ATOMKI PREPRINT

C/2 (1986)

DETERMINATION OF GOLD ACCUMULATION IN HUMAN TISSUES CAUSED BY GOLD THERAPY USING X-RAY FLUORESCENCE ANALYSIS

J. Bacsó, I. Uzonyi and B. Dezső

Submitted to International Journal of Applied Radiation and Isotopes

August, 1986

Institute of Nuclear Research of the Hungarian Academy of Sciences
Debrecen, P.O.Box 51, H-4001, Hungary

DETERMINATION OF GOLD ACCUMULATION IN HUMAN TISSUES CAUSED BY GOLD THERAPY USING X-RAY FLUORESCENCE ANALYSIS

József BACSÓ[I], Imre UZONYI and Balázs DEZSŐ[II X]

Institute of Nuclear Research of the Hungarian Academy of Sciences,
H-4001, DEBRECEN, HUNGARY
[X]Department of Pathology, University Medical School, Debrecen.

Abstract.

Human autopsy tissues from five patients with rheumatoid arthritis, who had been treated with aqueous solution of gold and from an untreated control cadaver with the same disease were analysed by X-ray fluorescence spectrometry using a conventional Si(Li) detection system. The gold and zinc concentrations of tissues were determined and compared to some results available in the literature. Correlation has been found between Zn and Au concentrations in heart, lung, kidney and liver tissues.

Key words: rheumatoid arthritis, trace element, gold therapy, gold toxicity, Zn- Au-correlation

Introduction

Most of the heavy metals essential for life (Mg, Ca, Cr, Fe, Co, Cu, Zn, Mo, etc.) have low or medium atomic numbers[1]. These elements have their biological effect partly as the key components of metal-enzymes.

The concentrations of these elements in the human body cover a wide range of 10^{-3} to 10^{-8} g/g [2]. The biological importance of many heavy elements (Al, V, Si, Ti, Ge, Rb, Ag, Cd, Sb, Au, Hg, Pb, Bi) has not yet been clearly established. Their concentrations in the human body are approximately 10^3 to 10^4 times lower then those of the elements essential for life [3]. The toxicity of some of these heavy metals (Cd, Hg, Pb) has been demonstrated at low concentrations, but knowledge of some other elements of the same group (Ge, Au, Bi) is unsatisfactory in this respect[3]

The treatment of rheumatoid arthritis and arteriosclerosis by injecting colloidal or aqueous solution of gold has been common for more than half a century. For example,in 1929, Forestier gave an account of five patients treated successfully in this manner [4]. For curing the diseases mentioned, various gold salts are used, but their influence on the human body is not entirely clear. About 60 to 70 % of the quantity of gold taken into the body is retained[8,9]. Therefore from medical point of view, it is important to know the extent of gold accumulation in various tissues Within the scope of a comprehensive investigation of trace elements in human tissues, van Rinsvelt, Lear, and Adams have observed gold accumulation in the tissues of a patient treated for arteriosclerosis with colloidal gold solution[5]. Recently Kazuhiro has demonstrated excessive gold accumulation in human monocytes and their morphological and functional demage associated with gold treatment [10]

In general terms the concentration of gold in human tissues is very low[2]. Therefore, any method used for the investigation of this element must be very sensitive. Because of the well-known advantages of energy dispersive X-ray fluorescence (XRF) analysis (simplicity of sample preparation, low detection limits, short analysis time, etc.), we have examined the possibility of using this method for the measurement of gold concentrations in human tissues.

It is interesting to note that five patients suffered antemortem from rheumatoid arthritis died at the Department of Internal Medicine of University Medical School, Debrecen, after having been treated with aqueous solution of gold (Na-aurothiomalate, Tauredon, Byk Gulden Konstanz). This paper contains the method and results of Au concentration determinations in some tissues from five patients and from a control individual as well as average values from literature[2].

Experimental:

The autopsy tissue samples from five patients of rheumatoid arthritis treated with gold and from an untreated control person having heart attack with the same disease were provided by the Department of Pathology , University Medical School, Debrecen. For this paper, the treated patients are indicated as "A", "B", "C", "D" and "E", denoting female rheumatoid arthritics of ages 22 y, 51 y, 42 y, 43 y and 51 y, respectively. Body weights were 51 kg, 56 kg, 61 kg, 53 kg and 58 kg, respectively. The quantities of gold administered by intramuscular injection were 0.22 g, 0.46 g, 0.28 g, 1.05 g and 0.5 g, respectively. The untreated control is indicated as "F", (female with the age of 55 y and with the body weight of 60 kg). The autopsy and histological examinations

confirmed the diagnosis of rheumatoid arthritis in these cases as well as the cause of death (panmyelophtisis in the treated patients and cardiac insufficiency for the untreated cadaver).

In the course of routine, post mortem, histological examinations, the tissues were initially formaline-fixed. For the XRF analysis the fixative was then removed by washing the tissues with phosphate- buffer pH 7.4. The tissue samples from heart, kidneys, liver, spleen and lung of each patient were chopped into small pieces with a tissue-sectioner (SC-2, Sorval Inc., USA). After drying to constant weight at 105 °C the tissues were crushed to fine powders in a mortal then pressed into pellets of 10 mm diameter and 0.13 g/cm^2 thickness using a hydraulic press (600 MPa) for one minute. For the XRF measurements, ^{125}I radioactive isotope exciter of 185 MBq (~5mCi) activity and a Si(Li) detection system (ATOMKI) were used. The energy resolution (FWHM) of the detector was 170 eV at 5.9 KeV (Mn K_α)[6]. The pulses, emerged from detector after amplification , were analyzed and recorded with a 4096 channel ICA-70 (KFKI) analyzer, and for further calculation, a TPA-i type minicomputer (KFKI) was used. A schematic diagram of the experimental set-up is given in fig. 1.

The data processing was performed by a display oriented interactive programme (DISP-78, KFKI). After the subtraction of linear background, the corrections for the overlapping Zn K_β and the Au L_α peaks were made from the Zn K_β/Zn K_α ratio. This was measured separately under identical conditions using X-Ray Mix Powder (Chemplex Industries, Inc. USA) dopped with zinc.

For the calibration, the Au L net intensity versus gold concentration regression line was determined by making mixtures from the rest of the dryed tissues to be analysed, dopping them with known quantities of gold and measuring the XRF spectra (standard addition method) fig.2.

The calculated detection limits for gold (the concentration that correspond to an intensity equal to three times the root of the background under the Au L peak)were found less than 7 mg/kg at a measuring time of 1000 seconds under the condition described above.

Results :

A part of two X-ray spectra for liver and lung samples of "B" and "A" cadavers with rheumatoid arthritis together with the X-ray spectra for the same organs of control patient are shown in figures 3 and 4, respectively. The spectra demonstrate the overlaping of Zn K_{β} and Au L_{α} lines and the Au content in different organs of patients. The appropriate Au concentrations in tissue samples are: 95 ±4 mg/kg (B; liver) and 55±4mg/kg (A; lung)

The numerical values for zinc and gold concentrations determined in the various tissues are listed in the table I. It is quite clear from these data that the concentration of gold in heart, kidney, liver, lung, spleen and bone marrow of treated patients are markedly elevated and the Zn concentrations are decreased as compared to the corresponding values for control. The literature values[2] for the gold concentrations in these tissues are also much lower then those determined in the samples from the gold treated patients.

As shown in figures 5-9 the Zn concentration values were plotted in function of Au concentration measured for heart, lung, kidney, liver and spleen, respectively. For comparison the average Zn values taken from literature[2] and the biological range of Zn (dushed line) as well as the value measured in control person (F) for appropriate organs have also been depicted (y-axis) in these figures.

It is interesting to note that the Zn concentration decreases with

the increase of Au concentration. As if the Au, accumulated in organs, replaced the Zn almost for every cases in some organs examined. Correlations appear to exist between these elements which seems to be negative and linear in tissue of heart, lung, and kidney (figures 5-7). Other then linear (if any?) correlation was found in liver (fig. 8) and no specific Zn-displacement could be demonstrated in spleen (fig. 9) in patients treated ante mortem with aqueous solution of gold. These correlation data mentioned above might be important from view of the gold toxicity. The different relationship between Zn and Au concentrations in different organs might reflect the functional specificity or complexicity of organs as well as the different storage form of Zn in tissues. Although, our control cadaver with rheumatoid arthritis did not show lower Zn values in tissues examined, it is important to note that rheumatoid patients without on gold containing drugs or penicillamine usually have a slightly decreased plasma Zn-concentration[12].

On the basis of our data demonstrating the specific exchange of Zn to Au in certain organs obtained from gold-treated patients it is conceivable that these tissues as biopsy specimens or some other one (i. e hair) might be just as well useful for carrying out XRF analysis to check the gold toxicity in living patients suffering from different gold-sensitive diseases

Conclusion

The results of the XRF analysis show that the gold highly accumulates in the tissues of patients suffering from rheumatoid arthritis treated with aurothyomalate comparing to those of an untreated control patient with the same disease. The extent of gold accumulation was found to be different for the various tissues. It

appears that the extent of Au accumulation is proportional to the quantity of gold administrated. These fatal cases investigated herein call attention again to the gold toxicity since death associated with gold treatment is well known from the literature as well [7,11]. The gold therapy should only be used under continuous and appropriate medical control. For such control many nuclear analytical methods (XRF, PIXE, INAA) can be used. Furthermore, as we have shown almost all tissue is suitable to evaluate the systemic intoxication of gold, probably including the scalp hair as well. In this case the result may have diagnostic value. The investigation of Zn and Au concentration variations along hair strand of living patient, is thought, to be useful as a diagnostic tool.

The biochemical and physiological consequence of the Zn loss with gold accumulation in tissues depicted herein will be discussed in an other paper.

Literature:

1. COTZIAS G. C.; Proc. First Ann. Conf. Trace Substances Environ. Health, edited by D. D. Hemphill /Columbia, Missouri/ ,1967. p. 5.

2. IYENGAR G. V., KOLLMER W. E., BOWEN H. J. M.; The elemental composition of human tissues and body fluids, /Verlag Chemie, Weinheim, New York/ 1978.

3. VALKOVIC V.; Trace element analysis, /Taylor and Francis Ltd, London/ 1975.

4. FORESTIER J.; Bull. Med. Soc. Med. D Hosp. de Paris, 53 1929 p.323.

5. Van RINSVELT H., LEAR R. D., and ADAMS W. R; Nucl. Instr. Meth. 142 1947. p.171.

6. BACSÓ J., KALINKA G., KERTÉSZ ZS., KOVÁCS P., and LAKATOS T.; High resolution Si(Li) X -ray spectrometer with high throughput rate, ATOMKI Kozl. 24 1982. p. 133.

7. KALMÁR ZS., NAGY P., KISS A., TAMÁS E., DEZSŐ B.; Lethal panmyelophthitis during treatment by gold preparations, Orvosi Hetilap, 125/9 1984. p. 513.

8. The Cooperative Clinics Committee of the American Rheumatism Association: A controlled trial of gold salt theraphy in rheumatoid arthritis, Arthritis Rheum., 16 1973. p. 353.

9. PYAI L. P.; Basic theraphy of rheumatoid arthritis in the light of long-term clinical follow up studies, Ter. Arkh., 53 1981. p. 67.

10. KAZUHIRO U.; Gold induced changes in the morphology and functional capabilities of human monocites, Arthritis Rheum., 22 1979.1352.

11. GUMPEL J. M.; Death associated with gold treatment: a reassessment, Brit. Med J., 1 1978. p. 215.

12. BALOGH Z., EL-GHOBAREY A.F., FELL G.S., BROWN D.H., DUNLOP J. and DICK W.C.; Plasma Zn and its relationship to clinical symptoms and drug treatment in rheumatoid arthritis, Ann. Rheum. Dis. 39 1980. p. 329.

Table 1. Zinc and gold concentrations and ±SD in different tissues of gold treated patients and control. The literature data contain the average value for gold and zinc and range of concentration for zinc.

Abreviations: <: the Au concentration is bellow the detection limit (7 mg/kg)

-: no sample was analysed.

		MARROW	SPLEEN	LIVER	HEART	LUNG	KIDNEY
A	Zn	-	33±1	26±1	19±1	37±1	10±1
	Au	-	18±4	38±4	63±4	55±4	116±4
B	Zn	50±1	28±1	27±1	27±1	28±1	43±1
	Au	56±6	75±4	95±4	43±4	57±5	65±3
C	Zn	589±3	55±3	50±3	37±3	64±2	47±6
	Au	22±2	39±2	25±3	17±1	19±4	97±4
D	Zn	-	58±3	-	-	49±2	38±2
	Au	-	194±5	-	-	37±4	107±9
E	Zn	-	81±5	206±6	-	73±5	147±6
	Au	-	36±4	24±3	-	30±4	45±4
F	Zn	235±5	70±1	134±2	89±6	59±2	-
	Au	<	<	<	<	<	<
Lit.	Zn		74	243	121	69 8	185
(2)	Zn		63-113	159-379	86-414	48 1-95	155-375
	Au		<1	<1	<1	<1	<1

Legend to figures:

Fig. 1. Schematic diagram of the used XRF experimental setup.

Fig. 2. Au calibration line for dried body tissues.

Fig. 3. A fragment of X-ray spectrum of human liver from patient B
(F: control liver from the untreated cadaver)

Fig. 4. A fragment of X-ray spectrum of human lung from patient A
(F: control lung from the untreated cadaver)

Fig. 5. Zn concentration vs. Au concentration in human heart samples.
(The capitals mark the patients)

Fig. 6. Zn concentration vs. Au concentration in human lung samples.
(The capitals mark the patients)

Fig. 7. Zn concentration vs. Au concentration in human kidney samples.
(The capitals mark the patients)

Fig. 8. Zn concentration vs. Au concentration in human liver samples.
(The capitals mark the patients)

Fig. 9. Zn concentration vs. Au concentration in human spleen samples.
(The capitals mark the patients)

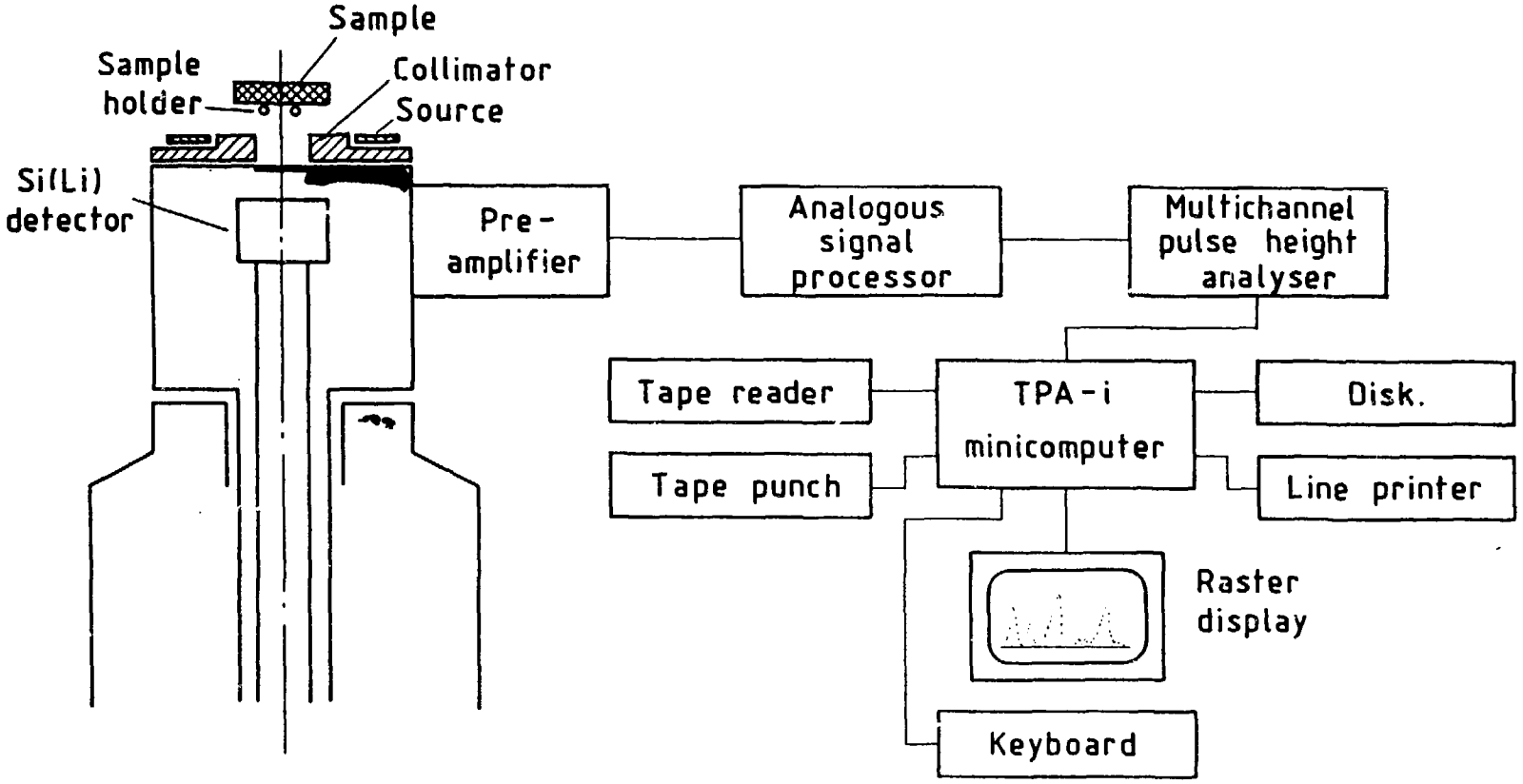
Sample
Sample holder
Collimator
Source
Si(Li) detector
Pre-amplifier
Analogous signal processor
Multichannel pulse height analyser
Tape reader
TPA-i minicomputer
Disk.
Tape punch
Line printer
Raster display
Keyboard

Fig. 1.

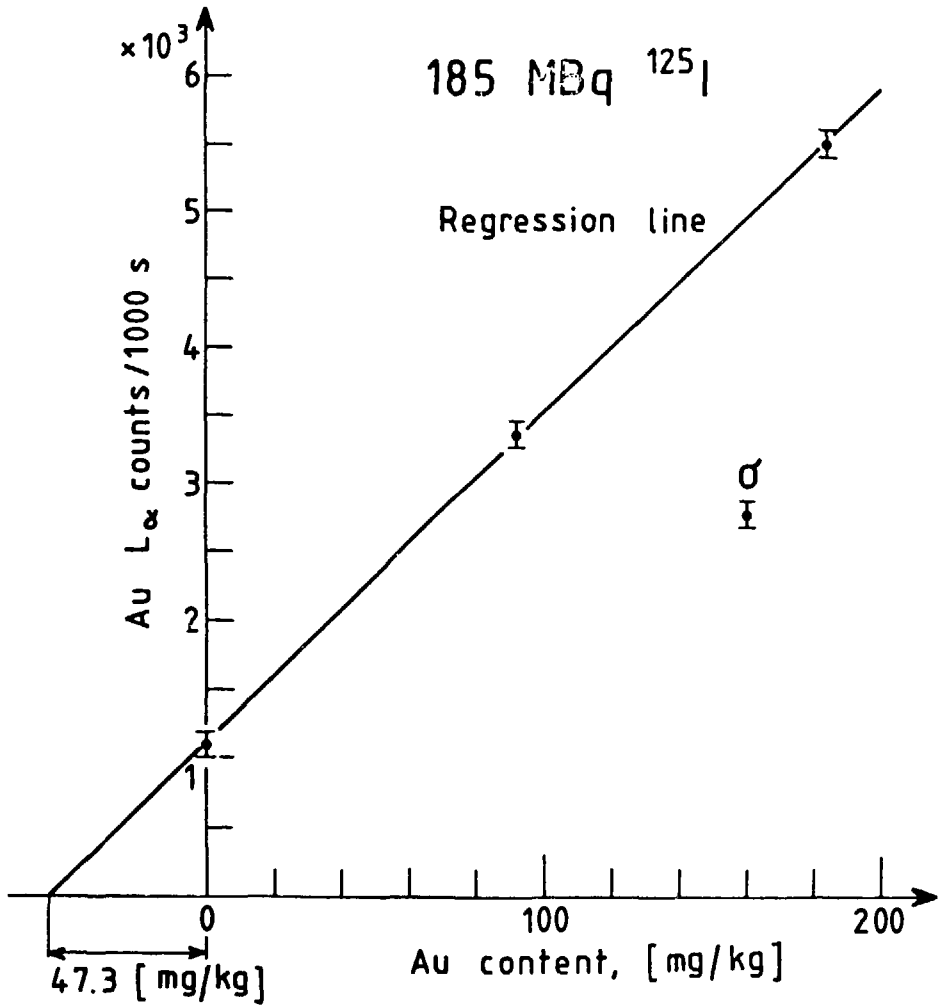
185 MBq 125I
Regression line
x 10 3
6
5
4
3
2
1
Au Lα counts/1000 s
σ
0
100
200
47.3 [mg/kg]
Au content, [mg/kg]

Fig. 2

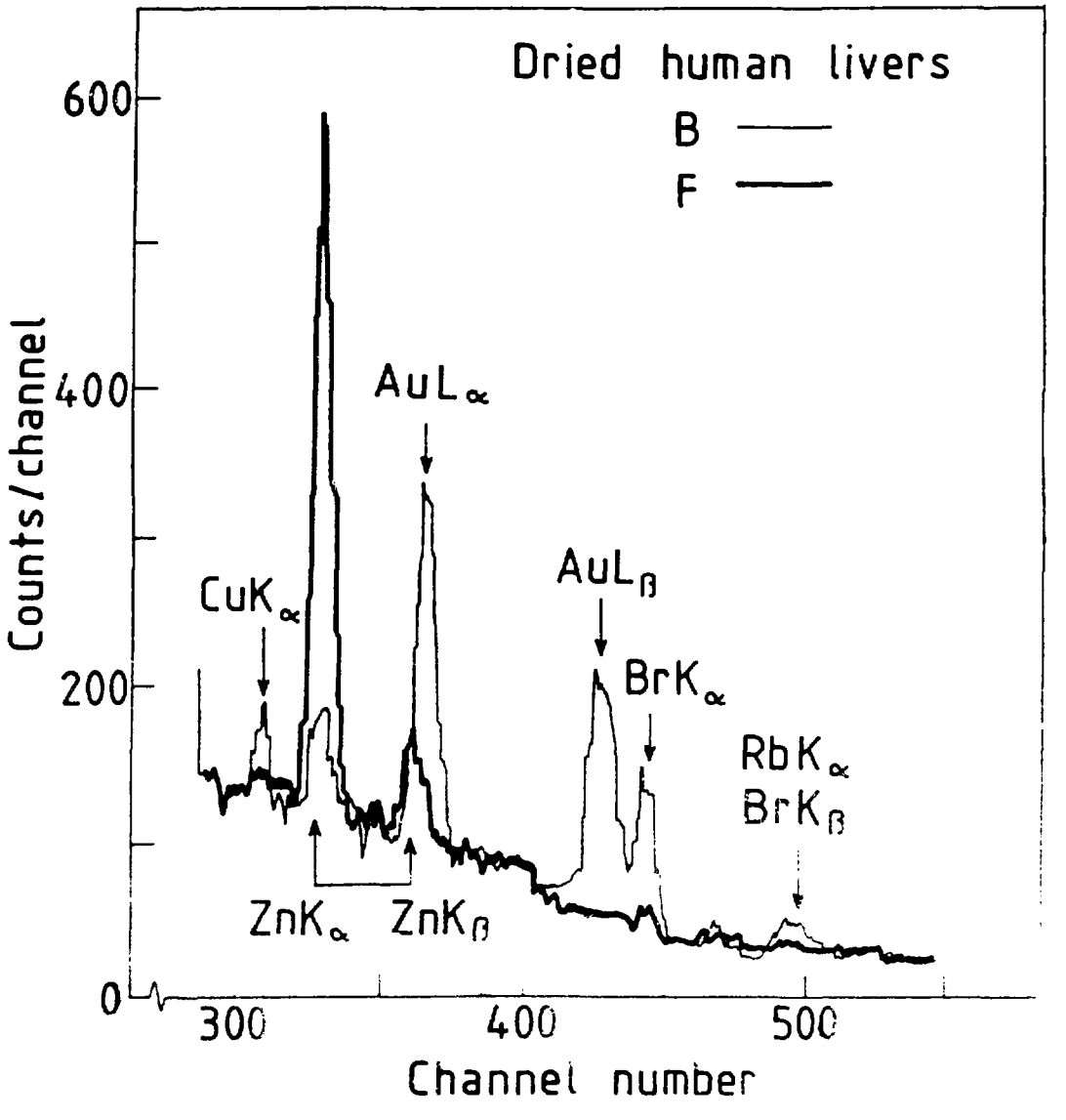
Dried human livers
B
F
600
400
200
0
Counts/channel
CuK$_\alpha$
AuL$_\alpha$
AuL$_\beta$
BrK$_\alpha$
RbK$_\alpha$
BrK$_\beta$
ZnK$_\alpha$
ZnK$_\beta$
300
400
500
Channel number

Fig. 3

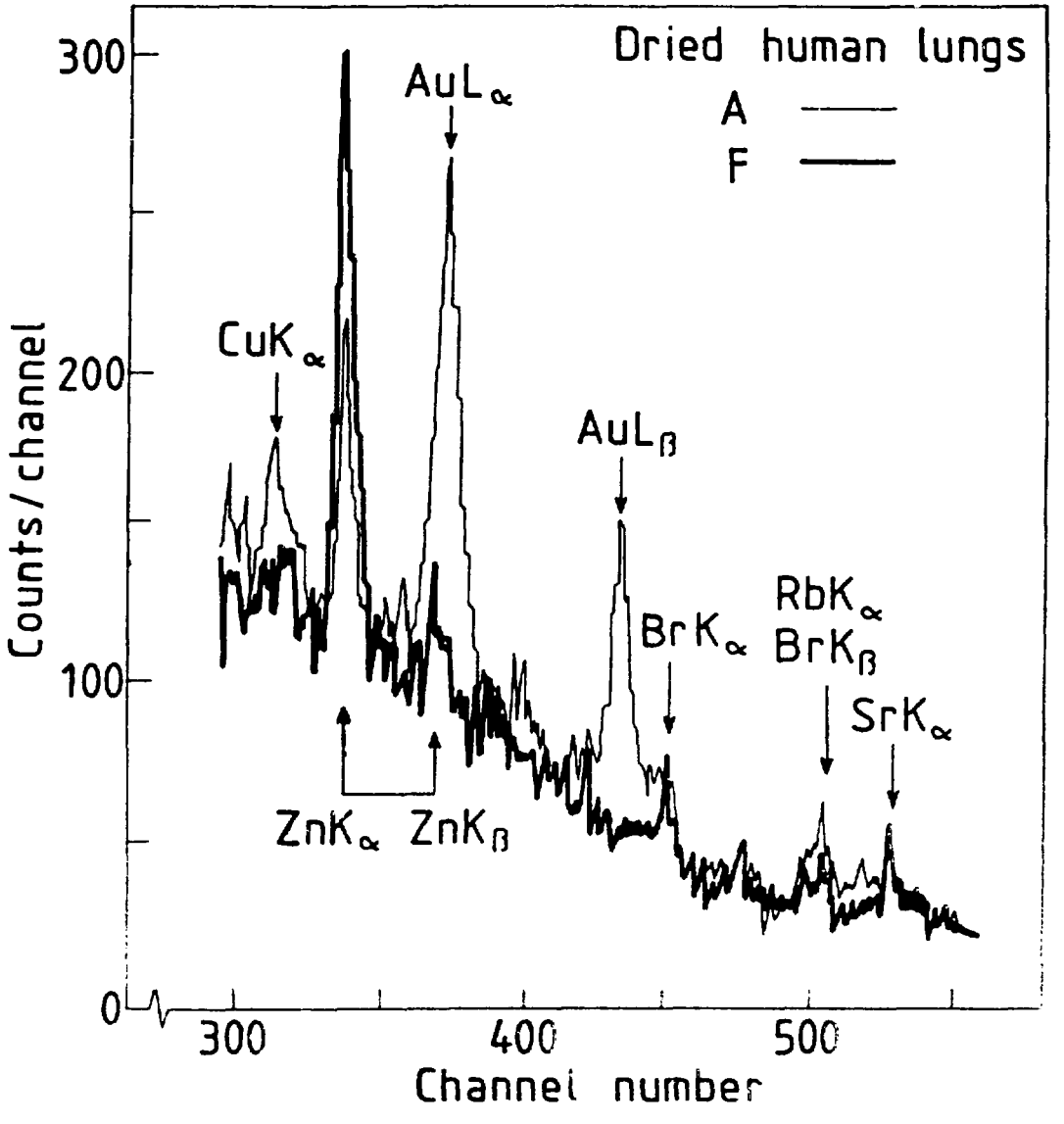
Dried human lungs
A
F
AuL$_\alpha$
CuK$_\alpha$
AuL$_\beta$
BrK$_\alpha$
RbK$_\alpha$
BrK$_\beta$
SrK$_\alpha$
ZnK$_\alpha$
ZnK$_\beta$
Counts / channel
300
200
100
0
300
400
500
Channel number

Fig. 4

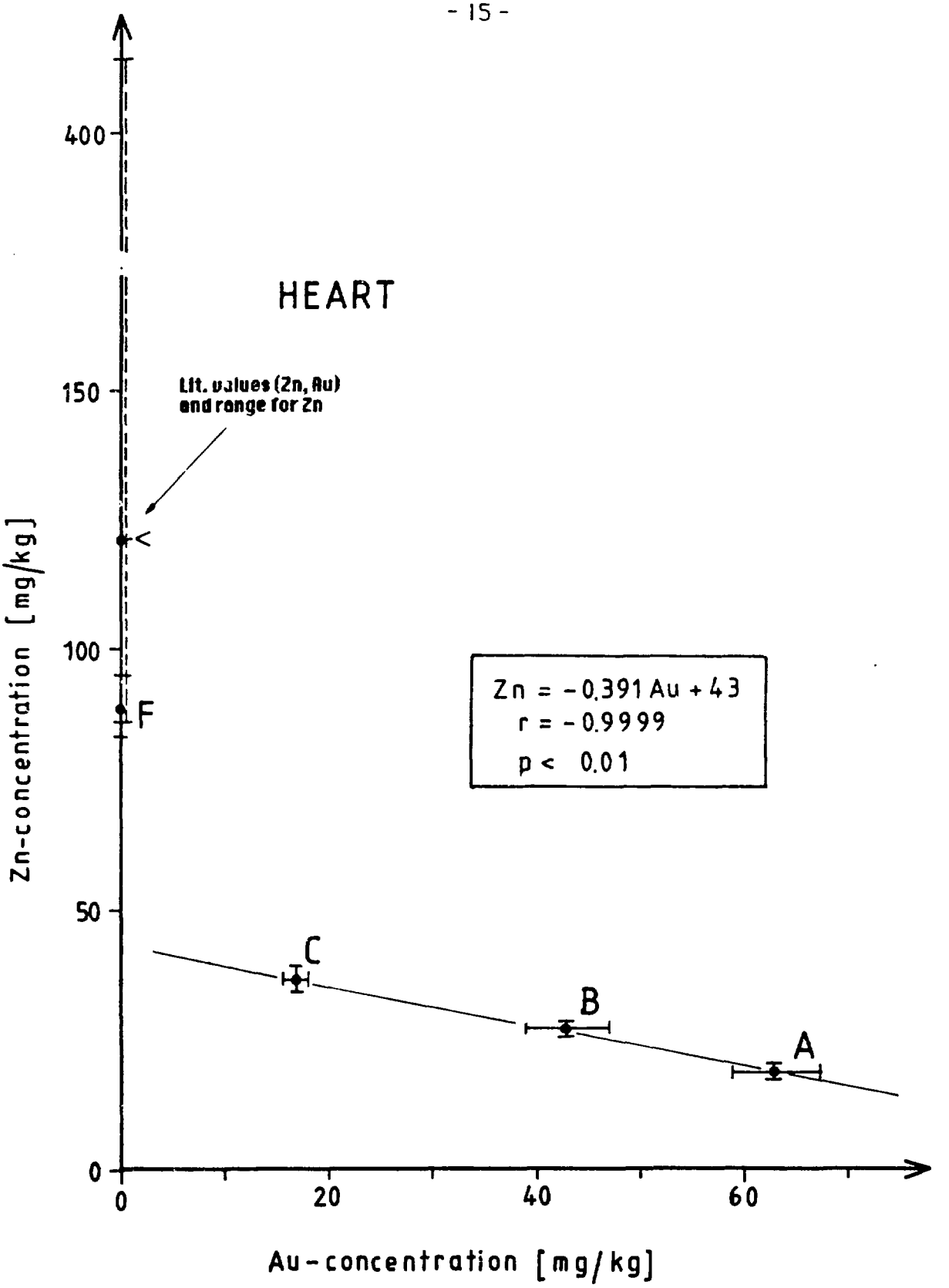
HEART
Lit. values (Zn, Au) and range for Zn
Zn = -0.391 Au + 43
r = -0.9999
p < 0.01
Zn-concentration [mg/kg]
Au-concentration [mg/kg]
400
150
100
50
0
0
20
40
60
A
B
C
F

Fig. 5.

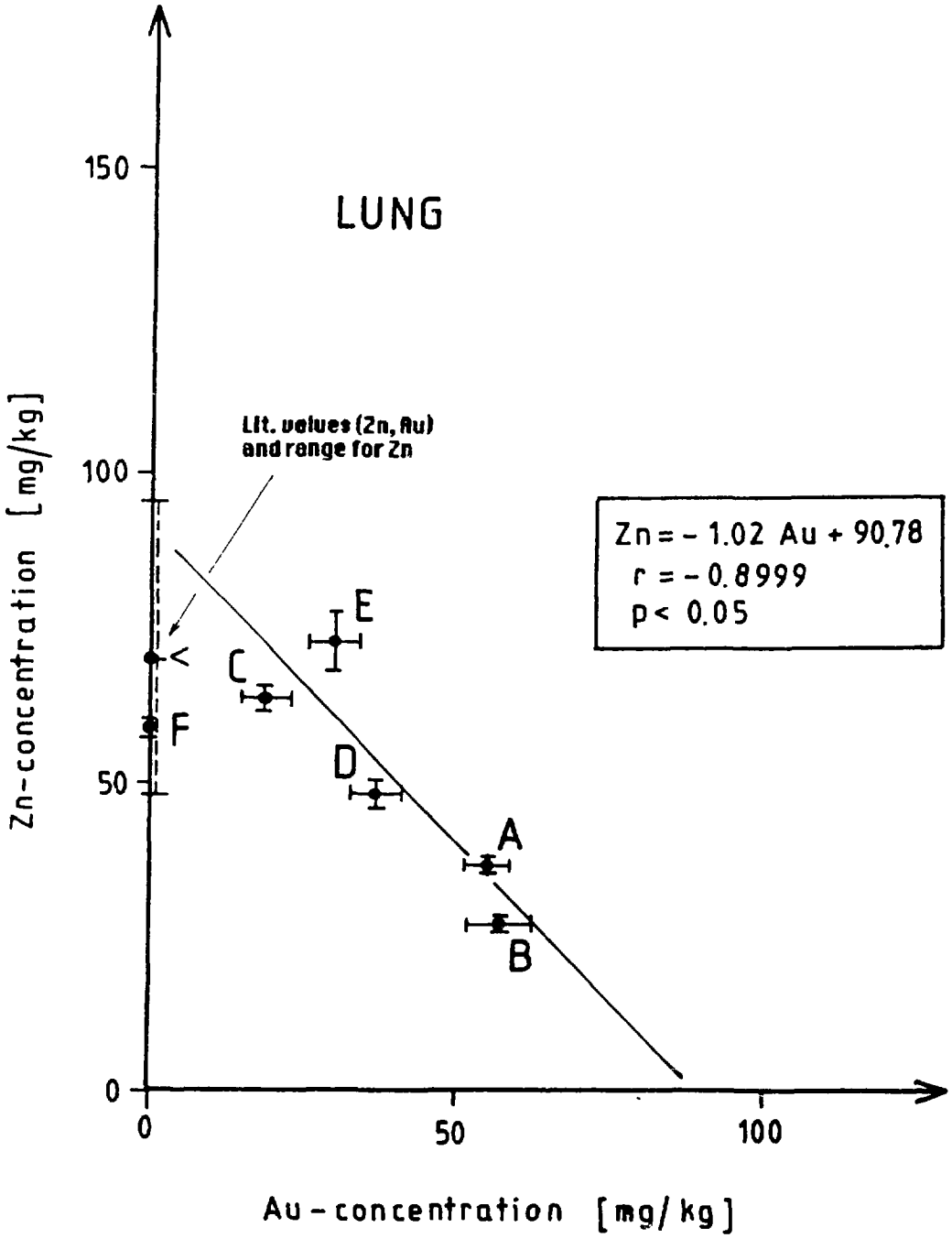
LUNG
Lit. values (Zn, Au) and range for Zn
Zn = - 1.02 Au + 90.78
r = - 0.8999
p < 0.05
Zn-concentration [mg/kg]
Au-concentration [mg/kg]
150
100
50
0
0
50
100
A
B
C
D
E
F

Fig. 6.

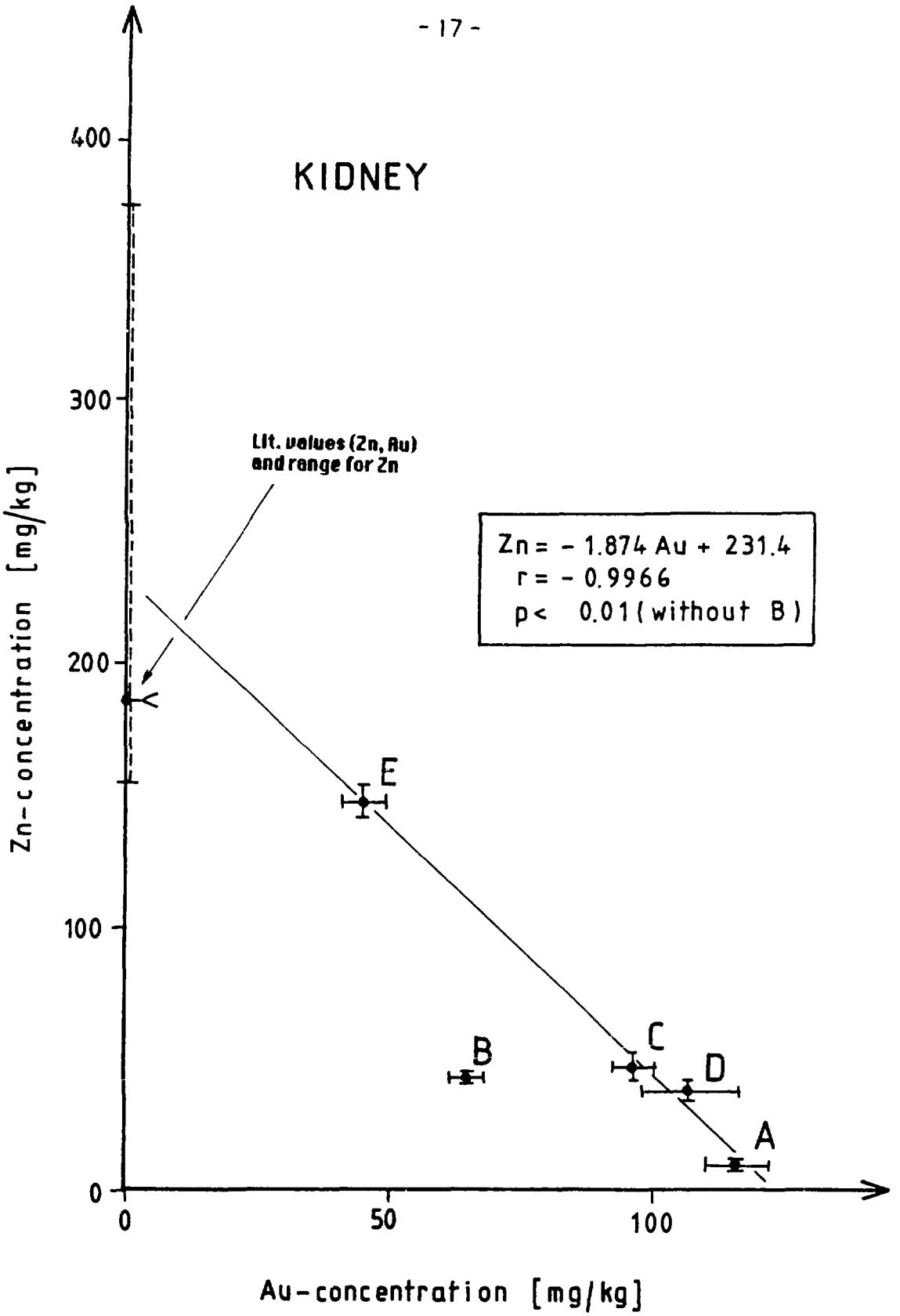
KIDNEY
Lit. values (Zn, Au) and range for Zn
Zn = - 1.874 Au + 231.4
r = - 0.9966
p < 0.01 (without B)
Zn-concentration [mg/kg]
Au-concentration [mg/kg]
400
300
200
100
0
0
50
100
A
B
C
D
E

Fig. 7

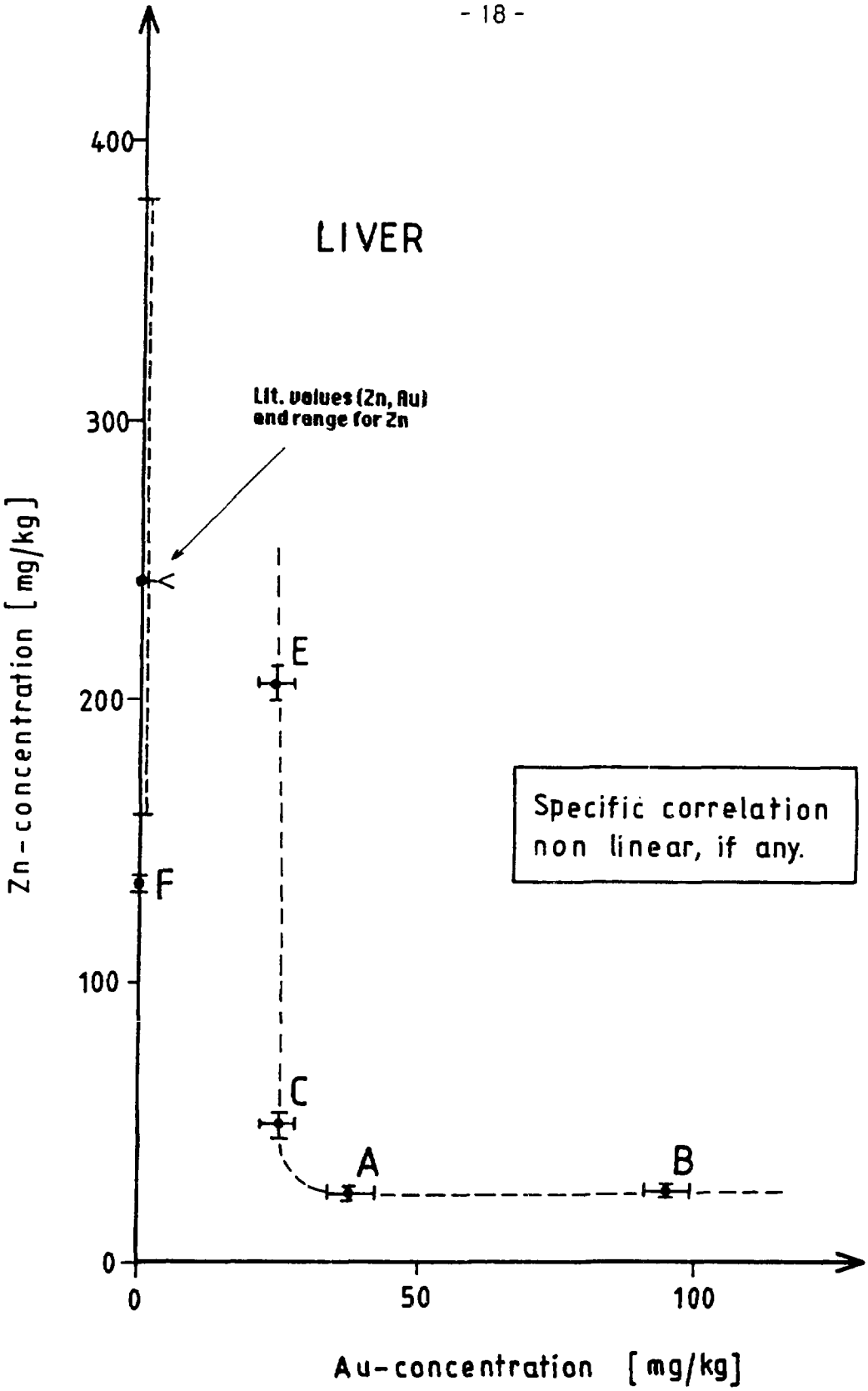
LIVER
Lit. values (Zn, Au) and range for Zn
Specific correlation non linear, if any.
Zn-concentration [mg/kg]
Au-concentration [mg/kg]
400
300
200
100
0
0
50
100
A
B
C
E
F

Fig. 8.

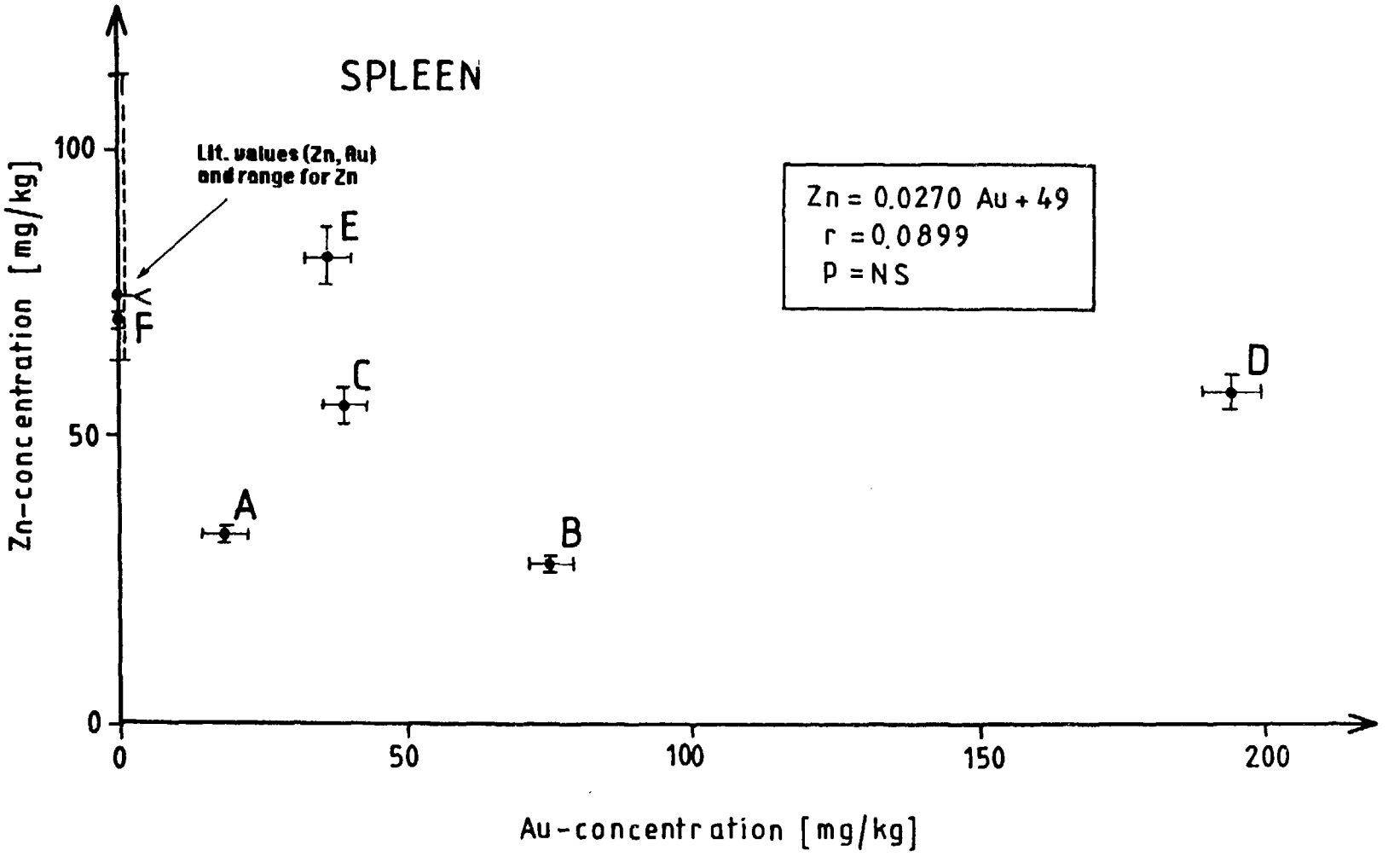
SPLEEN
Lit. values (Zn, Au) and range for Zn
Zn = 0.0270 Au + 49
r = 0.0899
P = NS
A
B
C
D
E
F
Zn-concentration [mg/kg]
Au-concentration [mg/kg]
0
50
100
150
200

Fig. 9

Kiadja a
Magyar Tudományos Akadémia
Atommagkutató Intézete
A kiadásért és szerkesztésért felelős
Dr. Berényi Dénes, az intézet igazgatója
Készült a Kinizsi Szakszövetkezet
Nyomdájában