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## Simultaneous determination of arsenic, manganese and selenium in human serum by neutron activation analysis

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Danish Atomic Energy Commission  
Research Establishment Risø

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Simultaneous Determination of  
Arsenic, Manganese and Selenium  
in Human Serum  
by Neutron Activation Analysis

by E. Damsgaard, K. Heydorn, N. A. Larsen and B. Nielsen

June 1973

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Simultaneous Determination of Arsenic, Manganese and Selenium  
in Human Serum by Neutron Activation Analysis\*

by

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Abstract

A procedure for the simultaneous determination of arsenic, manganese, and selenium in human serum is described, and its performance characteristics are evaluated with particular reference to manganese. Blank values depend critically on irradiation containers, and only one type of polyethylene ampoule gave a sufficiently low limit of detection. Other suspected sources of random and systematic errors are investigated; they did not, however, jeopardize precision or accuracy of analytical results. Careful studies of the sampling techniques with actual as well as simulated serum revealed considerable contamination with arsenic and manganese under normal hospital conditions; in addition, loss of added dimethylselenide was observed even during deep-freeze storage of serum. A detailed sampling procedure with special precautions against contamination is described, and is shown to yield meaningful results for normal, human serum. Results tend to agree with the lowest values reported in the literature, and the analytical precision is sufficient for determination of the standard deviation of the distribution of all 3 elements in human serum.

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\* This work was presented in part at the 2nd Symposium on the Recent Developments in Neutron Activation Analysis, Cambridge, 1971

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## INTRODUCTION

As a continuation of a recent study<sup>1)</sup> of arsenic, manganese and selenium concentrations in autopsy materials from uraemic patients, it is of interest to analyse serum from living persons in order to examine the effect of the dialysis treatment, the diet and the degree of uraemia.

Our previous method for the simultaneous determination of arsenic, manganese and selenium in biological materials by neutron activation analysis<sup>2)</sup> is adequate for the detection of the lowest reported levels of arsenic and selenium, but the extremely low concentrations reported for manganese in serum<sup>3)</sup> require an improvement of the separation procedure for this element.

In existing procedures for the determination of manganese in serum or in plasma by neutron activation analysis, radiochemical separation is achieved by precipitation as the tetraphenylarsonium permanganate<sup>4)</sup>, by solvent extraction with sodium diethyldithiocarbamate<sup>5)</sup> or by ion exchange<sup>6)</sup>. Manganese has also been determined by Ge(Li)-spectroscopy after removal of the activities of sodium, potassium, phosphorus and the halides<sup>7)</sup>.

In the present study a clean separation of manganese was achieved within minutes by extraction with diethylammonium diethyldithiocarbamate in chloroform<sup>8)</sup>, followed by back-extraction into hydrochloric acid.

Incorporation of this procedure into our previous method provided sufficient precision to permit studies of various factors affecting the analytical results for arsenic, manganese and selenium in normal human serum.

## ANALYTICAL METHOD

Our previous method for the simultaneous determination of arsenic, manganese and selenium in biological materials calls for a one-hour irradiation of a one-gram sample followed by radiochemical separation and measurement of the activities of  $^{76}\text{As}$ ,  $^{56}\text{Mn}$  and  $^{81\text{m}}\text{Se}$  in scintillation detectors. The chemical yield is determined by added  $^{54}\text{Mn}$ , respectively re-irradiation of the separated arsenic and selenium samples.

Only minor modifications of the detailed procedure given in<sup>2)</sup> are required for the determination of arsenic and selenium in serum, but the final removal of sodium with hydrated antimony pentoxide was replaced by an extraction with diethylammonium diethyldithiocarbamate for the determination of manganese.

### Irradiation

Gelation of serum occurs at a reactor irradiation dose of 3-4 Mrad, and a reduction of irradiation time below 45 minutes in the pneumatic tube system is therefore advisable.

No problems were encountered at irradiation times up to 30 minutes with sample volumes of 4 to 5 ml.

### Determination of Arsenic and Selenium

The possible presence of bromine in concentrations up to 20 ppm<sup>9)</sup> in serum necessitates correction for the contribution of <sup>82</sup>Br to the 559 keV peak of <sup>76</sup>As used for the calculation of arsenic content. This correction was made by computer stripping of the 777 keV peak of <sup>82</sup>Br in the sample spectrum by means of a <sup>82</sup>Br reference spectrum.

The use of a Ge(Li) semiconductor detector instead of the NaI(Tl) scintillation detector obviates the need for such correction. The precision achieved by counting for 80 minutes in the scintillation detector, however, required counting for 4 hours with a 45 cm<sup>3</sup> Ge(Li) detector, which is not acceptable.

Hence, the analytical procedures for arsenic and selenium are unchanged and require no further specification.

### Determination of Manganese

A drastic increase of the sensitivity for manganese is brought about by changing three important characteristics of our previous method.

The radionuclidic purity of <sup>56</sup>Mn in the separated manganese sample is greatly improved by the extraction into chloroform with diethylammonium diethyldithiocarbamate. The only other activity from irradiated serum that was observed in the extract is <sup>69m</sup>Zn.

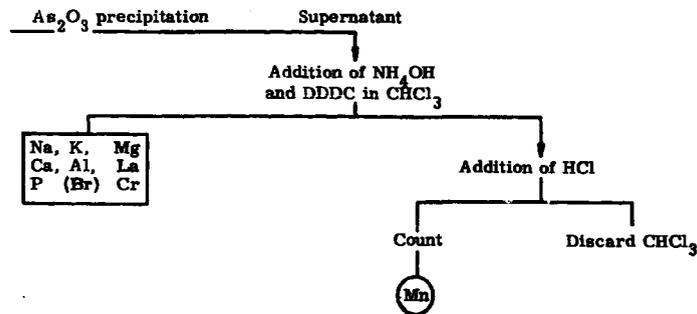
The chemical yield of manganese is increased from 4% to about 80% by concentrating the separated manganese in 1 ml of hydrochloric acid before counting. This back-extraction of manganese from the chloroform phase was essentially complete when concentrated acid was used.

The counting efficiency is increased by counting in a well-type scintillation detector instead of using a solid detector.

The increased sensitivity allowed a reduction by two orders of magnitude of the <sup>54</sup>Mn added for yield determination.

### Procedure

The following description is an overlay to our previous procedure so that common features are omitted; a schematic presentation is shown below.



### Reagents

Ammonium hydroxide 25%

Chloroform

Diethylammonium diethyldithiocarbamate (DDDC)

prepared by slowly mixing under cooling of 100 grams of diethylamine and 50 grams of carbondisulphide, both diluted with 50 ml of ethanol. The precipitate of DDDC is recrystallized from 40 ml of ethanol.

### Carriers

Mn-carrier, 10 mg/ml as Mn(II) in 4 M nitric acid, containing 0.02 μCl/ml of <sup>54</sup>Mn

### Irradiation

Serum samples of about 4.5 ml in 5 ml polyethylene ampoules\* are stored in a deep freezer, and left to thaw at room temperature before activation.

The polyethylene ampoule is heat-sealed and irradiated together with comparator standards for 30 minutes.

### Decomposition

Of the irradiated serum, 4.0 ml is transferred and decomposed as previously described.

The <sup>54</sup>Mn carrier sample set aside for yield determination contains 1000 μl of Mn-carrier in a half-dram polyvial.

\* Atomic Industrial Company, Tokyo

### Separation of Manganese

To the supernatant from the arsenic sulphide precipitation is added ammonium hydroxide to a pH of 7-8. Manganese is extracted by stirring for 1 minute with 5 ml of freshly prepared 3% w/v of DDDC in chloroform. The organic phase is transferred to a centrifuge tube, and the extraction is repeated with another 5 ml of DDDC solution.

The combined chloroform phases are washed 3 times with water, and after addition of 1.1 ml of hydrochloric acid manganese is back-extracted by stirring for 1 minute. The acid layer is transferred to a half-dram polyvial which is then closed and ready for counting.

### Counting of Manganese-56

A comparator standard is made by transferring 500  $\mu$ l of the irradiated Mn-comparator to a half-dram polyvial and diluting with water to 1.0 ml.

The manganese sample is counted for 40 minutes live time not later than 5 hours after pile-out in a 3" x 3" well-type scintillation detector at a gain of 6.7 keV/channel. The comparator standard is counted for 4 minutes under the same conditions.

### Counting of Manganese-54

The separated manganese sample and the  $^{54}\text{Mn}$  carrier sample are counted not earlier than 24 hours after pile-out under the same conditions as above.

The chemical yield averaged 80%.

## ANALYTICAL EVALUATION

The error associated with an analytical result, produced by applying a particular analytical method to an actual sample, represents the combined effect of imperfections in the analytical procedure, and the influence of conditions not specified in the analytical method. The two contributions may be referred to as inherent errors and circumstantial errors.

Inherent errors are estimated from the performance-characteristics of the analytical method, which serve to ascertain the applicability of the method to the solution of a specific, analytical problem. Performance-characteristics are intrinsic properties of the analytical method and do not depend on external conditions.

Circumstantial errors include all other sources of error and are required to evaluate the precision and accuracy of results. They must be determined separately for each set of circumstances, under which actual samples are analysed.

In thermal neutron activation analysis, thermal neutrons are inherent, whereas fast neutrons are circumstantial. Interference from other elements activated by thermal neutrons must therefore be included in the performance-characteristics of the method. The influence of interfering nuclear reac-

tions caused by fast neutrons on the accuracy of the results must be determined separately for each irradiation facility.

### Interference

The analytical procedure for arsenic and selenium is completely unchanged, and separation factors are therefore the same as in our previous method. Effective values are slightly changed, because of the reduction of irradiation time to 30 minutes, and revised interferences for both As and Se are listed in the tables 1 and 2, although the values for As are the same as in our original method.

For manganese, separation factors are greatly improved, and complete separation from Na and K is achieved. Experimentally determined interferences were therefore limited to those from Br and Ga, given in table 3.

Correction for bromine interference of the values for arsenic in serum lowered results by an average 0.3 ng/ml.

The contribution of all other interfering elements to the results for arsenic, manganese, and selenium in serum is well below the limits of detection, even at their highest reported<sup>10)</sup> concentrations.

### Blank

The polyethylene vials used for the irradiation of tissue samples in our previous method had been found to release up to a few nanograms of manganese during irradiation, and they are therefore not suitable for the irradiation of serum.

Cleaning with nitric acid instead of with redistilled water reduced the release of manganese from these vials by more than a factor of 2, but the variation in blank values is not acceptable. A 3% hydrogenperoxide solution proved just as effective as nitric acid, and less variation in blank values was observed.

A significant positive correlation between the total amount of manganese in the polyvial and the quantity released to the redistilled water blank was found and a search was made for polyethylene vials with lower manganese content.

One such container, which was made available to us\*, contained about half as much manganese, of which only about 1% was released during irra-

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\* Courtesy of Dr. Nic Spronk, Vrije Universiteit, Amsterdam

Table 1

Interferences in the determination of arsenic

Interfering element	Separation factor S	Effective value f	ppm of element ~ ± 1 ppb of As
Cu	$2.3 \times 10^{-5}$	0.29	150
Br	$1.1 \times 10^{-3}$	0.09	10
Sb	$5.3 \times 10^{-3}$	0.72	0.25
W	$7.2 \times 10^{-3}$	-0.29	0.5

Table 2

Interferences in the determination of selenium

Interfering element	Separation factor S	Effective value f	ppm of element ~ ± 1 ppb of Se
Cl	$2.0 \times 10^{-5}$	-0.01	5000
Br	$5.6 \times 10^{-4}$	-0.01	>40
Mo	$2.1 \times 10^{-5}$	0.07	600
Ba	$0.9 \times 10^{-5}$	0.15	>300
Dy	$3.7 \times 10^{-7}$	1100	2
Au	$1.9 \times 10^{-1}$	-0.45	0.01
Hg	$1.4 \times 10^{-2}$	0.09	0.7

Table 3

Experimentally determined interferences in manganese analysis

Interfering element	Radioactive tracer	Activity $\mu$ Cl	Mass $\mu$ g	Separation factor S	Effective value f	ppm of element ~ ± 1 ppb of Mn
Ga	Ga-72	200	7	$2.7 \times 10^{-5}$	$4.0 \times 10^{-4}$	90,000
Br	Br-80m	100	3	$7.1 \times 10^{-4}$	$1.3 \times 10^{-3}$	1,000

Table 4

Manganese blank values for different irradiation containers

Type	Irradiation container Supplier	Total Mn rg	Blank value		
			Volume ml	Number of analyses Concentration of Mn ng/ml	
Polyvidal $H_2O_2$ cleaned $HNO_3$ cleaned	Olympic Plastics Company	$24 \pm 7$	1.1	15	$1.1 \pm 0.7$
		$22 \pm 8$		10	$0.5 \pm 0.1$
		$18 \pm 5$		12	$0.4 \pm 0.4$
Polyvidal Polyethylene ampoule	Dr. Nic Spronk Atomic Industrial Co.	$14 \pm 3$	1.1	10	$0.15 \pm 0.06$
		$19 \pm 11$	4.5	10	$0.02 \pm 0.01$

diation. No correlation between total manganese and blank values was observed, and a correction of values for manganese in serum by 0.15 ng/ml would presumably yield acceptable results, although the observed variation in blank values would reduce their precision.

Finally a polyethylene ampoule of undetermined manganese content was investigated, and a significant release of sodium during irradiation was observed. However, the release of manganese was near the limit of detection of the method for the analysis of serum. At the same time the release of arsenic and selenium was also below their limit of detection, and this container was therefore selected for the irradiation of serum samples.

In contrast to the other polyethylene containers, total manganese content of the chosen polyethylene ampoule could not be determined by instrumental neutron activation analysis, because of its high sodium content. The irradiated ampoule was therefore brought into solution by alternate charring with hot sulphuric acid and oxidation with 50% hydrogen peroxide. The manganese contents, determined after radiochemical separation, showed considerable variation between individual ampoules, but their average was not lower than for containers with considerably higher blank values.

Results of these investigations are summarized in table 4, from which it is clearly brought out that the total manganese content of irradiation containers is no useful guide to the selection of containers with low blank values.

The three polyethylene containers are shown in figure 1 in approximately true size.

### PRECISION AND ACCURACY

#### Precision of Results

Random variations in neutron fluence between sample and comparators give rise to a standard deviation of 5% for arsenic and selenium, where the chemical yield is determined by re-irradiation.

For manganese the corresponding standard deviation is only 3.5%, the contribution from yield determination by the counting of <sup>54</sup>Mn being included in the counting statistics.

The calculated precision of a single determination represents the overall effect of counting statistics and random activation errors. The absence of other significant sources of variation has been demonstrated previously for arsenic and selenium by comparing calculated standard deviations of results with their observed variation.

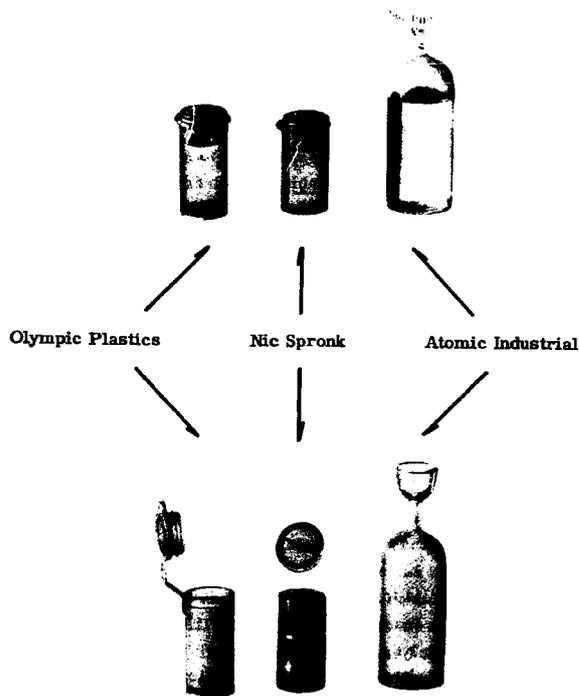


Fig. 1. Polyethylene containers used for the irradiation of serum  
Scale 1:1

For arsenic the contribution to the overall precision from the correction of arsenic values for bromine interference is included in the counting statistics.

For manganese the calculated precision was checked by repeated analysis of dried animal blood, supplied by the International Atomic Energy Agency<sup>11)</sup>. The concentration of manganese in this material is two orders of magnitude lower than in the plant materials analysed with our previous method, although still by far exceeding that of human serum.

The agreement between the observed and calculated standard deviations in table 5 does not indicate the presence of unknown sources of variation in the present analytical method for the determination of manganese.

Table 5

Analytical results for manganese in IAEA dried animal blood

Number of results	Standard deviation		Mean value ppb	Source
	calculated	observed		
6	6	5	150 ± 2	This work
16*	-	16	151 ± 4	IAEA**

\* Pooled results from 3 different laboratories

\*\* D. Merten, personal communication, 1971

#### Limit of Detection

The detection limits for arsenic and selenium in serum are based on the standard deviation from counting statistics only, using the lowest values among the samples analysed.

For manganese the limit of detection is determined by the concentration of <sup>54</sup>Mn tracer required for a satisfactory measurement of chemical yield. In the present method the <sup>54</sup>Mn concentration was chosen to ensure a precision of better than 1% for the chemical yield, and the resulting limit of detection is practically the same as the manganese blank value.

The limit of detection for manganese is inversely proportional to the relative standard deviation of the chemical yield, so that a lower concentration of <sup>54</sup>Mn could be useful in the analysis of redistilled water blanks. However, if a limit of detection below 0.005 ppb is desired, yield determi-

nation by re-irradiation gives superior precision, and no <sup>54</sup>Mn tracer is added.

This modification was actually used in the analysis of redistilled water blanks for the investigation of the origin of sample contamination. In this case the chloroform extract is evaporated instead of being extracted with hydrochloric acid, and the residue dissolved in nitric acid.

The a priori detection limits according to Currie<sup>12)</sup> are given in table 6 for all three elements in serum.

Table 6

Limits of detection and accuracy  
for the determination of As, Mn, and Se in serum

Element	Detection limit	Bias	Blank value
As	0.25 ppb*	-1%	< 0.2 ppb
Mn	0.02 ppb	-1%	0.02 ± 0.01 ppb
Se	10 ppb	+2%	< 0.2 ppb

\*Assuming absence of bromine interference

#### Accuracy of Results

The use of a different irradiation container for serum than for the comparator standards gives rise to a systematic error because of the vertical flux gradient in the reactor. This error has been estimated to yield a 1% negative bias for arsenic and manganese and a 2% positive bias for selenium. For the actual concentrations of these elements in serum, these errors are below the limits of detection, and no correction has been made in the results.

For manganese, accuracy was confirmed by the excellent agreement between our results for dried animal blood, and those of 3 other laboratories submitting their results to IAEA for intercomparison. Two of these laboratories used neutron activation analysis, while the third used atomic absorption; their pooled results are given in table 5 together with our uncorrected - results.

Blank Correction

The blank values for arsenic and selenium are below the detection limits, whereas for manganese this may not be the case. The origin of the manganese blank should therefore be investigated.

Duplicate samples of freshly made redistilled water were taken from the same bottle at the same time and transferred to polyethylene ampoules in a clean room. One sample, serving as a reference, was irradiated and analysed for manganese in the usual manner. The other was frozen and surrounded by solid carbon dioxide during an entire one-hour irradiation. After irradiation the frozen sample was removed from the container, and the surface layers were allowed to melt away. Only a solid core of ice which had not been in contact with the container wall was used for analysis.

The difference between the two results was 0.0035 ng/ml, which represents the contribution to the blank value from the irradiation container. Although probably significant, its contribution is definitely below our limit of detection for manganese in serum.

The greater part of the manganese blank value must represent an actual presence of manganese in the redistilled water samples, the origin of which is not known. It would be misleading to apply corrections on this basis, and consequently analytical results for all three elements in human serum are presented without blank correction.

Nuclear Interference

Nuclear transmutation brought about by fast neutrons during sample irradiation gives rise to interference from elements with atomic number one or two higher than the element to be determined.

The magnitude of this interference was estimated from the (n, p) and (n,  $\alpha$ ) cross sections given by Roy and Lawton<sup>13</sup>, and the highest values were found for the reactions  $^{56}\text{Fe}(n, p)^{56}\text{Mn}$  and  $^{81}\text{Br}(n, p)^{81}\text{Se}$ . The interferences from iron and bromine on the results for manganese and selenium were therefore determined experimentally.

Samples of specially purified  $\text{Fe}_2\text{O}_3^*$  were irradiated along with dried Mn comparator standards within and without a 0.5 mm thick cadmium box in the same irradiation facility as used for serum samples. Instrumental determinations of  $^{56}\text{Mn}$  were made with a scintillation detector as well as with a semiconductor detector.

\* 0.3 ppm of manganese, courtesy of Ulf Jacobsen

Table 7

Interference from nuclear transmutation

Element determined	Interfering reaction	Error of 1 ppb produced by
As	$^{76}\text{Se}(n, p)^{76}\text{As}$	~ 3000 ppm Se
	$^{79}\text{Br}(n, \alpha)^{76}\text{As}$	~ 4000 ppm Br
Mn	$^{56}\text{Fe}(n, p)^{56}\text{Mn}$	660 + 20 ppm Fe
	$^{59}\text{Co}(n, \alpha)^{56}\text{Mn}$	~ 2000 ppm Co
Se	$^{81}\text{Br}(n, p)^{81}\text{mSe}$	106 + 3 ppm Br

~ Estimated from ref. 13

+ Experimentally determined

Ammoniumbromide and Se comparator standards in aqueous solutions were irradiated under similar conditions, and after radiochemical separation by reduction of Se with hydrazine hydrate,  $^{81\text{m}}\text{Se}$  was determined as previously described.

Table 7 shows the two experimentally determined interferences together with other estimated interferences based on a thermal to fast neutron ratio of 44, which had been determined earlier by routine measurements of Co and Ni monitors.

On the basis of these values it was concluded that nuclear interference on the results for arsenic, manganese and selenium in serum is well below the limits of detection, even at the highest reported<sup>10</sup> concentrations of interfering elements.

From a measured value of nuclear interference the fast neutron cross section may be calculated from the thermal cross section of the element to be determined. With an activation cross section for manganese of  $13.2 + 0.1$  barn<sup>14</sup> the cross section for  $^{56}\text{Fe}(n, p)^{56}\text{Mn}$  was  $1.04 + 0.03$  millibarn, which is in excellent agreement with reported<sup>15</sup> values for the Watt fission spectrum.

For the reaction  $^{81}\text{Br}(n, p)^{81\text{m}}\text{Se}$  the cross section ratio was  $0.46 \times 10^{-3}$ , but the cross section for the formation of  $^{81\text{m}}\text{Se}$  from selenium is not accurately known. With a value of 30 mb<sup>14</sup> the fission neutron reaction cross section is 0.014 mb, which is in good agreement with the only reported experimental determination of 0.012 mb<sup>16</sup>.

SAMPLE PREPARATION

All steps preceding the irradiation of a serum sample should be taken with the utmost care to ensure that the composition of the in vitro sample analysed truly represents the in vivo condition of the patient.

Contamination from equipment in contact with the sample, as well as loss of specific components by volatilization or adsorption, may take place during sample preparation, and an investigation of such errors must be made before the reliability of results can be judged.

The preparation of a serum sample requires the following steps:

Blood samples taken by venipuncture are collected in a centrifuge tube and allowed to clot. After separation from the clot, serum is transferred to the irradiation container and stored in a deep freezer until activation.

Contamination Control

The most critical element from the point of view of contamination is manganese, and routine sampling techniques applied to redistilled water produced levels of manganese comparable to those found in serum.

The influence of individual steps were therefore studied by changing the procedure one step at a time, and it was observed that

- a) separation of serum by centrifugation gave less contamination than mere standing on the table,
- b) transfer of serum with Pasteur pipettes gave less contamination than decanting.

These observations led to the conclusion that environmental contamination in the sampling room is of major concern, and the sample preparation was moved to an isolated room with limited access.

The study was now repeated, and all variations gave results similar to the best values previously obtained.

However, the level of contamination was still significantly above the detection limit for manganese in serum, and further improvement was therefore desirable.

Contamination from the platinum cannula was disregarded, the first milliliter of a sample being deliberately spilled, and the Pasteur pipettes were flushed with redistilled water immediately before use. The centrifuge tubes and the polyethylene ampoules were for practical reasons cleaned in advance, and their contribution to sample contamination was therefore investigated.

Table 8

Manganese contamination in simulated serum samples

Centrifuge tubes		Manganese contamination pg/ml	
Material	Cleaning	Set 1	Set 2
Pyrex	Hospital	54.9	26.9
Pyrex	Risø	28.9	7.6
Polypropylene	Risø	45.2	13.9
Lusteroid	Risø	43.9	8.7

Set 1: Polyethylene ampoule cleaned at Risø and stored 2 months at the hospital.

Set 2: Polyethylene ampoule cleaned at Risø the day before sample preparation.

Simulated sample preparations with freshly made redistilled water were carried out with different centrifuge tubes and polyethylene ampoules, cleaned the day before, as well as with polyethylene ampoules cleaned two months earlier. All samples were prepared by the same person on the same day, and results are presented in table 8.

Clearly, prolonged storage of cleaned containers under normal hospital conditions is not acceptable; on the other hand, all results with freshly cleaned containers from Risø were below our limit of detection for manganese in serum.

Analysis of the same samples for arsenic showed contamination from the centrifuge tube cleaned at the hospital, while contamination from other centrifuge tubes was below the limit of detection.

It was concluded that with proper precautions during sample preparation, contamination of serum samples can be reduced below the limits of detection for our analytical method.

## Sampling Procedure

### Equipment

Platinum cannulae with gold-plated brass heads, Simonsen & Weel

After cleaning, repeatedly flushed with redistilled water, and placed in similarly treated glass tubes, stoppered with hydrophobic cotton; sterilized in a steam autoclave.

Pyrex centrifuge tubes, round-bottomed, 15 ml

After cleaning, immersed in a 3% hydrogenperoxyde solution for at least 3 hours, rinsed with redistilled water, and dried upside-down on tissue-paper at 100°; stored individually in sealed polyethylene bags.

Polyethylene ampoules, 5 ml, Atomic Industrial Co.

Rinsed with redistilled water, and dried upside-down on a tissue-paper at room temperature in a dust-free place; stored individually in heat-sealed polyethylene bags.

Pasteur pipettes, Harshaw, disposable

Stored in original box until use.

Parafilm M covers, 3M

Cut when required.

### Serum Sampling

Venipuncture is made with a sterile platinum cannula through normal, clean skin, no disinfectants being applied. The first milliliter of blood is discarded, and about 10 ml is collected in a centrifuge tube, cut out from its protective polyethylene bag.

The centrifuge tube is immediately covered with Parafilm, and the blood is allowed to clot. After clotting, serum is separated by centrifugation at 800 g (2000 rpm) for 10 minutes.

In a dust-free room a Pasteur pipette is flushed repeatedly with redistilled water and used to transfer the serum to a polyethylene ampoule, cut out from its protective bag. The Parafilm cover is only removed from the centrifuge tube to allow introduction of the pipette, and the polyethylene ampoule is closed with Parafilm, as soon as it has been filled with about 4½ ml serum.

### Storage

As soon as possible after completion of the transfer operation the sample is frozen, and the polyethylene ampoule is placed in a larger polyethylene receptacle.

Storage takes place in a freezer at -20° until the time of analysis.

## Adsorption Losses

Losses during sample preparation are even more difficult to control than contamination, because they depend on the actual chemical form of the elements in serum.

The effect of adsorption may be studied by preparation of the same sample, using containers of different materials. This was done with redistilled water, and results for manganese in table 8 indicate no difference between the lowest values for pyrex and Lusteroid centrifuge tubes. Analysis for arsenic of the same samples showed the same agreement between results, in spite of the very different adsorption characteristics of the two container materials.

Loss of manganese from dilute aqueous solutions during storage in polyethylene containers was observed in our previous study<sup>2)</sup>, and it is therefore important that storage of serum samples takes place in the frozen state.

Although the results for aqueous samples with inorganic manganese are not strictly applicable to serum samples, adsorption errors are probably overestimated rather than underestimated.

With the chosen sampling procedure, and with less than 2 hours between the transfer of serum and freezing, no detectable adsorption errors are therefore envisaged.

## Volatilization Losses

Both arsenic and selenium form volatile compounds, which may be partially or completely lost during sample preparation. Such losses have in fact been reported for arsenic<sup>17)</sup>, but the identity of the compound was not established.

Selenium is known to occur in the organism in the form of dimethylselenide, which is excreted by respiration. Its presence in blood must therefore be taken into account, and the recovery of added dimethylselenide was determined under simulated sampling and storage conditions.

A small quantity of dimethylselenide was mixed with 5.5 ml of human serum in a centrifuge tube, and a 1.0 g sample was taken for the analysis of initial selenium content. The remaining solution was covered with Parafilm, and after 30 minutes 4.4 g was transferred to a polyethylene ampoule as described in the sampling procedure. The ampoule was closed with Parafilm, and 2 hours later transferred to a freezer for storage at -20°.

Selenium contents were determined by instrumental neutron activation analysis with 17.4 seconds  $^{77m}\text{Se}$  as indicator. Irradiation for 5 seconds in the pneumatic tube system and counting for 30 seconds after 45 seconds' decay gave a total analysis time of less than  $1\frac{1}{2}$  minutes, during which the sample remained in the frozen condition.

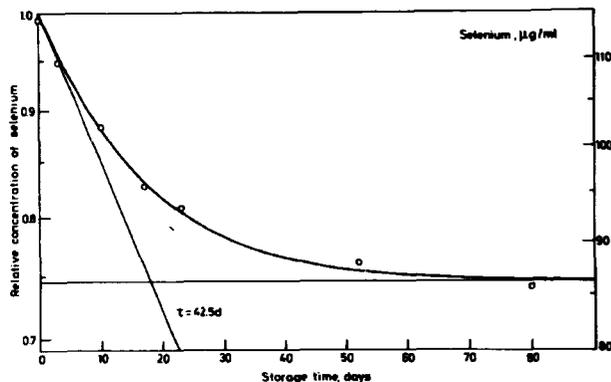


Fig. 2. Loss of dimethylselenide from serum during storage at  $-20^{\circ}$

The initial concentration of selenium was 115  $\mu\text{g}/\text{ml}$ , and the concentrations at different times during storage at  $-20^{\circ}$  are shown in figure 2. No detectable loss of selenium took place during sampling, but considerable losses occurred during storage, by diffusion through the container walls.

The results in figure 2 indicate concurrent losses of dimethylselenide by diffusion and by decomposition. Assuming that both are first-order reactions, we estimated their rate constants from the data by iteration; the equivalent half-lives were 6 weeks for diffusion and 2 weeks for decomposition.

Similar experiments at room temperature showed a diffusion rate greater than the rate of decomposition, so that the storage at low temperature gives a considerable reduction of the loss of selenium from dimethylselenide in the sample.

Actual storage periods of between 1 and 4 weeks give losses of 10-20%, but these losses will be below the limit of detection for selenium, unless more than half of the total selenium found in serum is originally present as dimethylselenide.

## RESULTS

The goal of the present study is to establish levels of arsenic, manganese and selenium in normal, human serum. Considerable care must therefore be exercised in the selection of normal persons, from whom samples are taken.

Selection was made from persons admitted to the surgical wards of Departments I and V of the Copenhagen Municipal Hospital, for either simple fractures or cerebral concussion. Physical examination should indicate no abnormality other than the trauma causing admission, and persons receiving drugs, including analgesics and antibiotics, were excluded.

Normal blood pressure and normal values for hemoglobin and serum creatinine were required, and tests for hemoglobin, protein and glucose in urine should be negative.

Final selection of the normal group as well as the actual sampling by venipuncture in the antecubital fossa were in all cases carried out by one of the authors; all samples were taken in duplicate.

The 1971 series was undertaken with what was believed to be an absolutely satisfactory sampling procedure, essentially as described in this report. Individual analytical results together with their calculated precision are presented in tables 9 and 10.

The average precision for arsenic was 0.17 ng/ml, and for manganese 0.04 ng/ml; in both cases the variation between duplicate results far exceeded what could be ascribed to known sources of variation. These results are therefore not representative of actual concentrations of these elements in human serum, but rather of uncontrolled variations in sampling conditions.

After careful study of the sampling process as described in this report, a new series was undertaken the following year.

For selenium, the average precision of 7 ng/ml is in good agreement with the observed variation between duplicate results, and no additional sources of variation are therefore suspected. No correlation between storage time and selenium concentration could be detected, and variations between samples are consequently expected to reflect actual variations of selenium concentrations in normal, human serum.

Table 9

Results for arsenic and manganese in human serum of the 1971 series

Identification	Arsenic ng/ml	Manganese ng/ml
270755	2.06 ± 0.17	0.45 ± 0.03
	2.11 ± 0.35	0.56 ± 0.04
081250	0.31 ± 0.18	0.66 ± 0.06
	1.11 ± 0.17	0.45 ± 0.03
180149	1.40 ± 0.15	0.51 ± 0.07
	0.70 ± 0.14	0.63 ± 0.05
150248	0.36 ± 0.25	0.60 ± 0.04
	0.24 ± 0.14	
190745	1.34 ± 0.14	0.61 ± 0.04
	1.35 ± 0.16	0.41 ± 0.03
230645	1.54 ± 0.20	0.87 ± 0.05
	2.22 ± 0.21	2.40 ± 0.10
230641	0.22 ± 0.18	0.38 ± 0.03
	0.65 ± 0.10	0.39 ± 0.03
170241	1.02 ± 0.15	0.35 ± 0.03
	1.21 ± 0.14	0.48 ± 0.03
040940	2.00 ± 0.21	0.58 ± 0.04
	2.65 ± 0.30	0.61 ± 0.05
260226	2.74 ± 0.40	0.40 ± 0.04
	2.34 ± 0.20	0.39 ± 0.03
120923	2.49 ± 0.17	0.46 ± 0.03
	2.76 ± 0.48	0.83 ± 0.04
110212	0.27 ± 0.13	0.62 ± 0.04
	0.29 ± 0.22	0.56 ± 0.04

The 1972 series was carried out with the same sampling procedure as the previous series, but in more secluded surroundings and with freshly cleaned sample containers, which were covered during practically the entire procedure to protect against dust. Individual analytical results for arsenic and manganese are presented in table 11.

For arsenic, the average, calculated precision of 0.11 ng/ml is now in very good agreement with the observed variation between duplicate results, and no correlation between storage time and arsenic concentration was detected. The reported arsenic concentrations are therefore expected to be truly representative of normal, human serum.

For manganese, the average, calculated precision of 0.03 ng/ml could not account for the observed variation between duplicate results. However, in a separate paper<sup>18)</sup> it is shown that the residual variation is so small that it does not significantly influence either the mean value or the standard deviation of the distribution of actual manganese concentrations in normal, human serum.

Means and their standard errors for all three elements, calculated from the results for 11 different adults of established normality in the tables 10 and 11, are given in table 12.

In addition, an Index of Determination was calculated from the average precision of a single analytical result,  $\sigma_a^2$ , and the observed sample variance,  $(\sigma_p^2 + \sigma_a^2)$ , with  $\sigma_p^2$  as the population variance,

$$\text{Index} = \frac{\sigma_p^2}{\sigma_p^2 + \sigma_a^2} = 1 - \frac{\sigma_a^2}{(\sigma_p^2 + \sigma_a^2)}$$

These indexes express the sufficiency of the analytical method for the measurement of the three elements in human serum.

Finally, the last column in table 12 lists the relative standard deviation as the square-root of the population variance in percent of the mean.

Table 10

Concentrations of selenium in normal, human serum

Age and sex	Hemoglobin g/l	Creatinine mmol/l	Selenium ng/ml	Weighted mean
16 ♀	132	0.07	82 ± 6	88
			111 ± 12	
26 ♀	113	0.07	84 ± 8	81
			78 ± 8	
30 ♀	117	0.08	107 ± 8	97
			89 ± 7	
48 ♀	132	0.10	101 ± 7	103
			104 ± 7	
20 ♂	129	0.10	96 ± 6	98
			101 ± 7	
23 ♂	146	0.11	79 ± 8	83
			88 ± 7	
26 ♂	133	0.11	69 ± 6	69
			69 ± 6	
30 ♂	139	0.08	101 ± 8	108
			111 ± 8	
31 ♂	138	0.12	85 ± 7	91
			101 ± 9	
45 ♂	140	0.11	87 ± 5	71
			82 ± 8	
59 ♂	151	0.10	84 ± 7	88
			94 ± 9	

Table 11

Concentrations of arsenic and manganese in normal, human serum

Age and sex	Hemoglobin g/l	Creatinine mmol/l	Arsenic ng/ml	Weighted mean	Manganese ng/ml	Mean
26 ♀	111	0.11	0.79 ± 0.11	0.73	0.33 ± 0.02	0.36
			0.66 ± 0.11		0.39 ± 0.03	
49 ♀	115	0.10	0.91 ± 0.13	0.87	0.67 ± 0.04	0.66
			0.84 ± 0.11		0.65 ± 0.03	
49 ♀	124	0.11	1.77 ± 0.12	1.82	0.67 ± 0.03	0.73
			1.90 ± 0.14		0.80 ± 0.04	
56 ♀	130	0.10	0.49 ± 0.09	0.46	0.80 ± 0.04	0.77
			0.44 ± 0.07		0.75 ± 0.03	
63 ♀	110	0.11	1.75 ± 0.13	1.65	0.78 ± 0.04	0.78
			1.57 ± 0.11		0.78 ± 0.03	
14 ♂	140	-	1.42 ± 0.11	1.42	0.35 ± 0.02	0.39
					0.45 ± 0.03	
20 ♂	154	0.10	0.81 ± 0.10	0.78	0.51 ± 0.03	0.48
			0.76 ± 0.09		0.45 ± 0.03	
47 ♂	134	0.09	1.54 ± 0.16	1.51	0.37 ± 0.04	0.41
			1.48 ± 0.16		0.44 ± 0.03	
50 ♂	122	0.11	0.83 ± 0.15	0.88	0.38 ± 0.03	0.44
			0.98 ± 0.22		0.50 ± 0.03	
51 ♂	129	0.11	0.97 ± 0.16	0.71	0.40 ± 0.02	0.41
			0.65 ± 0.08		0.43 ± 0.02	
66 ♂	137	0.12	1.11 ± 0.14	0.93	0.54 ± 0.05	0.49
			0.75 ± 0.14		0.44 ± 0.05	

## DISCUSSION

Table 12  
Summary of determinations of arsenic, manganese and selenium  
in normal, human serum

Element	Concentration ng/ml		Index of determination	Population standard deviation
	Mean	Standard error		
Arsenic	1.07	0.14	0.94	41%
Manganese	0.54	0.05	0.90	30%
Selenium	89	4	0.87	12%

Arsenic

Previous measurements of arsenic in plasma and serum were reported by Heydorn<sup>19)</sup> who found a logarithmic normal distribution of plasma concentrations in normal Taiwanese subjects. The levels in plasma from normal Danish subjects were below the limit of detection, and only a mean value of  $2.4 \pm 0.6$  ng/ml was reported.

The improved precision of our present results mainly comes from improved sampling conditions, which also result in reduced contamination of the samples. In addition, the <sup>82</sup>Br correction reduces the results by an average 0.24 ng/ml, so that the difference between the present 1971 series average and the above is only 0.8 ng/ml, which is not significant.

The 1972 series is more accurate, as well as precise, but the limited number of samples does not permit a distinction between the Gaussian and the log-normal distribution.

No correlation with other observed factors could be found.

Manganese

Previous measurements of manganese in serum or plasma are summarized in table 13.

The individual results reported by Guinn<sup>20)</sup> form a logarithmic normal distribution with a logarithmic standard deviation of 45%; the modal concentration is 19.5 ng/ml. This is in manifest disagreement with the expected distribution for an essential element like manganese, and there is little doubt that the results represent the levels of contamination encountered during sampling.

This variation in sampling conditions easily accounts for the very considerable differences between results reported by other investigators, with the exception of the lowest reported values for serum<sup>3)</sup> and for plasma<sup>4)</sup>; these in turn are in good agreement, and our present results are indistinguishable from both of them.

This indicates an absence of significant geographical variation, which allows us to pool all 37 results from the 3 investigations; the grand mean becomes 0.59 ng/ml with a relative standard deviation of 27%.

No correlation of Mn-concentrations with age, sex or other observed factors could be established from our present results.

Table 13

Concentrations of manganese in normal, human serum or plasma

Serum		Plasma		Investigation		Number of results
Mean ng/ml	Standard deviation	Mean ng/ml	Standard deviation	Reference	Year	
0.63	20%			Fernandez <sup>3)</sup>	1963	12
22*	-			Guinn <sup>20)</sup>	1965	98
		4.3	40%	Olehy <sup>6)</sup>	1966	12
		0.59	31%	Cotzias <sup>4)</sup>	1966	14
13	15%			Kanabrocki <sup>21)</sup>	1967	41
1.4	37%			Campero <sup>5)</sup>	1967	128
112	14%			Malikova <sup>22)</sup>	1970	20
		14	44%	Arsagova <sup>23)</sup>	1971	27
4.6	-			Maziere <sup>7)</sup>	1972	-
0.54	30%			This work		11

\* Median

Table 14

Concentrations of selenium in serum or plasma from normal adults

Serum		Plasma		Investigation		Number of individuals
Mean ng/ml	Standard deviation	Mean ng/ml	Standard deviation	Reference	Year	
100	-			Gofman <sup>25)</sup>	1964	39
102	16%			Guinn <sup>20)</sup>	1965	93
		144	20%	Dickson <sup>26)</sup>	1967	253
		111	21%	Levine <sup>27)</sup>	1970	4
59	27%			Behne <sup>28)</sup>	1972	4
98	18%			Kasperek <sup>24)</sup>	1972	164
		58	-	Maxia <sup>29)</sup>	1972	46
		102	18%	Rhead <sup>30)</sup>	1972	7
129	26%			Morss <sup>31)</sup>	1972	11
89	-			Maziere <sup>7)</sup>	1972	-
89	12%			This work		11

Selenium

Previous measurements of selenium in serum or plasma are summarized in table 14.

The individual results reported by Guinn<sup>20)</sup> form a Gaussian distribution with a relative standard deviation of only 16%, which is confirmed by later studies, and in good agreement with results for other essential elements like Cu and Zn<sup>24)</sup>. In fact, Bartlett's test reveals no disagreement between 7 reported values for the coefficient of variation, and the pooled variance gives an overall relative standard deviation of 19%.

While no significant difference between serum and plasma is seen, considerable differences between the mean values reported by different investigators are observed, indicating the presence of significant geographical variations.

Our present results are in good agreement with the overall geographical mean of  $99 \pm 10$  ng/ml; likewise our value for the relative standard deviation is not significantly different from the most probable value of 19% referred to above, but significantly smaller than that of manganese.

No significant correlation of Se-concentrations with age, sex, or other observed factors could be detected in our present results.

## CONCLUSION

Simultaneous determination of arsenic, manganese, and selenium in human serum can be carried out with satisfactory precision and accuracy by neutron activation analysis using radiochemical separation. As was the case with our previous method for the analysis of tissue, the present procedure is well suited for routine use in the analytical laboratory.

Meaningful results were, however, only achieved after careful evaluation and control of sampling conditions to reduce the level of contamination to insignificance; the sampling procedure is probably reliable only in the hands of experienced investigators.

## REFERENCES

- 1) N. A. Larsen, B. Nielsen, H. Pakkenberg, P. Christoffersen, E. Damsgaard, and K. Heydorn, Neutron Activation Analysis of Arsenic, Manganese and Selenium Concentrations in Organs of Uraemic and Normal Persons. In: Nuclear Activation Techniques in the Life Sciences, Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 561-568.
- 2) K. Heydorn and E. Damsgaard, Simultaneous Determination of Arsenic, Manganese, and Selenium in Biological Materials by Neutron Activation Analysis. In: Aspects of Research at Risø. Risø Report No. 256 (1972) 143-161.
- 3) A. A. Fernandez, C. Sobel, and S. L. Jacobs, Sensitive Method for the Determination of Submicrogram Quantities of Manganese and Its Application to Human Serum. Anal. Chem. 35 (1963) 1721-1724.
- 4) G. C. Cotzias, S. T. Miller, and J. Edwards, The stability of manganese concentrations in human blood and serum. J. Lab. & Clin. Med. 67 (1966) 836-849.
- 5) A. Campero, F. M. Graber, and H. R. Lukens, Neutron Activation Analysis of Biological Materials. In: Proceedings of the International Conference on the Utilization of Research Reactors and Reactor Mathematics and Computation, Mexico City, 2-4 May 1967 (CNM-R-2) Vol. I, 346-356.
- 6) D. A. Olehy, R. A. Schmitt, and W. F. Bethard, Neutron Activation Analysis of Magnesium, Calcium, Strontium, Barium, Manganese, Cobalt, Copper, Zinc, Sodium, and Potassium in Human Erythrocytes and Plasma. J. Nucl. Med. 7 (1966) 917-927.
- 7) B. Maziere, A. Gaudry, W. Stanilewicz, and D. Comar, Possibilités et Limites de L'Analyse par Activation Neutronique Multielementaire d'Echantillons Biologiques avec ou sans Separation de la Matrice Activable. Paper presented at International Conference on Modern Trends in Activation Analysis, Saclay, 2-6 October 1972. 20 pp.
- 8) H. Bode and F. Neumann, Extraktionen mit Lösungen von Diäthylammonium diäthylthiocarbamidat in organischen Lösungsmitteln. Z. analyt. Chem. 172 (1960) 1-21.

- 9) L. Plantin, Bestämning av brom i biologisk vävnad med aktiveringsanalys. Paper presented at 3die Nordiske Sporelement Konfernce, Århus, 14-16 September 1971. 1 pp.
- 10) L. R. Anspaugh et al., Compilation of Published Information on Elemental Concentrations in Human Organs in both Normal and Diseased States. UCRL-51013 (1971) 160+107 pp.
- 11) J. Heinonen and O. Suschny, The Reliability of Neutron Activation Analysis for Trace Elements in Biological Materials. In: Nuclear Activation Techniques in the Life Sciences, Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 155-173.
- 12) L. A. Currie, Limits for Qualitative Detection and Quantitative Determination. Anal. Chem. 40 (1968) 586-593.
- 13) J. C. Roy and J. J. Hawton, Table of Estimated Cross Sections for (n, p), (n,  $\alpha$ ) and (n, 2n) Reactions in a Fission Neutron Spectrum. AECL-1181 (1960) 36 pp.
- 14) A. I. Aliev, V. I. Drynkin, D. I. Leipunskaya, and V. A. Kasatkin, Handbook of Nuclear Data for Neutron Activation Analysis. Translated by B. Benny. (Israel Program for Scientific Translations, Jerusalem, 1970) 168 pp.
- 15) A. M. Bresesti, M. Bresesti, A. Rota, and R. A. Rydin, Threshold Reaction Excitation Functions Intercalibrated in a Pure Fission Spectrum. Nucl. Sci. Eng. 40 (1970) 331-348.
- 16) E. Steinnes, Cross-Sections of some (n, p) and (n,  $\alpha$ ) Reactions Induced by Reactor Fast Neutrons. Kjeller Report KR-123 (1967) 9 pp.
- 17) H. S. Satterlee and Gertrude Blodgett, Ultramicrodetermination of Arsenic by Gutzzeit Spot-Filtration under Vacuum. Ind. Eng. Chem. (Anal. Ed.) 16 (1944) 400-407.
- 18) K. Heydorn and Krista Nørgård, Analysis of Precision of Activation Analysis Methods. Paper presented at International Conference on Modern Trends in Activation Analysis, Saclay, 2-6 October 1972. 10 pp.
- 19) K. Heydorn, Environmental Variation of Arsenic Levels in Human Blood Determined by Neutron Activation Analysis. Clin. Chim. Acta 28 (1970) 349-357.
- 20) V. P. Guinn, H. R. Lukens, Jr., and L. A. Weinrich, Activation Analysis of Blood Serum. GA-6658 (1965) 50 pp.

- 21) E. L. Kanabrocki, L. F. Case, L. Graham, T. Fields, E. B. Miller, Y. T. Oester and E. Kaplan, Non-Dialyzable Manganese and Copper Levels in Serum of Patients with Various Diseases. J. Nucl. Med. 8 (1967) 166-172.
- 22) T. A. Malikova, Influence of Hydrotherapy on the Concentration of some Trace Elements in Serum and Blood from Patients with Residual Symptoms of Contagious Hepatitis. Voprosy Kuzortologii, Fizioterapii i Lechebnj Fizicheskoj Kultury 35 (1970) 212-217.
- 23) N. S. Arsagova, On the Nickel and Manganese Content in Patients with Uterine Cancer. Voprosy Onkologii 17 (1971) 53-56.
- 24) K. Kasperek, H. Schicha, V. Siller, L. E. Feinendegen, and A. Höck, Trace-Element Concentrations in Human Serum: Diagnostic Implications. In: Nuclear Activation Techniques in the Life Sciences, Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 517-526.
- 25) J. W. Gofman et al., Chemical Elements of the Blood of Man. Arch. Envir. Health 9 (1964) 105-109.
- 26) R. C. Dickson and R. H. Tomlinson, Selenium in Blood and Human Tissues. Clin. Chim. Acta 16 (1967) 311-321.
- 27) R. J. Levine and R. E. Olson, Blood Selenium in Thai Children with Protein-Calorie Malnutrition. Proc. Soc. Exp. Biol. 134 (1970) 1030-1034.
- 28) D. Behne and F. Diel, Relations between Carbohydrate and Trace-Element Metabolisms Investigated by Neutron Activation Analysis. In: Nuclear Activation Techniques in the Life Sciences, Bled, Yugoslavia, 10-14 April 1972 (IAEA, Vienna, 1972) 407-414.
- 29) V. Maxia, S. Meloni, M. A. Rollier, A. Brandone, V. N. Patwardhan, C. L. Waslien, and Said El Shami, Selenium and Chromium Assay in Egyptian Foods and in Blood of Egyptian Children by Activation Analysis. Ibid. 527-550.
- 30) W. J. Rhead, E. E. Cary, W. H. Allaway, S. L. Saltzstein, and G. N. Schrauzer, The Vitamin E and Selenium Status of Infants and the Sudden Infant Death Syndrome. Bioinorganic Chemistry 1 (1972) 289-294.
- 31) S. G. Morss, H. R. Ralston, and H. S. Olcott, Selenium Determination in Human Serum Lipoprotein Fractions by Neutron Activation Analysis. Analytical Biochemistry 49 (1972) 598-601.

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