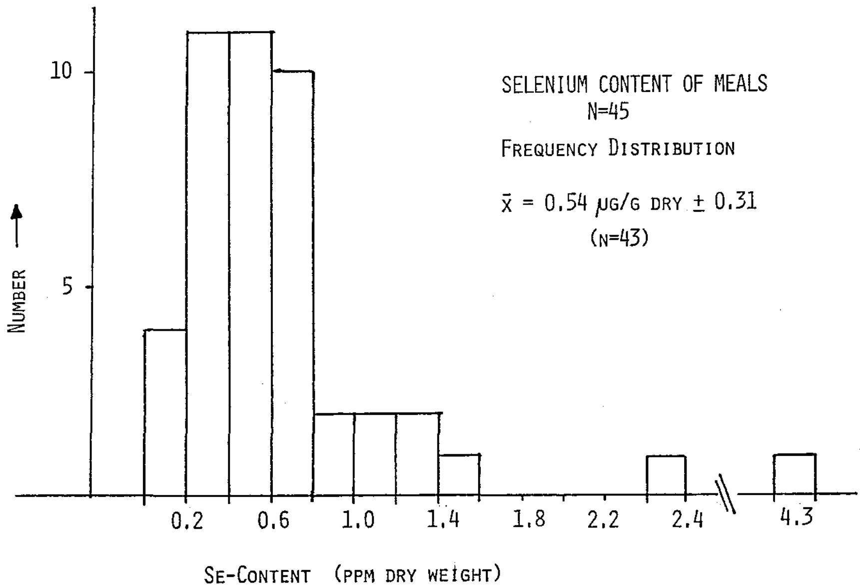


Fig. 1 Selenium content of meals

Se in serum and red blood cells

The population investigated was divided into age groups according to the classification used in the clinical examination. As shown in Fig. 3 an increase of the Se-content of serum and red blood cells (RBC) with age has been found. This increase amounts to a factor of 2 if the children and the adults aged 31 - 50 years are compared.

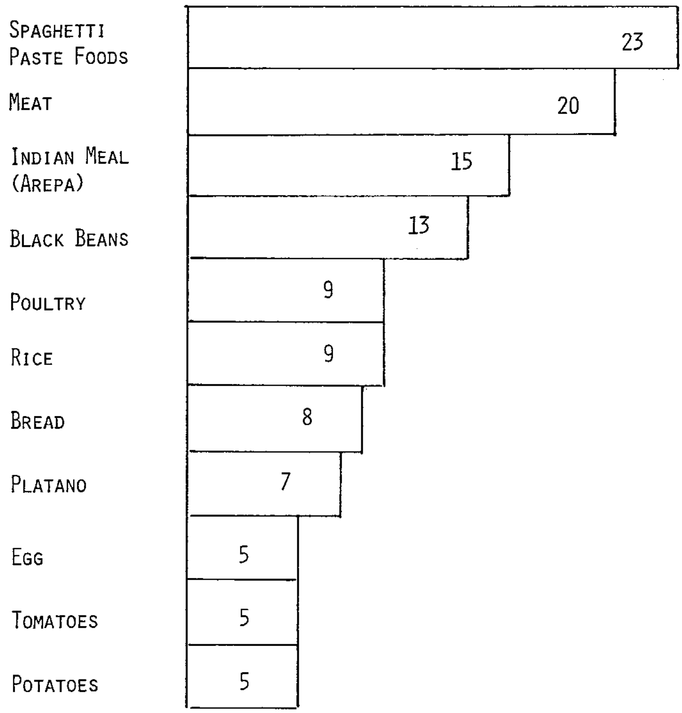


Fig. 2 A Occurence of different foods in 43 meals from Villa Bruzual

| | |
|---|---|
| SESAME | 2.8 |
| SORGO | 0.76 |
| RICE | 0.96 |
| GUAYABA | 1.82 |
| EGG WHITE | 6.2 / 28.8 |
| EGG YOLK | 2.6 / 6.2 |

Fig. 2 B Selenium content of different foods (µg/g dry)

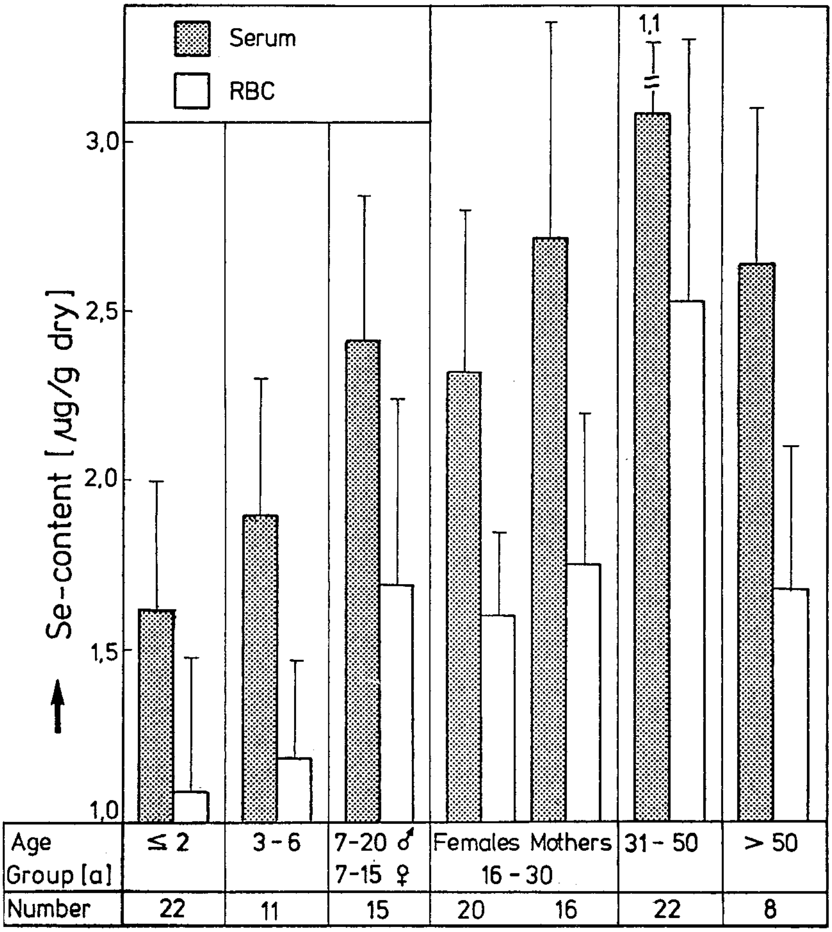


Fig. 3: Se-content of Serum and Red Blood Cells (RBC) Dependence of the age groups

The blood selenium values tend to decrease in the senior group and that might be due to a lower food consumption of elderly people. In general the RBC-selenium is always higher than the serum level if calculated on the wet weight basis. Another point of interest might be the RBC-serum ratio which seems to be nearly constant for all age groups.

This correlation has been proved including all persons as shown in Fig. 4. The correlation coefficient of 0.923 demonstrates a good relationship but at serum values greater than

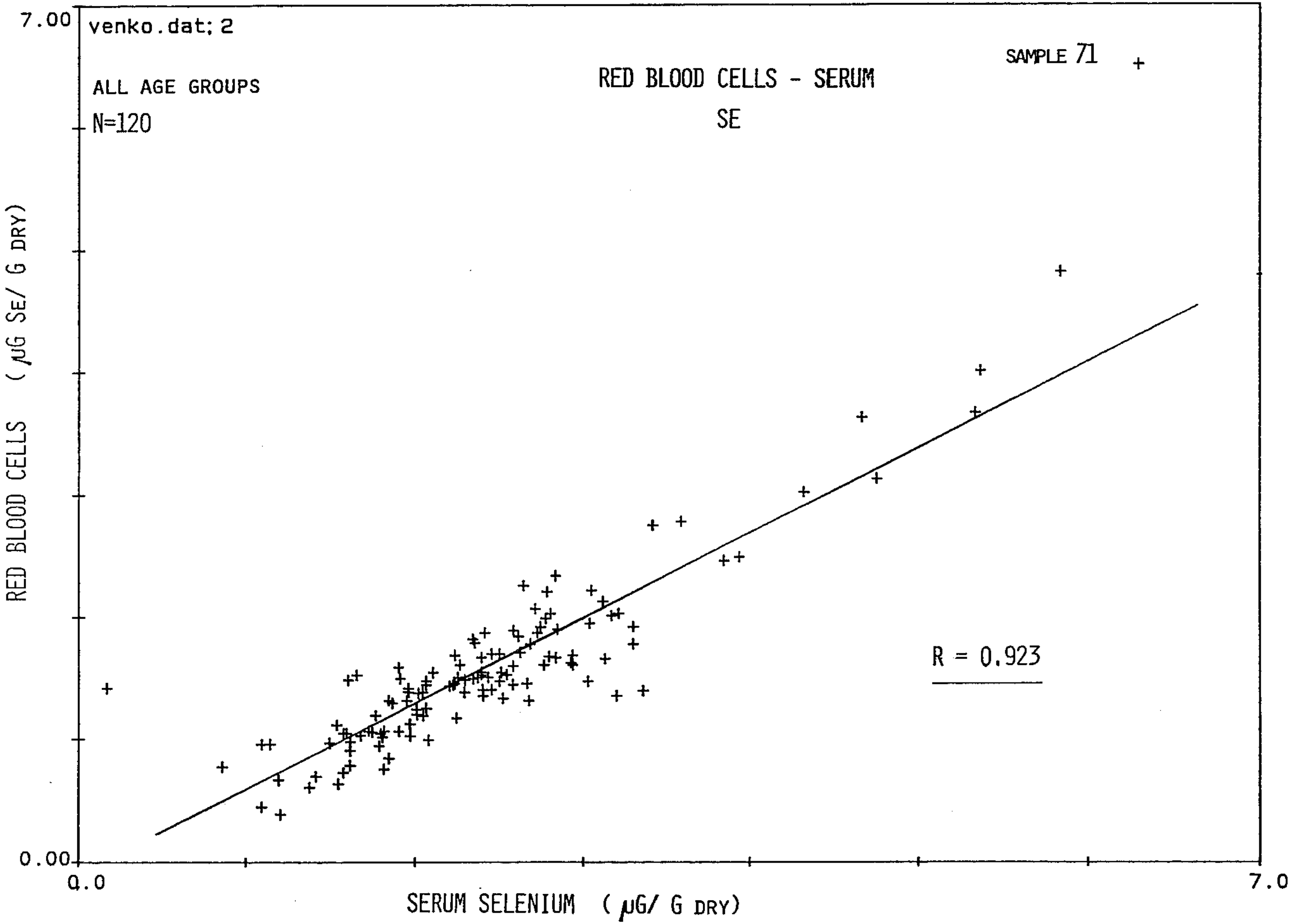


Fig. 4: Selenium in RBC as a function of the serum Se-content

| VENEZUELA VILLA BRUZUAL | Se (ng/g) $\bar{X} \pm s$ | Fe (µg/g) $\bar{X} \pm s$ | Zn (µg/g) $\bar{X} \pm s$ | Cu (µg/g) $\bar{X} \pm s$ | Rb (µg/g) $\bar{X} \pm s$ | Co (ng/g) $\bar{X} \pm s$ |
|---|---|---|---|---|---|---|
| MOTHERS 18 - 40a n = 17 | 265 ± 60 | 1.37 ± 0.58 | 0.83 ± 0.47 | 1.23 ± 0.20 | 0.22 ± 0.06 | 0.69 ± 0.52 |
| ADULTS 16 - 30a n = 20 | 217 ± 60 | 1.45 ± 1.20 | 0.74 ± 0.12 | 1.11 ± 0.53 | 0.21 ± 0.07 | 0.55 ± 0.18 |
| ADULTS 31 - 50a n = 28 | 314 ± 135 | 1.34 ± 0.40 | 0.84 ± 0.14 | 1.28 ± 0.33 | 0.19 ± 0.06 | 0.54 ± 0.23 |
| GERMANY BERLIN n = 8 | 92 ± 6.3 | 1.96 ± 0.18 | 0.83 ± 0.55 | n.d. | 0.196 ± 0.024 | 0.52 ± 0.28 |

Tab. 2   Serum trace element content of adults

4 μg Se/g dry the relative increase of selenium in the red blood cells appears to be higher and the ratio between the both blood fractions tends to increase. With respect to this findings it is interesting to note, that for the low selenium intake situation of New Zealand residents also a constant ratio of Se-RBC to Se-serum has been found (6). The difference lies in the value of the ratio which yield about 1.54 for New Zealand and about 2.0 for the high Se-intake situation of Villa Bruzual. From our data it might be assumed that in the case of a longterm and nearly constant selenium intake of a population a balance could be established which yields a constant ratio for the distribution of Se between both blood fractions. The deviation at higher Se-serum values in our case needs further examination.

Another objective of the study was to investigate the influence of high dietary selenium intake on the concentration of other essential trace elements.

The results of the determination of Co, Cu, Fe, Se and Zn in blood serum are summarized in Tab. 2. For comparison, the corresponding values of a control group in Berlin are given. The mean serum selenium content was found to be 3 times higher than those of the Berlin group, but there was no significant difference in the values of the other trace elements. Copper values were also found within the known normal range. The data suggests that there is no direct effect of the high dietary selenium status of Villa Bruzual on the serum level of the other trace elements investigated.

Breast milk

From 17 mothers in Villa Bruzual samples of mature milk were analyzed. The results of trace element analysis in Tab. 3 are expressed on the wet and the dry weight basis. A comparison with 45 samples from Berlin shows that the higher dietary selenium level is reflected in the breast milk as well.

| | Venezuela, Villa Bruzual | | | | Germany, Berlin |
|---|---|---|---|---|---|
| | Wet weight | | | Dry weight | |
| | $\bar{X} \pm S$ | | Range | $\bar{X} \pm S$ | $\bar{X} \pm S$ (N = 45) |
| Se (ng/g) N = 17 | 57.70 | 37.40 | 21 - 158 | 416 ± 236 | 121 ± 36 |
| Co (ng/g) N = 17 | 0.53 | 0.24 | 0.31 - 1.27 | 4.1 ± 2.3 | 4.8 ± 3.5 |
| Cr (ng/g) N = 17 | 4.20 | 2.25 | 1.0 - 10.3 | 33.1 ± 18.0 | 42 ± 22 |
| Cs (ng/g) N = 17 | 2.00 | 1.20 | 0.9 - 5.3 | 15.7 ± 10.3 | 30 ± 13 |
| Fe (µg/g) N = 16 | 0.66 | 0.34 | 0.21 - 1.30 | 5.1 ± 3.0 | 4.5 ± 2.5 |
| Rb (µg/g) N = 17 | 0.60 | 0.13 | 0.35 - 0.90 | 4.5 ± 1.3 | 5.4 ± 1.3 |
| Zn (µg/g) N = 16 | 1.14 | 0.60 | 0.27 - 2.18 | 8.6 ± 5.1 | 45.2 ± 18.0 |
| Hg (ng/g) N = 6 | 1.20 | 0.20 | 1.0 - 1.5 | 9.9 ± 1.7 | 13.3 ± 11.5 |

Tab. 3 Trace element content of breast milk

The breast-fed infants of Villa Bruzual receive about 3 times more Se compared to the Berlin infants. On the other hand, we found that the mean zinc content of breast milk samples from Villa Bruzual amounts to 1.14 µg/g wet or 8.6 µg/g dry weight. These values are significantly lower than those of the Berlin group. In consequence, the corresponding zinc intake of the infants meets only the lower limit of the recommended values.

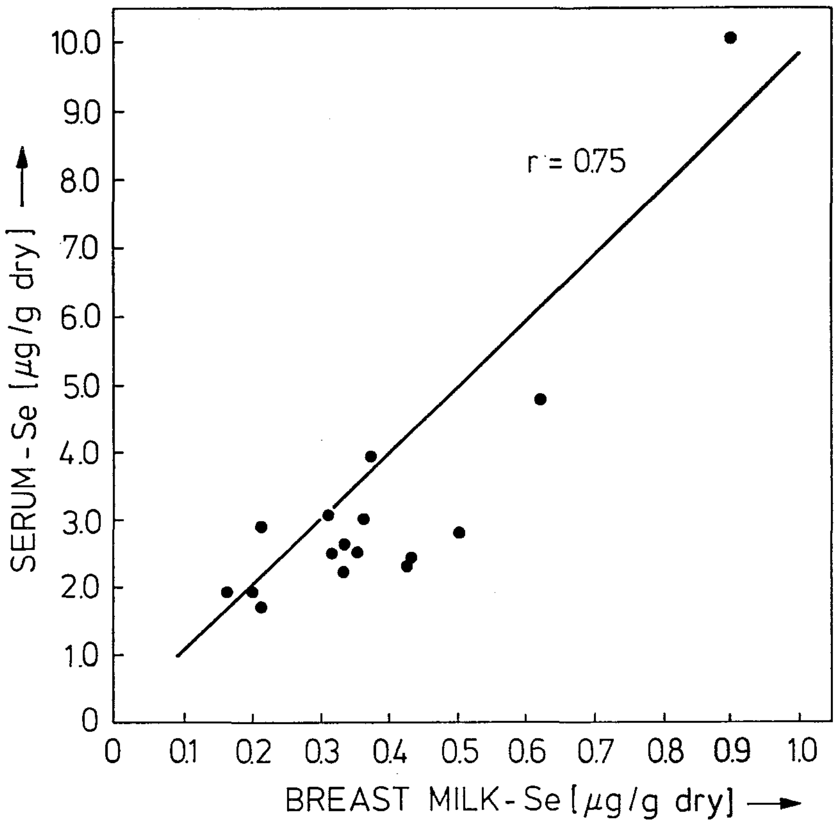


Fig 5: Serum Se-content as a function of Se in breast milk

A linear regression (Fig. 5) of serum selenium of the mothers versus their breast milk Se-content shows a positive correlation. However, from the point of view of statistical significance the number of cases has to be extended.

The serum zinc content of the mothers of Villa Bruzual (Tab.2) was found in the normal range suggesting that the low Zn-content of the breast milk is independent of the maternal Zn-status.

However, at this time, it remains an open question if the low Zn-content of the breast milk might be related to the high dietary selenium intake and the corresponding high Se-level of the breast milk.

It is known that high levels or chronic ingestion of mercury may lower the physiological activity of selenium due to the antagonistic relationships of both elements. Therefore, mercury was analyzed in the milk samples. Because of the low order of the values found, an influence of mercury was not taken into consideration. This was also supported by the Hg-level of the hair samples (Tab. 4), when they are used as monitor of the environmental burden.

( VILLA BRUZUAL, VENEZUELA)

| ELEMENT | MEAN (µG/G) | RANGE |
|---|---|---|
| Co | 0.074 | 0.008 - 0.390 |
| Fe | 54.2 | 11.3 - 270 |
| Hg | 0.86 | 0.09 - 7.0 |
| Se | 1,56 | 0.62 - 9.4 |
| Zn | 211 | 23 - 580 |

Tab. 4 Trace element content of hair (N=129)
2 cm segment close to the scalp

Hair

From all persons hair samples were taken in order to compare their trace element content with the Se-content of the blood fractions. Such kind of investigation is reasonable if the people do not wash the hair with sulfur containing shampoos

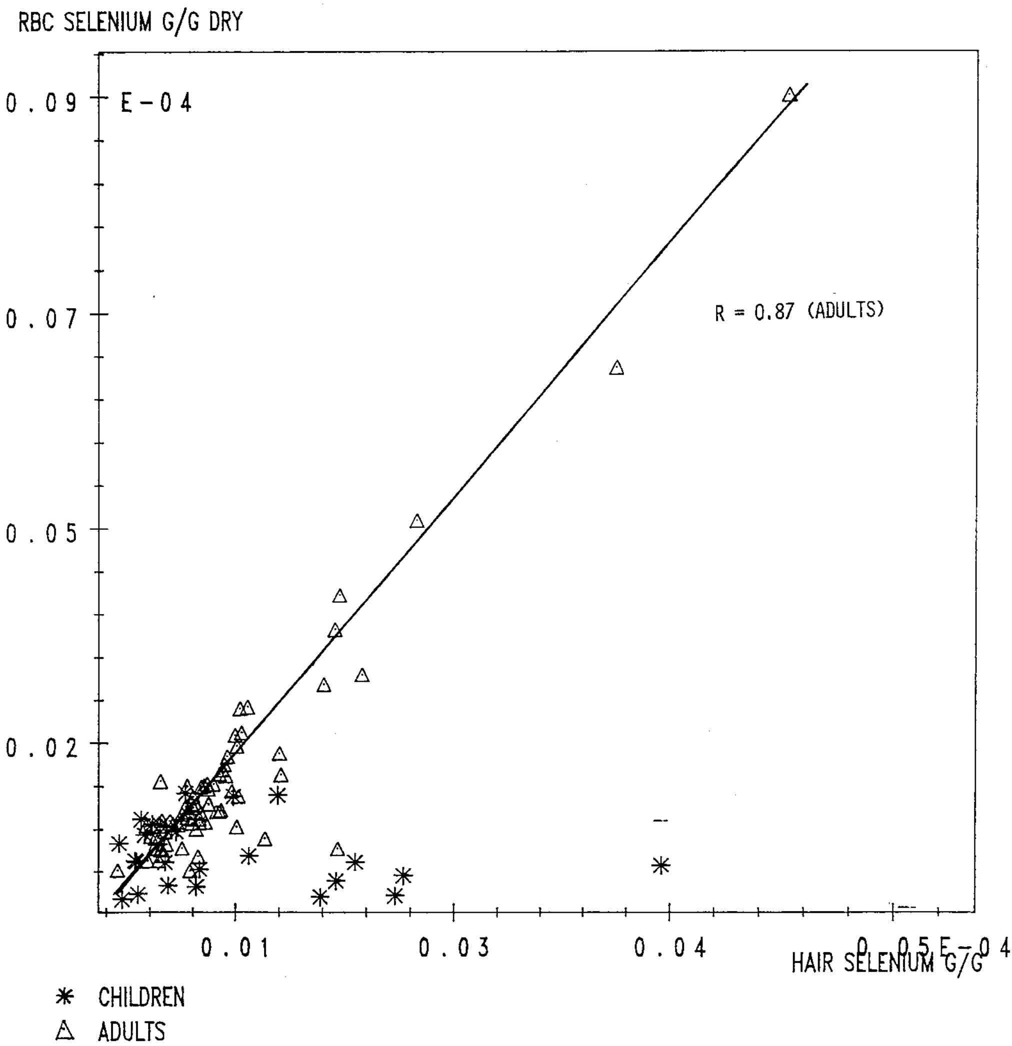


Fig. 6: Selenium content of red blood cells in relation to selenium in hair of children and adults

as it is the case in this region.

Hair receives its trace elements mostly from the blood and is able to uptake selenium in its matrix during keratinization. If the permanent dietary Se-level of a population group is reflected in the blood Se-level, a relation may also exist between hair concentration and diet. We regarded the red blood cells as a measure of the long term Se-status of the organism and tried to correlate their content with that of the hair, as shown in Fig. 6. Different results were obtained for children and adults. For adults a linear regression yielded a significant positive correlation but no correlation was found for the children.

To obtain some idea of the relation between Se in hair and the Se-intake the data of the seleniferous area of Villa Bruzual were compared with data from China (7) . The chinese values were obtained from regions of medium and low dietary Se-ingestion. The comparison in Tab. 5 shows clearly the connection between the blood- and hair Se-content at the different dietary levels.

| | SELENIUM INTAKE | | |
|---|---|---|---|
| | HIGH | MEDIUM | LOW |
| SE - LEVEL | VENEZUELA | CHINA | |
| BLOOD $\bar{x}$ (µG/ML) | 0.420 | 0.095 | 0.021 |
| HAIR $\bar{x}$ (µG/G) | 1.56 | 0.359 | 0.074 |

Tab. 5 Dependence of the blood and hair selenium level on the selenium intake

The factor of about 3.5 between the blood and hair content in all 3 cases may not be a casual factor, but it describes an equilibrium between the Se-concentration in the organs and the compartments of the body.

Summary

From the study of high dietary Se-intake the following results were obtained:

1. The Se-levels in blood, breast milk and hair were influenced.
2. The Se-content of serum and RBC is age-dependent.
3. The level and the distribution of Se between serum and RBC are positively correlated. The ratio between the Se-content of serum and RBC was constant in the range of 1 to 4 µg Se/g dry serum, but above that value the RBC-Se increases much more than the corresponding serum-Se.
4. No deviation from the normal serum levels of other trace elements including Co, Cu, Fe, Rb and Zn was observed.
5. The Se-content of breast milk is positively correlated with that of the serum of the mothers. In contrast to the elevated Se-level ( $\bar{x}$ = 416 ng/g dry ) the Zn-content of the breast milk ( $\bar{x}$ = 8.6 µg/g dry ) was found to be below the normal range. If the high Se-intake may cause the low Zn-content of the breast milk remains an open question.
6. Hair might be a good monitor to investigate the dietary Se-ingestion of different population groups under certain conditions ( permanent dietary Se-level, exclusion of the use of sulfur containing shampoos).
7. Clinical symptoms of high Se-intake as nausea, pathological nails or loss of hair occured only in a few cases.

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