STUDY ON CORRELATION OF TRACE ELEMENTS IN HUMAN HAIR AND INTERNAL ORGANS BY NUCLEAR METHODS

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Abstract

Autopsy samples from 24 human males, aged 35-60 years were collected from Shanghai, People's Republic of China, to study possible relationships between the trace element content in hair and internal tissues. Samples of hair, kidney-cortex and lung were collected and analyzed. A radiochemical neutron activation analysis (RNAA) procedure was developed based on a simple group extraction scheme using zinc-diethyl dithiocarbamate and methyl-isobutyl ketone-iodide. The trace elements As, Cd, Hg, Cu and Zn were measured following this procedure. In addition, the elements Se, Na, Mg, Cl, Br, S, Cr, Co, and Rb were determined using instrumental neutron activation analysis (INAA). The reliability of the analytical procedures were checked analyzing several biological reference materials, such as IAEA-H-8 Horse Kidney, NBS-SRM-1573 Tomato Leaves and NIES-5 Human Hair. The results showed a larger concentration variability for As, Cd and Hg than for Cu, Se and Zn. Some significant positive correlations were found for some elements among different tissues.

1. INTRODUCTION

In recent years, physiological role of trace elements in living body has attracted a lot of researchers. The effects of trace elements on human health have also studied increasingly in China. The contents of trace elements in different tissues are affected by occupational and environmental exposure, daily nutrition, age, sex and various diseases. Such as the pathogeny of Kashan disease - a local epidemic in some area of northern China is established mainly by lack of selenium in local area [1]. The Chinese doctors have considered adding Se to table salt as a protective measure to prevent heart disease. The trace element in hair of longevo in Shanghai have been determined by X ray fluorescence analysis [2]. The results show that the contents of Mn, Fe, Cr and Ti in hair are obviously higher than ordinary old people. The trace elements in Chinese foodstuffs the dietary nutritional status and trace element in resident hair have been determined and evaluated recently [3-5]. However, most researches concentrated their efforts on analyzing the trace element constituents of patient or normal human hair, without the knowledge of correlation of trace elements between human hair and internal organs, so it is hardly to interpret the cause of diseases. On the other hand, the human hair has a number of characteristics as a practical indicator for evaluating the environmental pollutants on human health. However, serious uncertainty still exists for interpretation on hair analytical data in environmental health studies because of the lack of knowledge about whether there is a quantitative relationship between mineral concentrations of hair and internal body burdens.

As a part of the International Atomic Energy Agency (IAEA) with Co-ordinated Research Programme on The Significance of Hair Mineral Analysis As a Means for

Assessing Internal Body Burdens of Environmental Pollutants, the main aims of the present study are to assess the elements of As, Cd, Hg, Cu, Se and Zn contents of hair and internal organs of autopsy cases in order to:

(1) investigate whether the toxic elements As, Cd and Hg contents in hair can be used as an indicator of body burden of these elements

(2) to try to clarify whether there are any correlations between elements in same tissue

(3) to try to find the relationship between the trace elements of body burden and the smoking habit and residential area of the subjects.

For these purposes, 24 autopsy samples of hair kidney-cortex, liver and lung were collected and analyzed for As, Cd, Hg, Cu and Zn by RNAA, Se, Cl, Na, Mn, Mg, Br, S, Cr, Co and Rb by INAA and Mn, Fe and Ca by PIXE.

2. EXPERIMENTAL

2.1. COLLECTION AND PRETREATMENT OF SAMPLE

Twenty-four male autopsies between 35-60 years old were obtained from Institute of Forensic Medicine in China. All autopsy samples were identified clearly by pathologist and tissue samples of liver, lung, kidney-cortex and scalp hair were collected within 24-48 hours after accidental death of persons who were proven in good health before the accident. The knowledge concerning the residence, profession, length of service and smoking habits of each subjects were obtained. The liver samples were collected from the superior half of the organ, lung samples were collected from the lower half of the right, kidney-cortex were collected from the outer cortex of the whole left one and hair samples were collected from the occipital region. To remove external contamination, the surface of the soft tissues were cut off using titanium knife, diced into 1-2 cm cubes in a clean bench and stored in a freezer at temperature below -30°C until taken out for analysis.

2.2. HOMOGENIZATION

The brittle fracture technique (BFT) was used to homogenize all the samples in our laboratory. Details of this method was discussed by Iyengar [7]. Soft samples were placed in the teflon vessel along with the teflon ball and the lid closed tightly. The vessel was then cooled in liquid nitrogen for a few minutes and vibrated for five minutes at 3000 cycles per minute with a commercially available Mikro-Dismembrator. Hair samples taken only less than 5 cm from proximal end were cut into 2 mm pieces and washed according to the IAEA recommended procedure [16] with acetone, water, water, water and acetone again. After air drying over night at room temperature, hair samples were homogenized by brittle fracture technique in liquid nitrogen with Mikro-Dismembrator as described before.

2.3. RADIOCHEMICAL NEUTRON ACTIVATION ANALYSIS

A simple group separation scheme based on extraction for determination of element As, Cd, Hg, Cu and Zn in biological samples were developed for this programme [8]. Zinc diethyl-dithiocarbamate $Zn(DDC)_2$ and methyl-isobutyl-ketone-iodide were chosen as reagents. The elements could be extracted successively and quantitatively from strong mineral acids without adjusting pH of the solution, and separated into two groups suitable for gamma ray spectrometry. Samples of 100-200 mg dry weight were double sealed into polyethylene bags and irradiated in a swimming pool type reactor with a thermal neutron flux of 1×10^{13} $n \cdot cm^{-2} \cdot s^{-1}$ for 44 h. After a cooling period of 1-3 days, the samples were digested with microgram quantities of carrier in concentrated nitric acid and sulphuric acid at 150°C for 3.5 h in teflon bomb, then extracted as described on separation scheme (Fig. 1). The procedure of RNAA was described in detail elsewhere [8].

2.4. INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS (INAA)

100 mg of lyophilized samples of liver, kidney-cortex, lung and scalp hair were heat sealed into clean polyethylene envelops. The chemical standards of the various elements were prepared by dropping appropriate solution on clean filter paper and heat sealing in a polyethylene envelope for quantitative analysis. The samples and standards were successively placed in a pneumatic transfer rabbit system from SLOWPOKE reactor and swimming pool type reactor for short-period and long-period irradiation at Beijing Institute of Atomic Energy. Table I shows the conditions of experiment. The activities were measured by using an Ortec Ge(Li) detector coupled to a CANBERRA S-80 4096 channel pulse-height analyzer equipped to a PDP 11/34 computer system. The net peak area, statistic error and the content of elements in samples were printed out automatically based on the SPAN computer program [9].

TABLE I. THE CONDITIONS OF EXPERIMENT FOR INAA

Neutron flux ($n \cdot cm^{-2} \cdot s^{-1}$)	Irradiation time	Cooling time	Counting time	Group of nuclides
8×10^{11}	60 s	5 s	60 s	^{77m}Se
5×10^{11}	8 min	60 s	400 s	^{24}Na, ^{38}Cl, ^{56}Mn, ^{27}Mg, ^{37}S, ^{82}Br
1×10^{13}	44 h	2-3 weeks	3600 s	^{51}Cr, ^{86}Rb, ^{60}Co

2.5. PROTON INDUCED X RAY EMISSION ANALYSIS (PIXE)

Each powder sample (50 mg) was dried at 90°C temperature for 4 h then ashed in plasma ashing oven and later dissolved in 6N nitric acid. An internal standard (Yttrium) was then added into the sampie. The target was composed of depositing a final solution onto a cleaning Mylar filter. The targets were irradiated in a vacuum chamber. A 3.5 Mev proton beam was obtained from a van de Graaff accelerator, diffused by a thin aluminium foil and finally collimated to a 6 mm diameter beam spot on the target. Typical beams of the order of 11-21 nA were used. The X ray were detected by a 32 mm^2 Si(Li) detector located at 135° with respect to the beam direction in the horizontal plane and the spectra were stored in a PDP-II/34 on line computer for handing and analysis .

The analysis of the PIXE spectra was done using the FORTRAN program AXIL developed by Van Espen et al. The minor and trace elements of Pb, Br, Ca, Fe, Sr, P and Mn in hair and tissues have been determined by PIXE.

The reliability both for NAA and PIXE was checked by a determination of the elements of interest in reference materials Bovine Liver NBS-SRM-1577a, Horse Kidney IAEA-H-8, Tomato Leave NBS-SRM-1573 and Human Hair NIES-5. Most of results in this work agree with the certified values. The relative standard deviation for most of these elements was found to be less than 10% [8,9].

3. RESULTS AND DISCUSSION

Analytical results for 24 cases of autopsy subject and some other information about the smoking habit and residence are given in Table II-IV and Figs 2-2[illegible].

The values are reported on dry weight bases in mg/kg. In these Tables, the number of samples (N), arithmetic mean (AM), standard deviation (SD) and geometric mean (CM) are reported for each element in each tissue. The range (minimum (MIN) and maximum (MAX) values) , and the median (MED) are also presented.

Mercury Hg is one of the important toxic elements this CRP. Chronic exposure to mercury vapour may affect other tissues besides the neuro system. From the results showed in Table II. The contents of Hg show a large variability both for hair and internal tissues. The level of Hg content is higher in hair (0.41-3.22 mg/kg) than in kidney-cortex (0.09-1.64 mg/kg), liver (0.09-0.93 mg/kg) and lung (0.01-0.20 mg/kg). It seems to be in accordance with the results for the order in concentration of Hg from 36 subjects in Sweden presented by Muramatsu [10]. The contents of hair Hg are usually depended on populations consuming methylmercury from fish. The contents of hair Hg for 32 area of thirteen countries have been presented by Airey [11]. The mean value of hair Hg in our work (1.18 mg/kg) is comparable to that of people residing in Italy (1.5 mg/kg).

TABLE II. ANALYTICAL RESULTS OF CHINESE AUTOPSY SAMPLES (mg/kg dry weight)

		Hair	Kidney cortex	Liver	Lungs
As	N	24	24	24	24
	AM±SD	0.40 ±0.22	0.11±0.06	0.16±0.11	0.10±0.07
	GM	0.34	0.09	0.14	0.08
	MED	0.34	0.11	0.12	0.07
	MIN	0.08	0.02	0.06	0.02
	MAX	0.97	0.30	0.58	0.27
Cd	N	24	24	24	24
	AM±SD	0.20±0.14	116.18±74.1	8.60±5.32	1.90±1.30
	GM	0.16	96.64	7.14	1.40
	MED	0.17	90.7	6.58	1.89
	MIN	0.04	21.1	1.79	0.15
	MAX	0.59	320.3	21.00	5.68
Hg	N	24	24	24	24
	AM±SD	1.18±0.65	0.62±0.46	0.37±0.26	0.10±0.04
	GM	1.04	0.47	0.29	0.08
	MED	1.00	0.47	0.34	0.09
	MIN	0.41	0.09	0.09	0.01
	MAX	3.22	1.64	0.93	0.20
Cu	N	24	24	24	24
	AM±SD	10.07±2.14	13.42±3.39	34.11±13.65	9.79±3.77
	GM	9.84	13.04	31.27	9.14
	MED	9.68	12.75	36.55	9.38
	MIN	5.34	9.02	13.50	5.30
	MAX	14.70	22.80	61.80	18.50
Se	N	24	24	24	24
	AM±SD	0.62±0.24	3.65±0.82	1.45±0.38	0.93±0.32
	GM	0.61	3.53	1.41	0.86
	MED	0.63	3.67	1.40	0.85
	MIN	0.37	2.04	0.73	0.50
	MAX	1.43	5.36	2.29	1.55

TABLE II. (cont.)

		Hair	Kidney cortex	Liver	Lungs
Zn	N	24	24	24	24
	AM ± SD	206.65 ± 43.3	181.87 ± 71.5	209.74 ± 55.1	54.99 ± 11.16
	GM	202.13	173.11	203.38	53.86
	MED	203.15	181.25	200.20	55.45
	MIN	112.40	73.30	114.80	29.80
	MAX	293.3	316.00	377.00	81.00
Mn	N	21	19	18	6
	AM ± SD	3.54 ± 2.37	4.11 ± 1.50	6.02 ± 2.31	1.26 ± 1.49
	MAX	8.72	7.39	12.5	4.14
	MIN	0.37	1.81	1.96	0.14
	MID	3.49	4.08	6.04	0.85
Fe	N	21	24	24	24
	AM ± SD	40.8 ± 22.2	292 ± 133	835 ± 560	1342 ± 1041
	MAX	84.9	642	2212	5924
	MIN	13.5	105	131	370
	MID	32.5	265	872	1307
Ca	N	22	24	24	24
	AM ± SD	960 ± 491	774 ± 432	341 ± 589	435 ± 231
	MAX	2523	1843	2420	1070
	MIN	357	350	63	211
	MID	839	626	168	355
Cr	N	11	6	7	12
	AM ± SD	1.83 ± 0.86	0.50 ± 0.30	0.645 ± 0.369	4.61 ± 3.71
	MAX	3.24	0.99	1.37	14.23
	MIN	0.52	0.27	0.323	1.73
	MID	1.62	0.39	0.467	3.09

TABLE II. (cont.)

		Hair	Kidney cortex	Liver	Lungs
Br	N	5	5	5	5
	AM±SD	3.32±0.75	18.9±1.8	4.62±0.93	23.8±4.0
	MAX	3.31	19.9	4.49	22.9
	MIN	2.55	16.4	3.32	20.3
	MID	4.18	20.6	5.91	30.1
Co	N	9	11	11	12
	AM±SD	0.037±0.010	0.061±0.015	0.155±0.156	0.145±0.146
	MAX	0.053	0.088	0.234	0.561
	MIN	0.026	0.034	0.080	0.039
	MID	0.036	0.06	0.124	0.093
S	N	5	5	5	5
	AM±SD	36764±5114	8590±2830	5131±928	8862±2732
	MAX	42220	13000	6153	10560
	MIN	29500	5999	4212	4180
	MID	35700	7940	4737	10420
Mg	N	4	5	4	5
	AM±SD	155±19	718±95	676±49	428±82
	MAX	179	750	655	459
	MIN	135	614	625	288
	MID	157	826	740	486
Na	N	5	5	5	5
	AM±SD	218±99	9563±1263	2972±1399	10408±2147
	MAX	202	9180	2252	9940
	MIN	88.6	7990	2065	7771
	MID	332	10915	5408	13560
Cl	N	5	5	4	5
	AM±SD	1198±533	9758±1774	3228±1615	10814±1373
	MAX	909	9270	2746	11280
	MIN	827	7460	2163	9220
	MID	2080	11600	5623	12550

TABLE II. (cont.)

		Hair	Kidney cortex	Liver	Lungs
Rb	N		11	12	11
	AM±SD		37.5±7.6	46.7±16.8	30.4±4.60
	MAX		52.5	73.7	38.4
	MIN		26.3	19.8	24.5
	MID		34.8	51.6	29.4

TABLE III. CORRELATION BETWEEN HAIR AND INTERNAL TISSUES FOR TRACE ELEMENTS OF 24 MALE AUTOPSY CASES

Elements	Tissues[a]	r-value[c]	p-value[b]
As	Kidney cortex	0.751	<0.01
	Liver	0.161	>0.05
	Lung	0.130	>0.05
Cd	Kidney cortex	0.418	<0.05
	Liver	0.05	>0.05
	Lung	-0.017	>0.05
Hg	Kidney cortex	0.175	>0.05
	Liver	0.028	>0.05
	Lung	-0.082	>0.05
Cu	Kidney cortex	-0.434	<0.05
	Liver	-0.247	>0.05
	Lung	0.026	>0.05
Se	Kidney cortex	0.635	<0.01
	Liver	0.639	<0.01
	Lung	0.570	<0.01
Zn	Kidney cortex	0.041	>0.05
	Liver	-0.092	>0.05
	Lung	-0.188	>0.05

[a]n = number of hair-tissue pairs; there are 24 pairs for every element

[b]r = value is degree of correlation in linear regression analysis

[c]p = value of probability of correlation

TABLE IV. INFORMATION ABOUT THE 24 MALE CASES AUTOPSIED

No.	Age	Residence	Profession	Smoking habits (cigarettes/day)
502	55	urban	manager	5-20
503	35	urban	assembler	non-smoker
504	60	urban	retired worker	non-smoker
505	57	urban	cook	more than 20
606	41	urban	steersman of barge	5-20
607	46	urban	electrician	non-smoker
608	51	suburban	pedlar	non-smoker
609	41	suburban	teacher	non-smoker
610	46	urban	builder	5-20
611	57	urban	retired worker	non-smoker
612	55	urban	electrician	5-20
613	59	suburban	boatman	5-20
614	52	urban	engineer	5-20
615	59	urban	retired worker	5-20
616	46	suburban	director of factory	non-smoker
617	46	urban	worker	5-20
618	52	urban	worker	5-20
619	42	urban	director of factory	5-20
620	42	urban	worker	more than 20
621	60	urban	officer	non-smoker
622	45	suburban	farmer	more than 20
623	54	suburban	worker	more than 20
624	57	suburban	farmer	5-20
625	41	urban	worker	non-smoker

New Zealand 1.3 mg/kg [11], France 1.33 mg/kg [12] and Iraq 1.4 mg/kg [12]. However, no significant relationships are found between Hg in hair and different tissues, even though the positive correlation between Hg concentrations in hair and kidney cortex has been reported by Y. Muramatsu [10].

Arsenic The contents of As show a large individual variability and there is almost difference of more than 10 fold in each sort of tissue. On contrary to the Bacso's results determined by XRF, the content of As in hair are lower than in kidney, liver and lung. We find the contents of As decrease in the order of hair, liver, kidney-cortex and lung. It is interesting to note the positive correlation between As concentration in hair and kidney-cortex (see Fig. 3) from statistic analysis for 24 cases of autopsy subject (correlation coefficient r = 0.751). The ratio of hair As to kidney As is 4.10 ± 2.0 in this work. However, no relationships are found between As in hair and liver or lung. In comparison with the literature, the average value of hair As (0.40 ± 0.22 mg/kg) is slightly higher than the results presented by other countries [11,12]. It may be correlated to the pollution and food structure

in Shanghai area. The As daily intakes (52.2 microgram) in Shanghai shown by Sun Laiyan [5] are higher than in other area of China and some west countries [13].

Cadmium

The results from Table II show a individual difference of more than 10-fold for Cd mean values of each sort of tissues is observed and the contents of Cd decreased in the order of kidney-cortex, liver, lung and hair. A difference of more than 500-fold of Cd mean values is observed between kidney-cortex and hair and a middle correlation is shown between these two tissues, (Fig. 10, $r = 0.418$, $p < 0.05$). However, no significant relationships are observed between hair and liver or lung. The concentration levels in this work are comparable to the results presented by other researchers for hair [14] and tissues [15].

Cigarette smoke is an important source of cadmium. If the 24 cases of subject are divided into three sub-groups according to their smoking habits (group A - non smoker, group B - 21 cigarettes/day and group C - more than 21 cigarettes/day) . It can be found that the concentrations of Cd are related to the smoking habits both for kidney-cortex and lung tissues (see Table IV and Fig. 21). The concentrations of Cd in kidney-cortex increase with the smoking habits. There is a significant difference between group A and group C. The mean value of Cd for group C is two-fold more than that for group A. A comparison of concentration of Cd in lung for non-smoker and smoker show a significant difference between group A and group B ($p < 0.01$). However, no significant differences are observed between group B and group C both for kidney-cortex and lung tissues for cadmium, and no significant effect on tissues As, Hg, Cu, Se and Zn could be found in smoker. The kidney damage may be due to the fact that cadmium competes with zinc for binding sites in various enzymes and other proteins. The differences of ratio Cd/Zn between kidney-cortex and kidney-medulla have been shown by M. Yukawa [16].

It is interesting to note that there is a significant relationship between concentration of element Cd and Zn in kidney-cortex for normal persons (Fig. 21, $r = 0.80$, $p < 0.01$, $n = 24$) . The mean value of ratio Cd/Zn in kidney-cortex is 0.60 ± 0.23. However, the exact mechanism in these detoxification reactions are still unknown, and no relationships between Cd and Zn in other tissues are found.

Copper

A difference of about three-fold is observed in Cu values for hair (5.34-14.7 mg/kg), kidney-cortex (9.02-22.8 mg/kg) and lung (5.30-18.5 mg/kg). The highest concentration of Cu are found in liver tissues (13.5-61.8 mg/kg). The mean value of Cu for hair in this study (10.1 mg/kg) is lower than Korean hair (31.2 mg/kg) and Japanese hair (18.0 mg/kg) but higher than Pakistan hair (9.6 mg/kg) and Bangladesh hair

(6.78 mg/kg) [17]. There are no significant relationships are observed between hair and tissues except kidney-cortex ($r = -0.434$, $p < 0.05$). However a significant relationship exists between element Zn and Cu in liver tissues ($r = 0.54$, $p < 0.01$).

Zinc Concentrations of Zn show a low variability in each sort of tissue except for kidney cortex. Zinc concentrations have a tendency to decrease in following order: liver, hair, kidney-cortex and lung. The arithmetic mean values of Zn are 206.6, 187.9, 209.7 and 55 mg/kg for hair, kidney-cortex, liver and lung respectively. It agrees with the results presented by Bacso (195, 187, 239 and 60.4 mg/kg) for hair, kidney-cortex, liver and lung respectively. However, no relationships are shown between the levels of Zn in hair and different tissues.

Selenium Se is one of essential elements for man to maintain health. The only known function of Se is the seleno-enzyme glutathione peroxidase (GSHPx) which protects bio-membranes against oxidative destruction. From the analytical results (Table II), which show the concentration of Se are higher in kidney cortex (2.04-5.36 mg/kg) than in liver (0.73-2.29 mg/kg), lung (0.50-1.85 mg/kg) and hair (0.37-1.43 mg/kg). It is interesting to find that there are significant relationships for concentration of Se between hair and all three internal tissues. The correlation coefficient r in linear regression analysis are 0.635, 0.639 and 0.570 for kidney-cortex, liver and lung respectively and the p-values are all less than 0.01 for the three tissues. On the basis of the experiment results, which show the information of hair-Se analysis for normal person may correlate to the individual Se state of internal body burdens, if the hair samples are not loss or contaminated by external environment during the sampling and analysis procedure.

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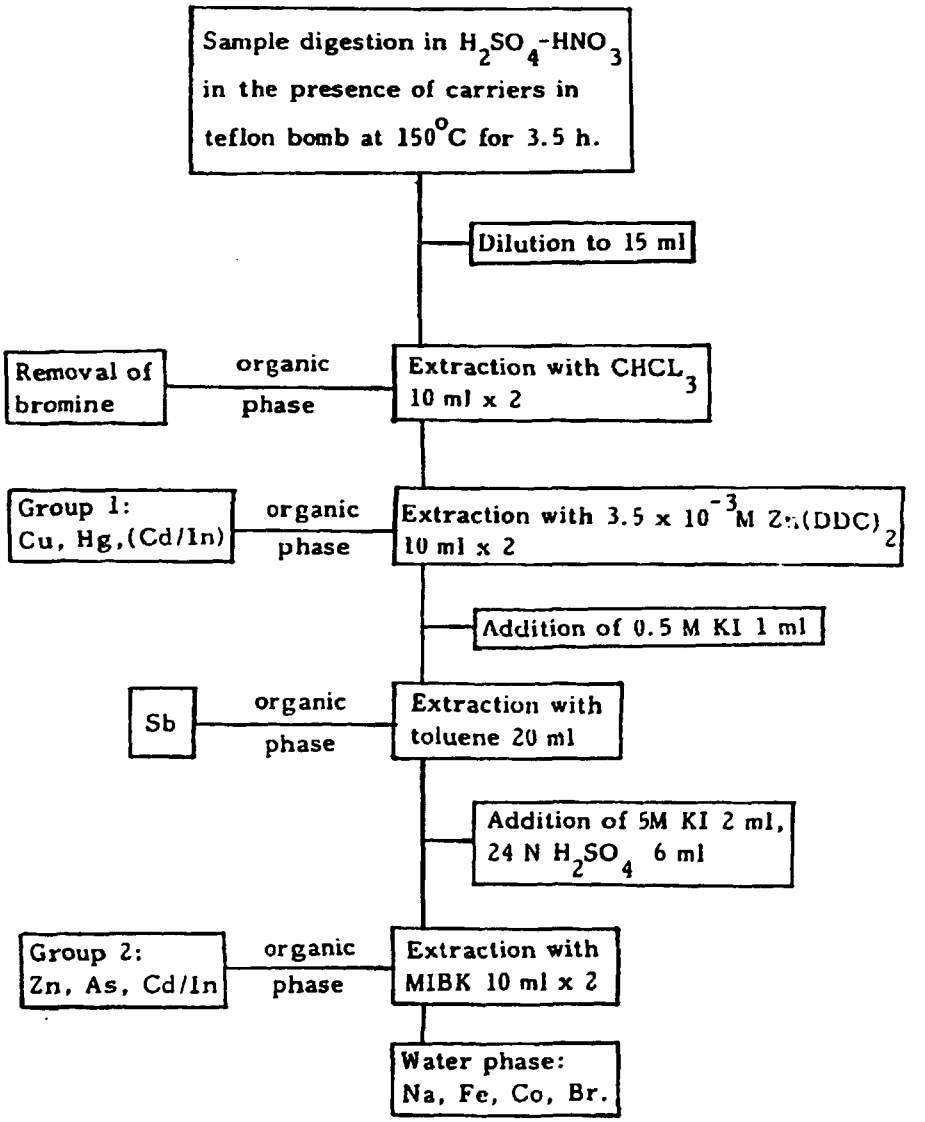

Figure 1. The radiochemical separation scheme for NAA

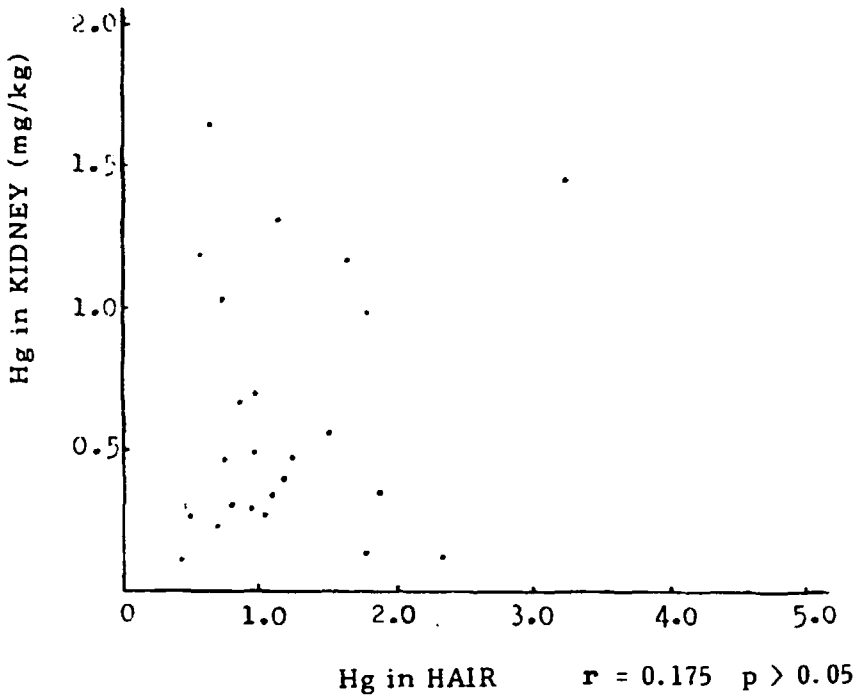

Figure 2. Scatter plot of Hg contents of hair and kidney cortex in 24 individuals

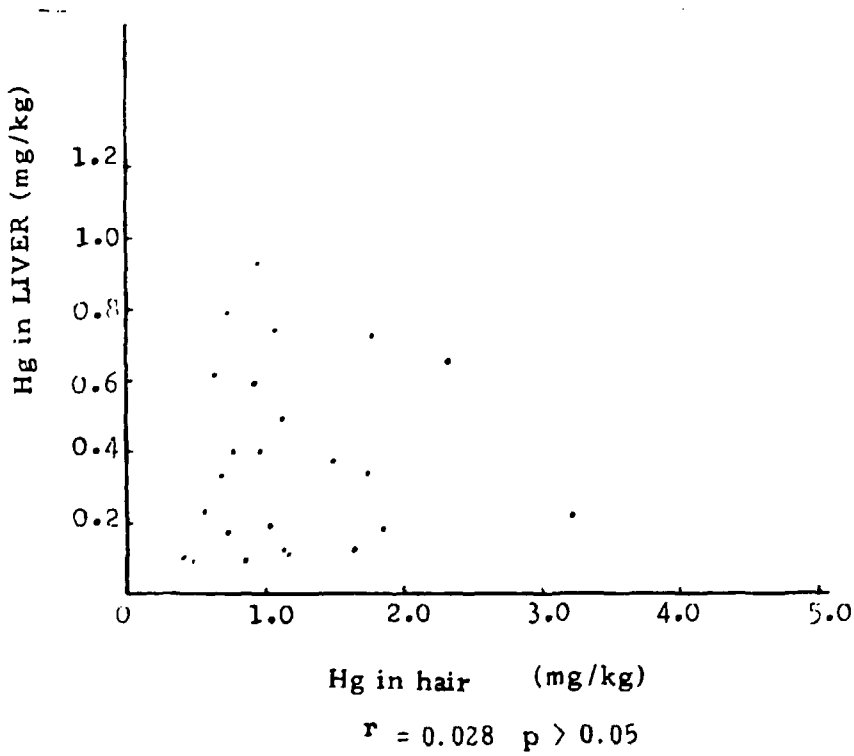

Figure 3. Scatter plot of Hg contents of hair and liver in 24 individuals

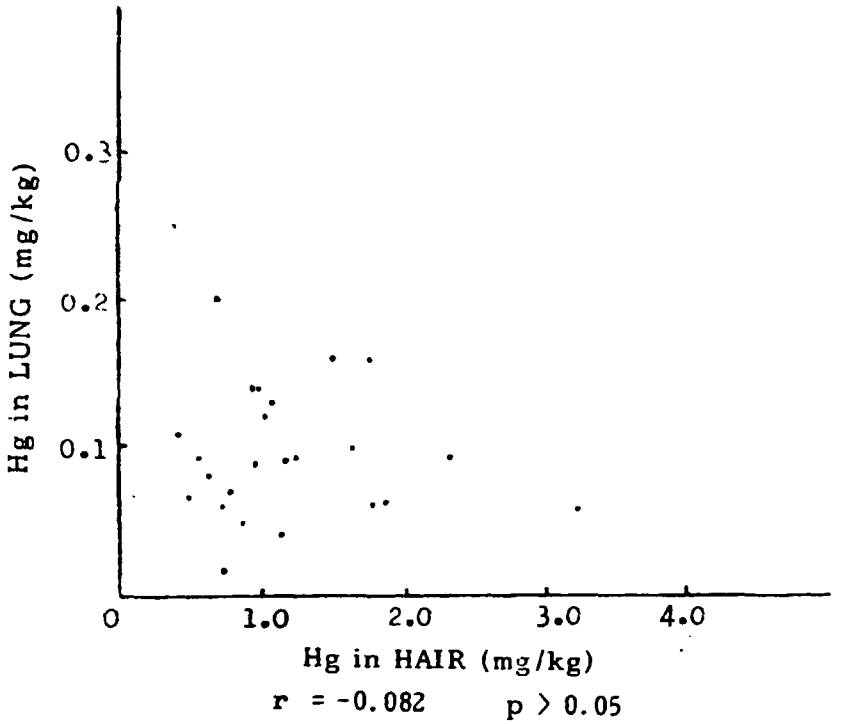

Figure 4. Scatter plot of Hg contents of hair and lung in 24 individuals

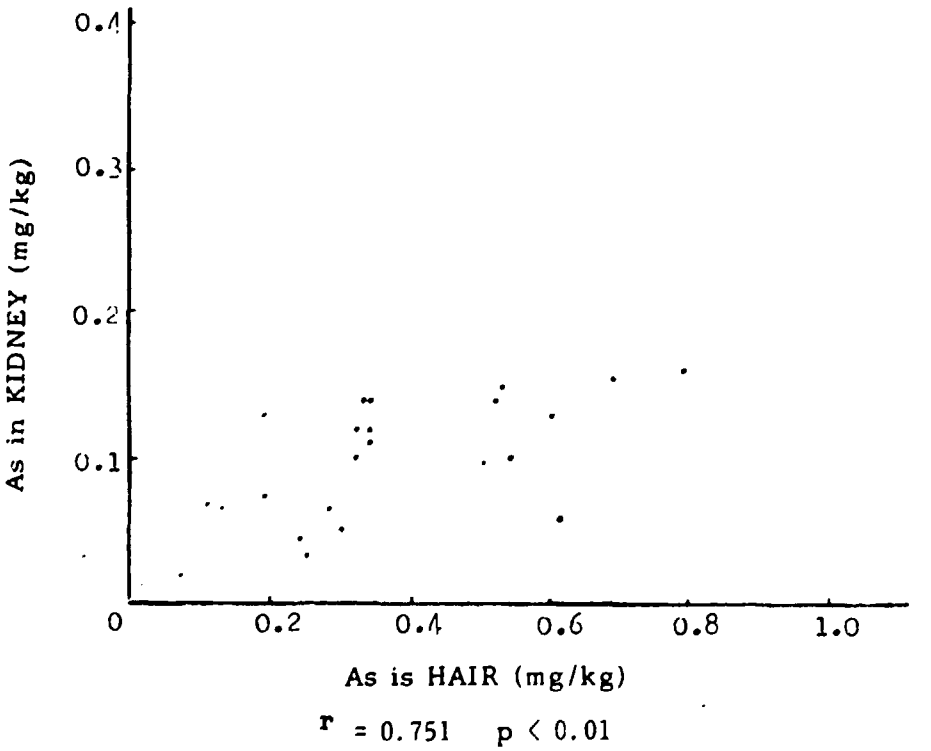

Figure 5. Scatter plot of As contents of hair and kidney cortex in 24 individuals

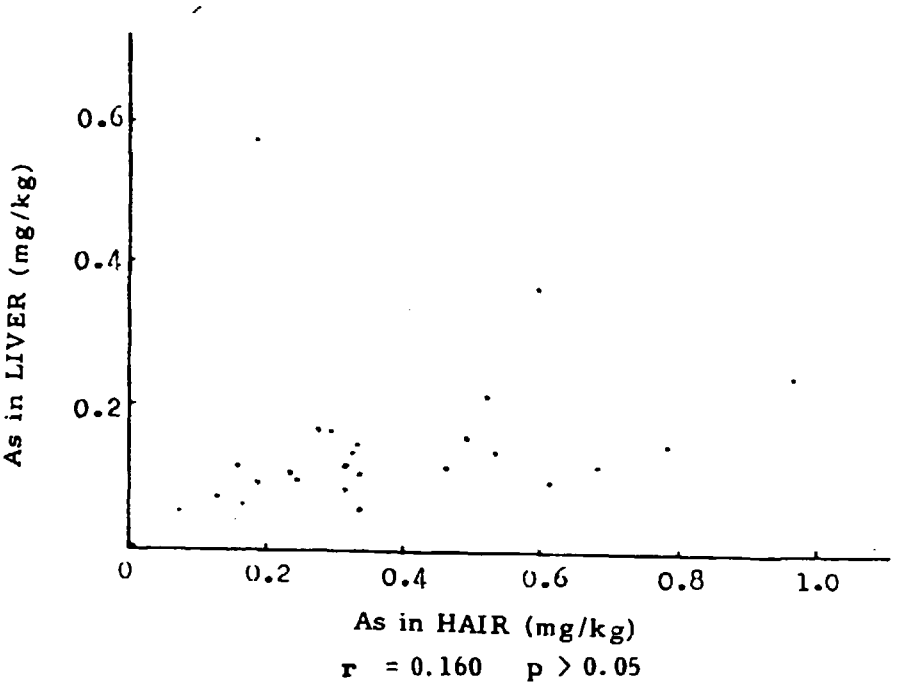

Figure 6. Scatter plot of As contents of hair and liver in 24 individuals

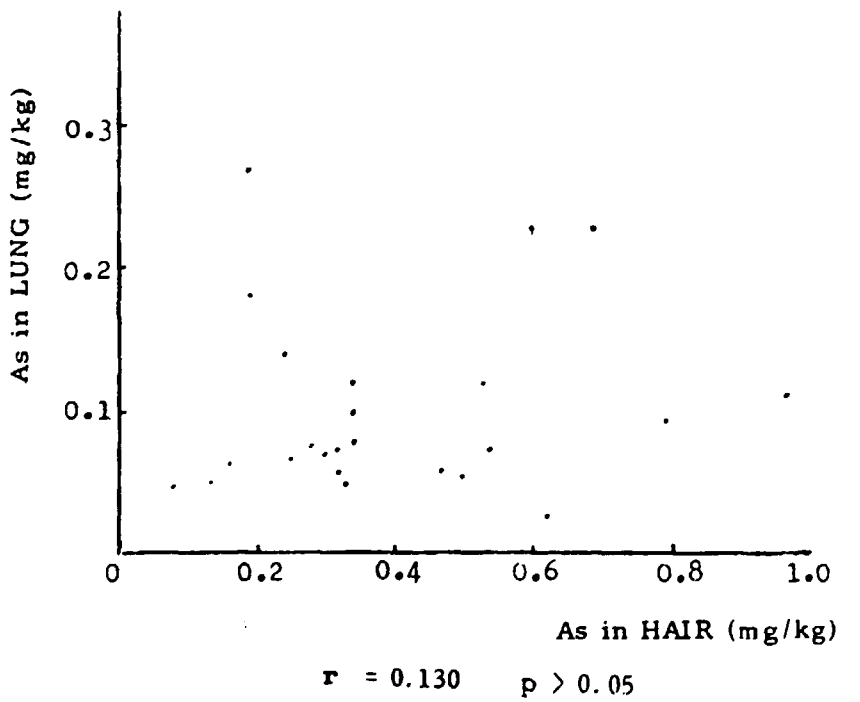

Figure 7. Scatter plot of As contents of hair and lung in 24 individuals

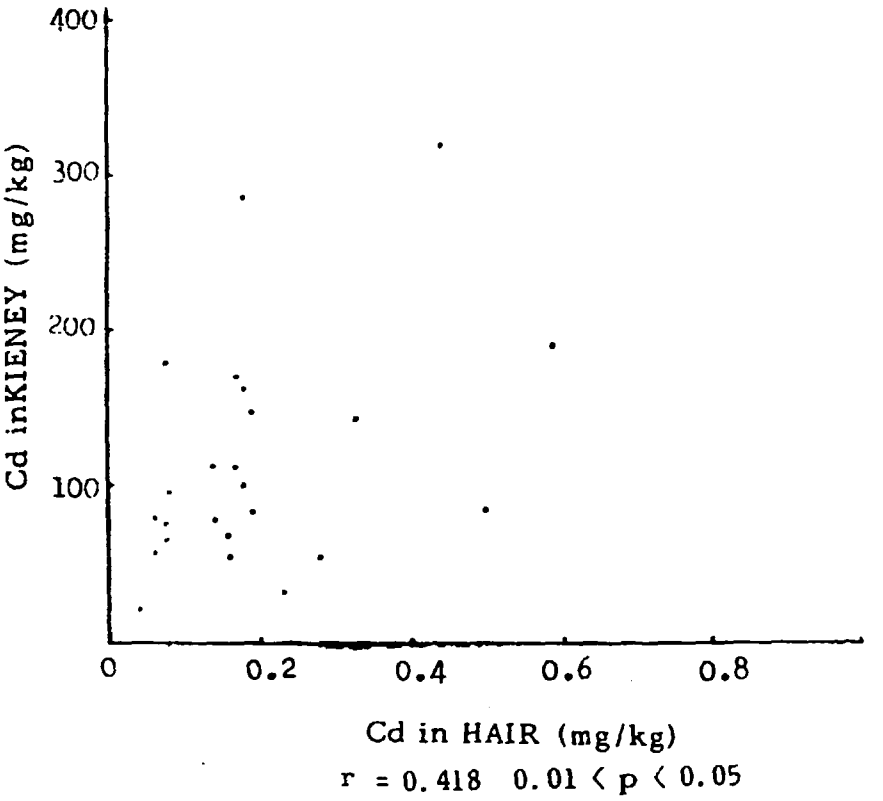

Figure 8. Scatter plot of Cd contents of hair and kidney cortex in 24 individuals

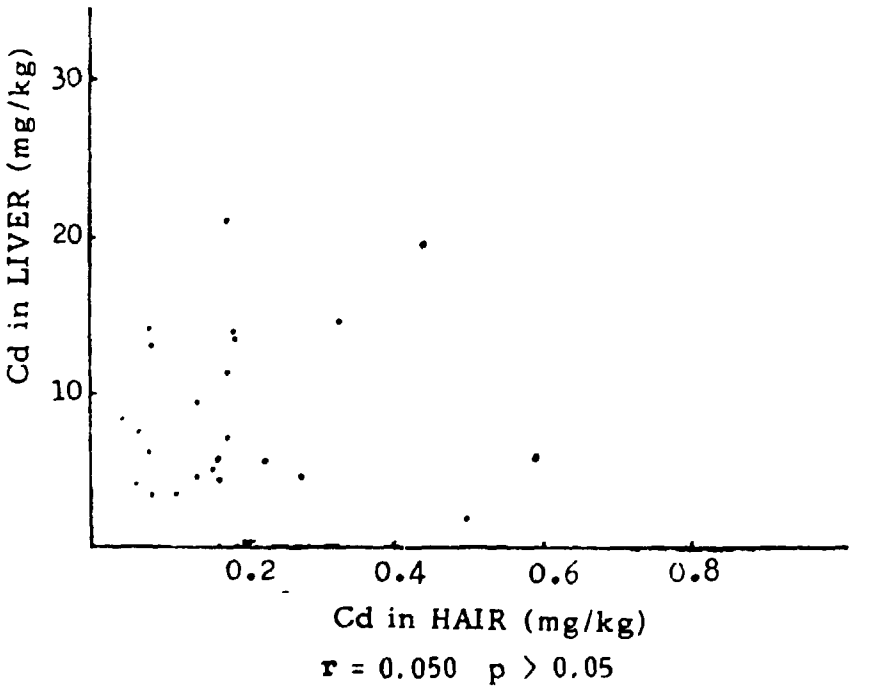

Figure 9. Scatter plot of Cd contents of hair and liver in 24 individuals

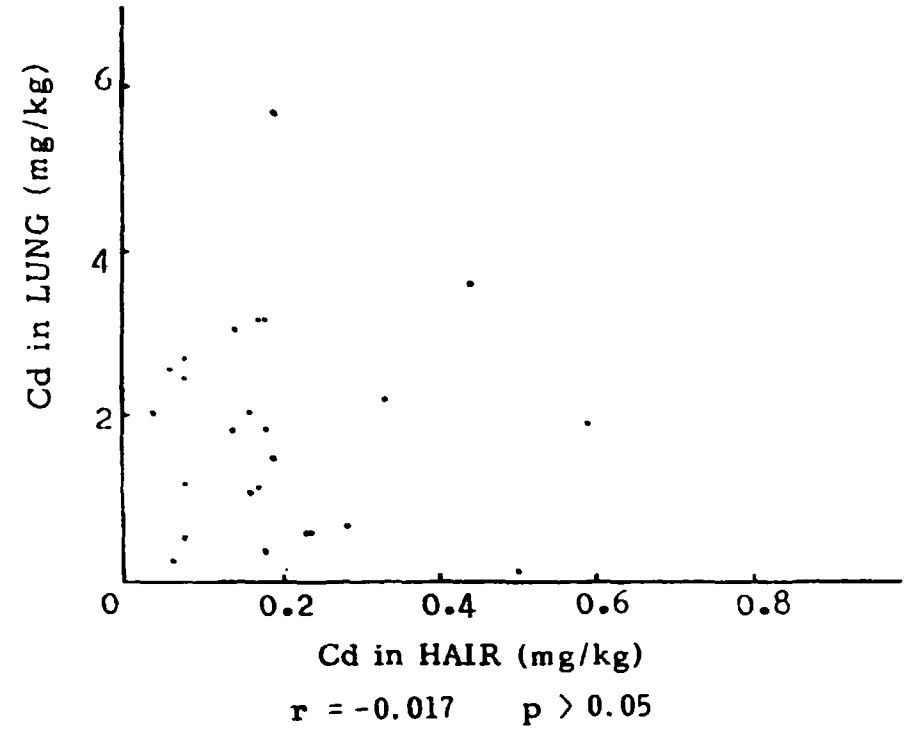

Figure 10. Scatter plot of Cd contents of hair and lung in 24 individuals

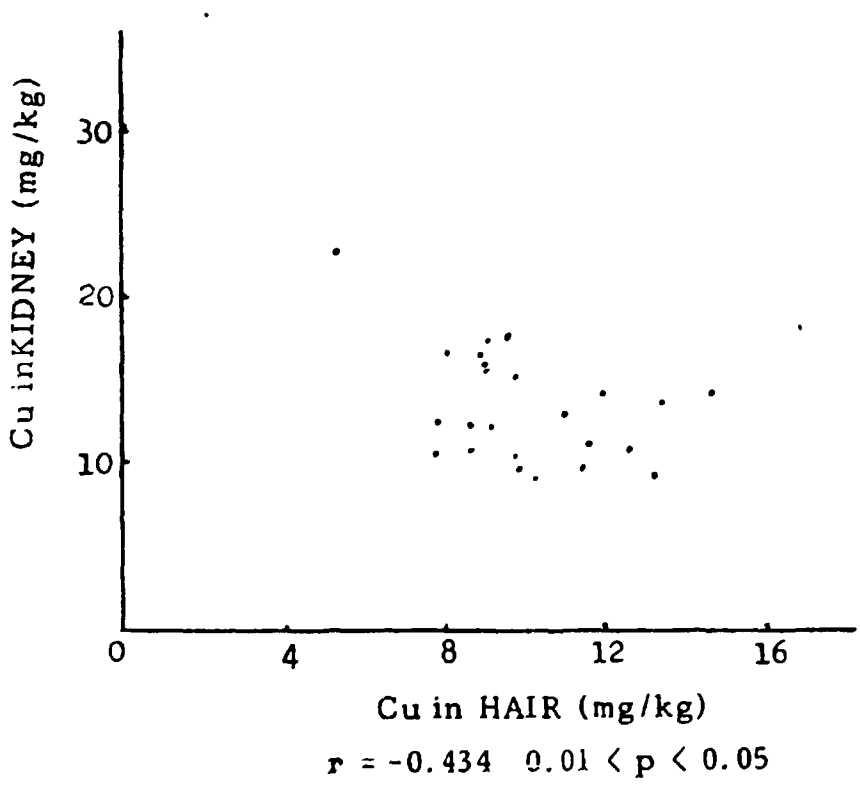

Figure 11. Scatter plot of Cu contents of hair and kidney cortex in 24 individuals

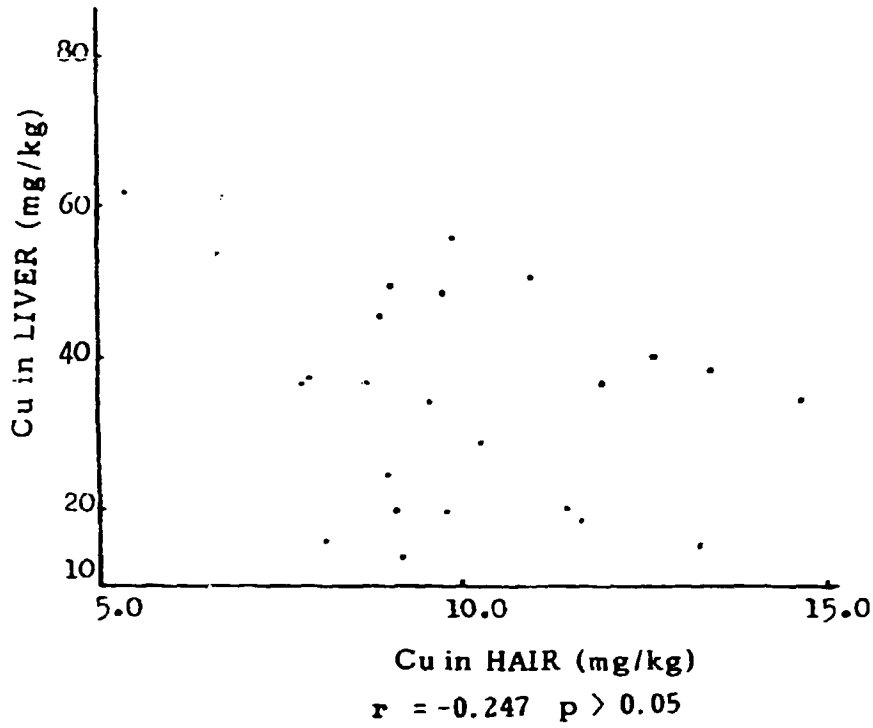

Figure 12. Scatter plot of Cu contents of hair and liver in 24 individuals

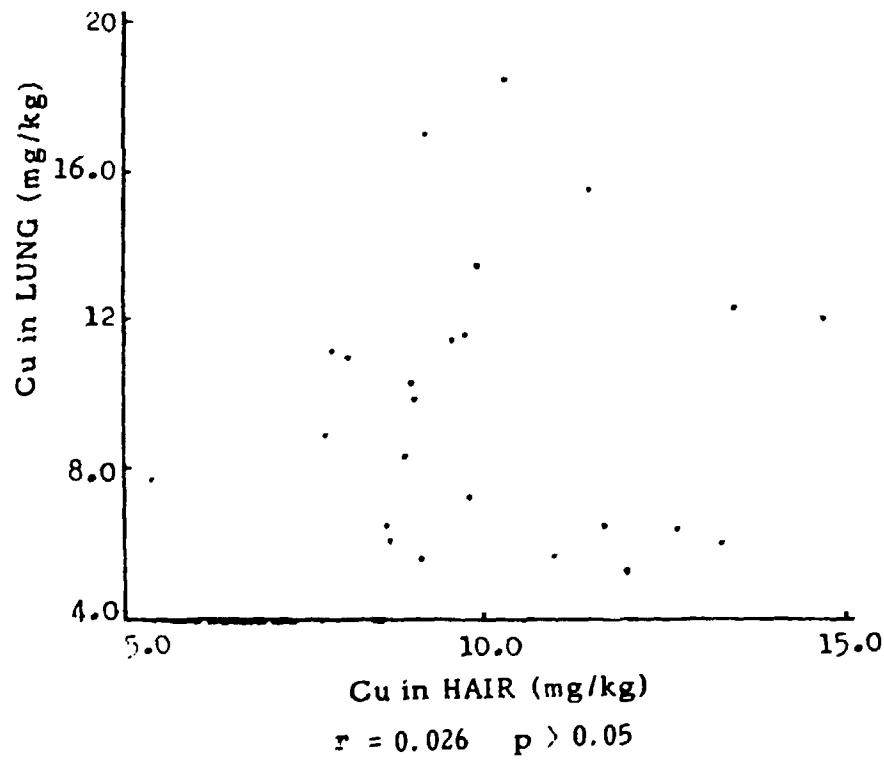

Figure 13. Scatter plot of Cu contents of hair and lung in 24 individuals

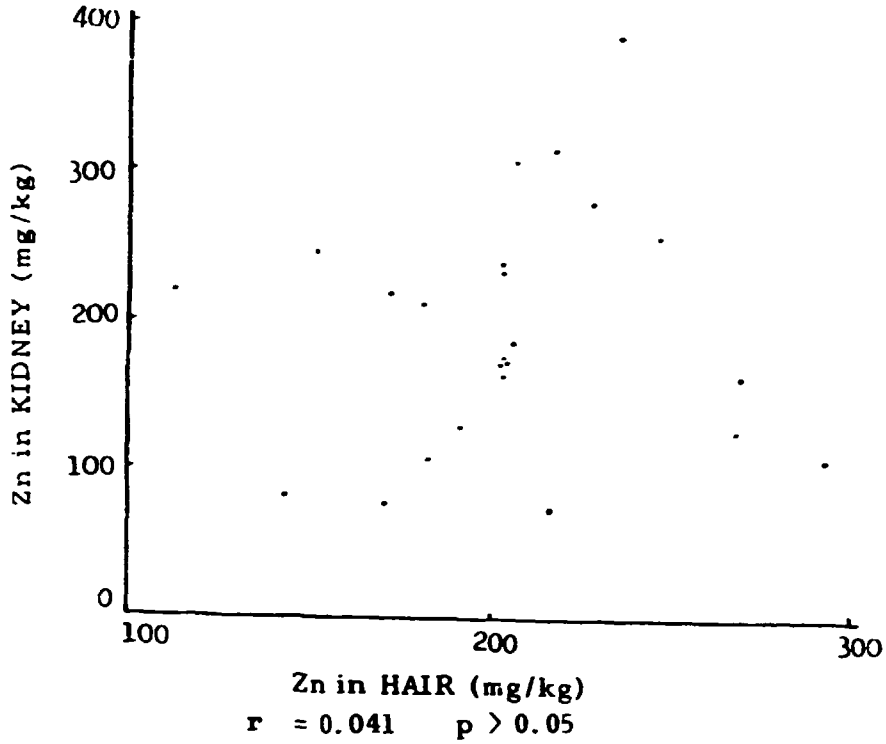

Figure 14. Scatter plot of Zn contents of hair and kidney cortex in 24 individuals

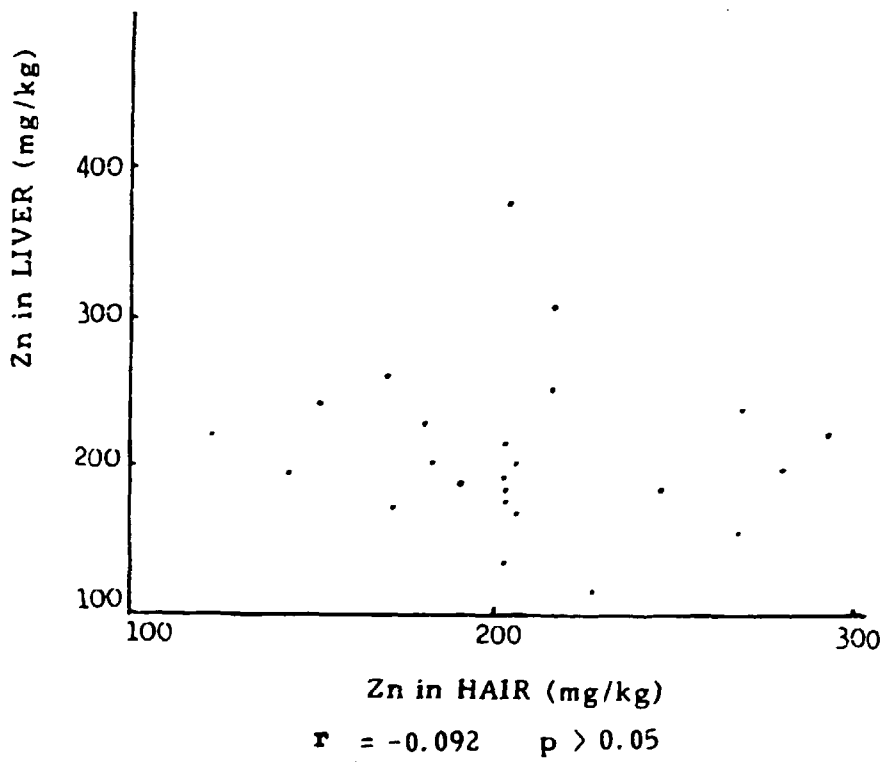

Figure 15. Scatter plot of Zn contents of hair and liver in 24 individuals

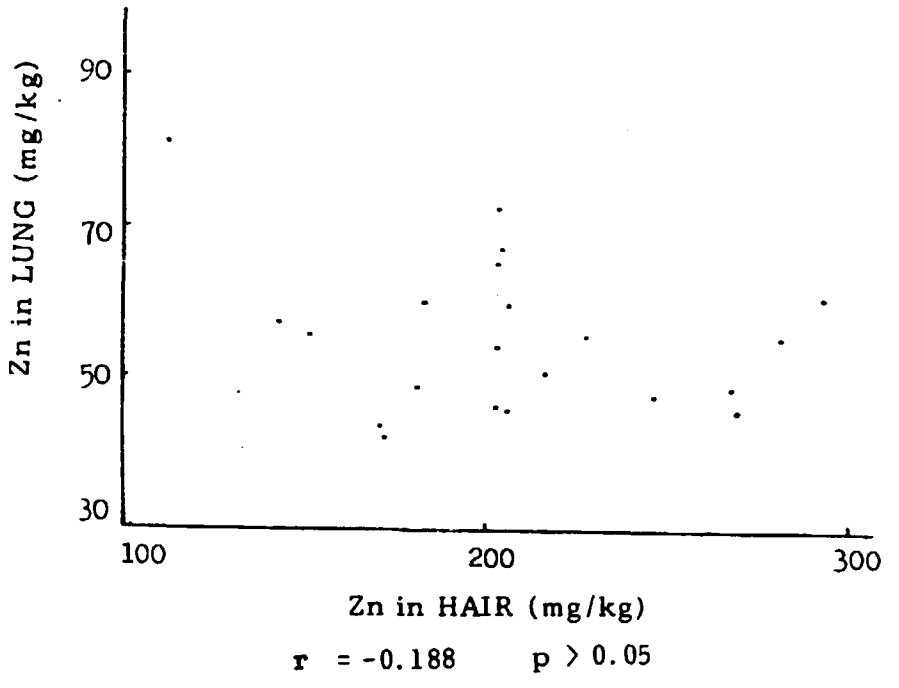

Figure 16. Scatter plot of Zn contents of hair and lung in 24 individuals

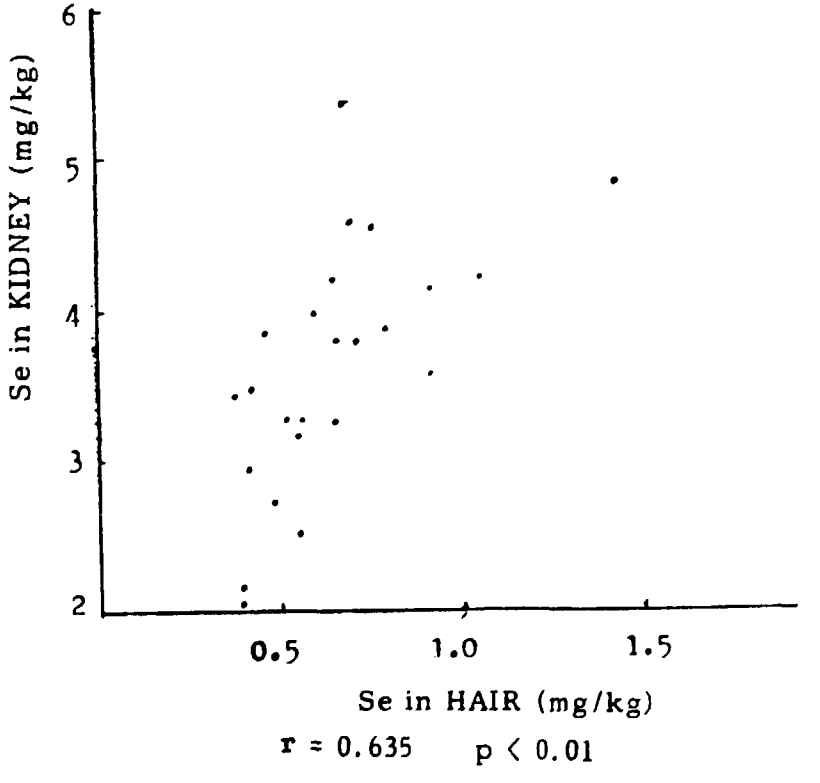

Figure 17. Scatter plot of Se contents of hair and kidney cortex in 24 individuals

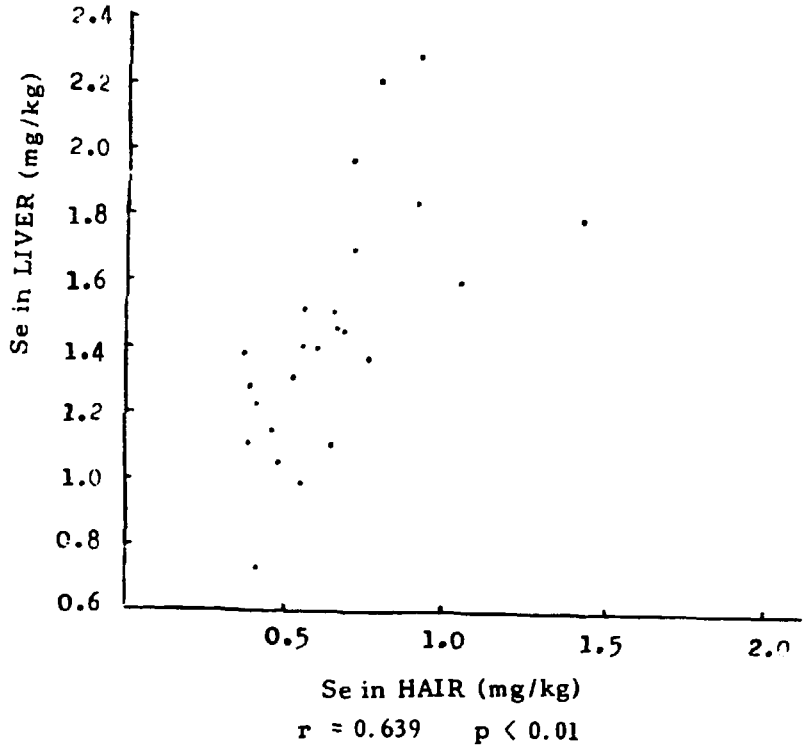

Figure 18. Scatter plot of Se contents of hair and liver in 24 individuals

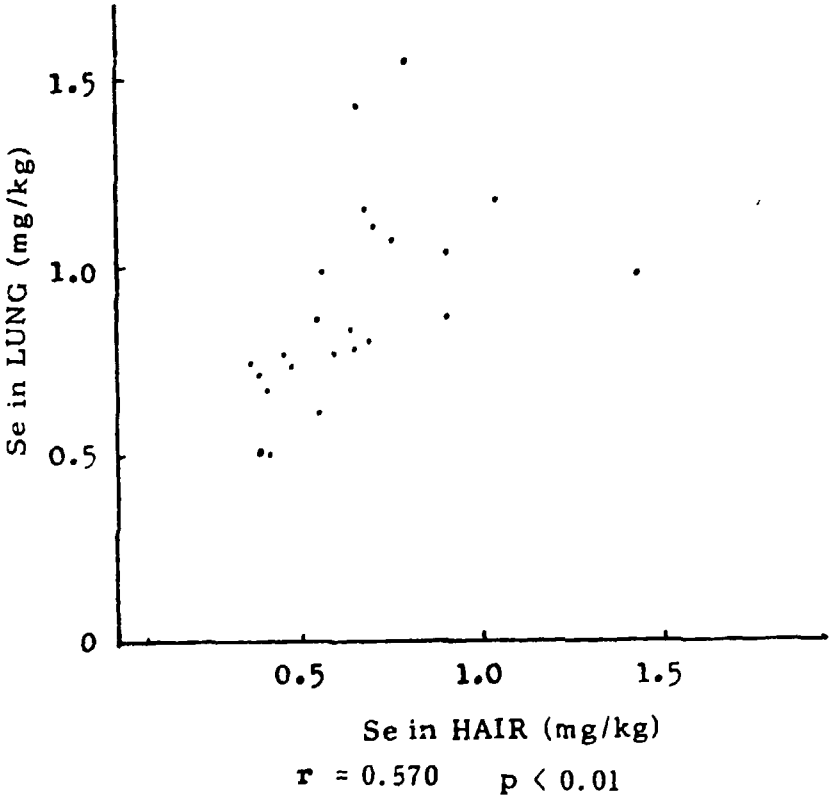

Figure 19. Scatter plot of Se contents of hair and lung in 24 individuals

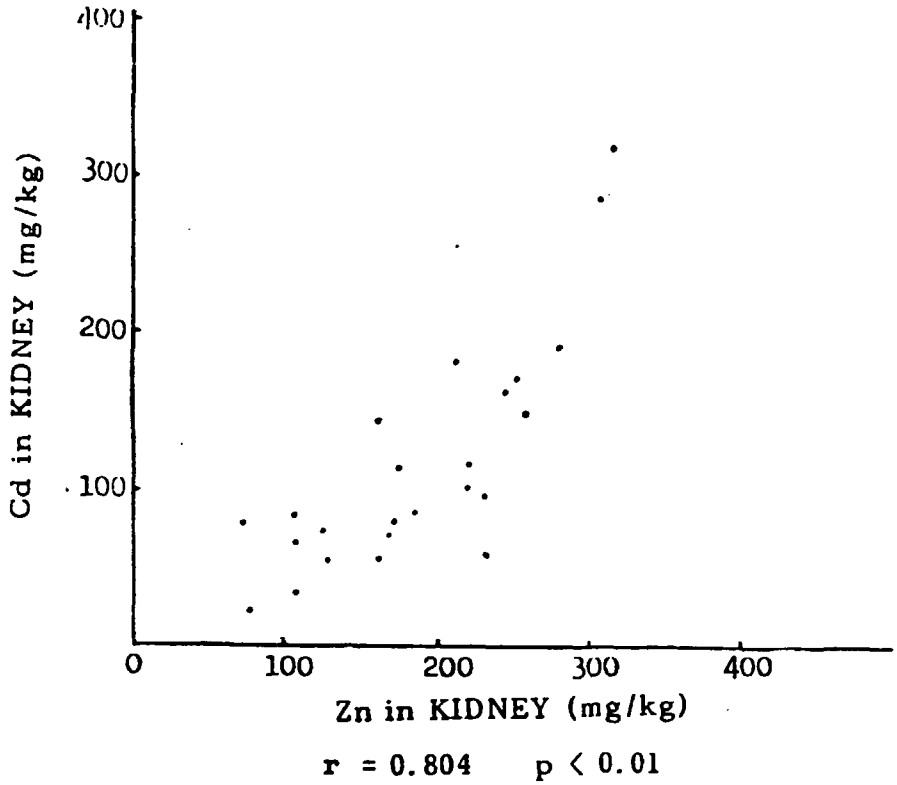

Figure 20. Scatter plot of Cd and Zn contents of hair and kidney cortex in 24 individuals

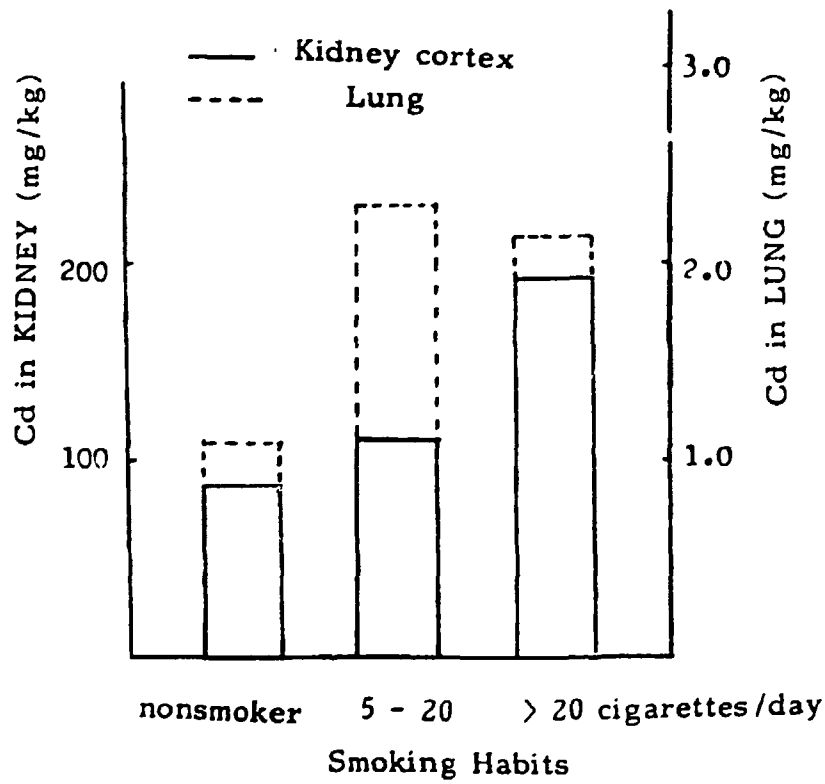

Figure 21. Influence of smoking habits on Cd concentration in kidney cortex and lung samples

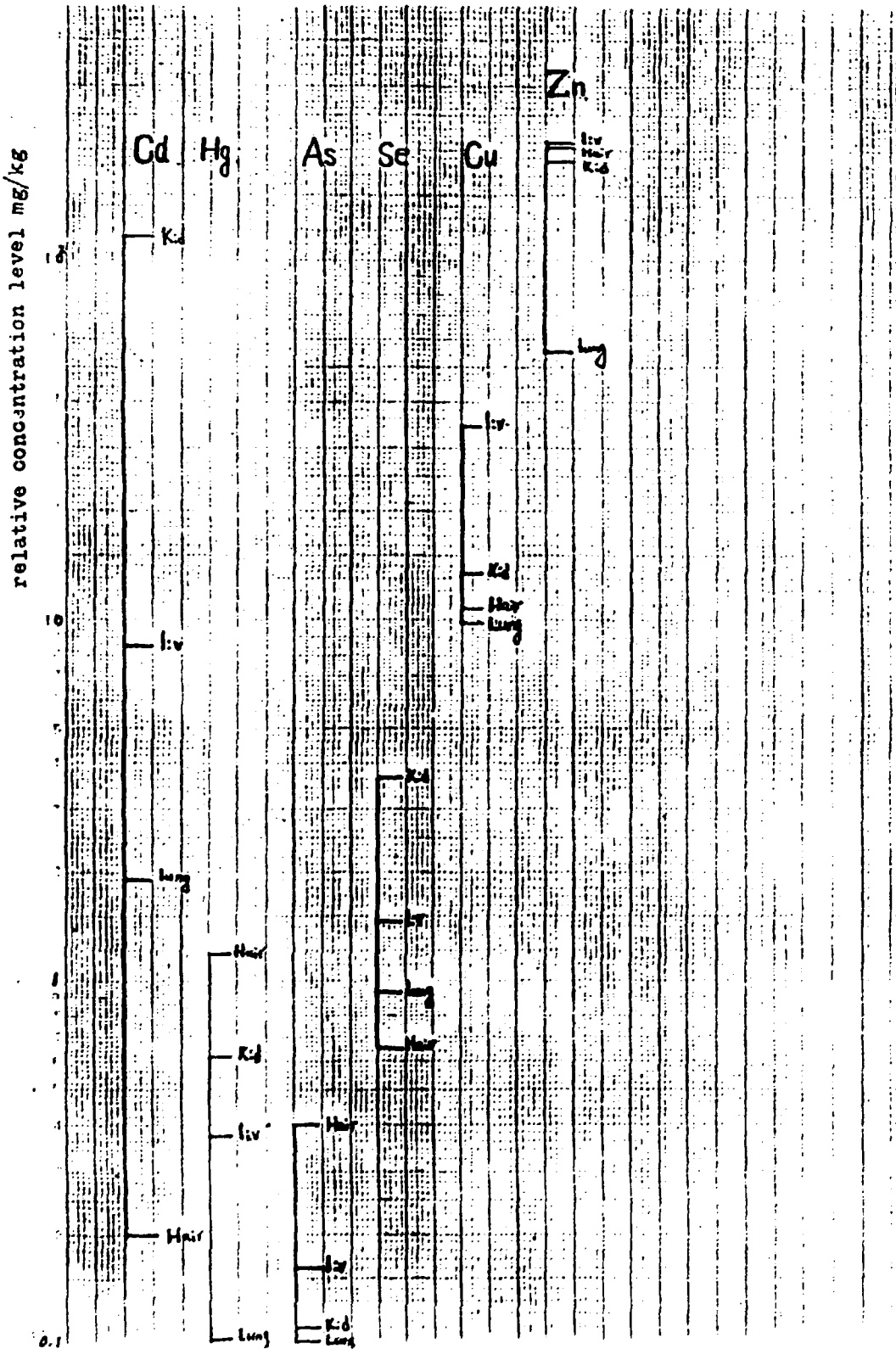

Figure 22. The comparison of levels of As, Cd, Hg, Cu, Se and Zn in human hair and tissues of autopsy