LEAD LEVELS IN HUMAN PLACENTAE AND FOETAL HEALTH

by

Ashok Kumar Khera

A thesis presented for the degree of

Doctor of Philosophy

in the

University of Aston in Birmingham

June 1981

Department of Pharmacy

University of Aston in Birmingham

Birmingham B4 7ET

SUMMARY

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Natural sources of lead as well as its distribution in the environment from its industrial uses have been discussed. Historical review of the excessive maternal lead exposure and its effect on reproduction are further discussed.

Lead levels in human placentae, foetal tissues, maternal and cord blood have been measured by the carbon furnace atomic absorption spectroscopy. The comparatively higher lead levels in still birth placentae when compared to placentae from normal births are possibly a reflection of higher calcium accumulation in the form of nodules in human placenta. The apparent low calcium levels in still birth bones further suggests the movement of calcium from foetus to mother. During this process lead may accumulate along with calcium in human placenta.

Further, the lead levels in human placenta do not correlate with either the maternal or cord blood lead levels. However, there is a significant positive correlation between maternal and cord blood lead levels suggesting that the human placenta does not operate as a complete barrier for lead and therefore in the case of excessive maternal lead exposure may damage the foetus.

KEY WORDS: LEAD, PLACENTA, FOETAL HEALTH

ACKNOWLEDGEMENTS

I am thankful to my teacher, Professor D. G. Wibberley, Professor of Medicinal Chemistry, for his guidance, advice, supervision, constructive criticism and encouragement throughout the course of this investigation.

The data presented in the thesis is on the samples received from Dr. D. I. Rushton of the Birmingham Maternity Hospital and Dr. J. G. Dathan (formerly of the Health and Safety Executive, Stoke-on-Trent). The author is thankful to both of them.

The author's previous postgraduate training in trace metal research at the Agricultural University of Norway, Oslo University and the Institute for Atomic Energy, Kjeller, Norway, was of great help. The author feels highly privileged in expressing his sincere thanks and deep gratitude to his teachers at these institutes (in particular Professors J. Låg and E. Steinnes) for their continual source of inspiration.

CONTENTS

1. INTRODUCTION

The adverse effects of excessive exposure of lead like anemia, renal damage and encephalopathy are well known. Women exposed to excessive lead in the pottery industries during the early 1900's were reported to produce more structurally and neurologically inferior infants. Other reports also suggested an increased rate of stillbirths and abortions among women lead workers.

Less well known are the toxic effects to relatively low concentrations of lead. One group of the general population which may be particularly vulnerable to the toxic effects of environmental levels of lead is the pregnant women and foetus. However, very little is known about the possible effects of prenatal lead burden of the embryo and the foetus respectively.

In order to attempt to clarify the extent of prenatal lead exposition the relatively easily obtainable investigation material is placenta, besides an investigation of this organ as a parameter of environmental exposure seems particularly appropriate because of the exposure time over a particular period of time. It could also be assumed that the lead level in the placenta in relation to defined period of exposure would be more representative than the blood lead level and be a better parameter as an environmental exposure. The human placenta is also of particular importance to the problem of whether pregnancy by itself leads to incorporated lead from other organs being mobilised and whether the placenta then acts as a secondary collector of lead.

The investigations made in this study were into the lead levels in human placentae, foetal tissues, blood lead levels of mothers and babies. The intention was to determine whether such analytical technique could provide a marker of exposure to lead and an indication of any teratogenic role of the metal.

1.1 Properties of Lead

1.1.1 Properties of Metallic Lead

Metallic lead is bluish white in colour and has a bright lustre. Important physical attributes of lead include high density, softness, flexibility and malleability, low melting point and low elastic limit. These properties as well as its high lubricity, low electric conductivity, high corrosion resistance, and high coefficient of expansion determine its widespread industrial applications. Some of the important physical properties of lead are summarized in Table 1.

Table 1

Physical Properties of Metallic Lead[1]

Atomic Number	82
Atomic Weight	207.2
Valence State	2 or 4
Stable Isotopes with relative abundance	204 (1.48)
	206 (23.6)
	207 (22.6)
	208 (52.3)
Melting Point	327.5^{o}C
Boiling Point (normal)	1740^{o}C
Thermal Conductivity	0.346 W/cm/^{o}C (25^{o}C)
	0.396 W/cm/^{o}C (100^{o}C)
Hardness, Moh's scale	1.5
Density	11.342 g/cm^{3} (25^{o}C)
Refractive Index	2.01 wavelength of 3890 Å
Reflectivity	62%
Surface Tension 350^{o}C	445 dyne/cm
450^{o}C	438 dyne/cm
1600^{o}C	310 dyne/cm
Crystal properties	Face centred cubic: Fm3m:
	A : 49505 A

Lead is commonly used for radiation protection because the gamma and X-rays are efficiently attenuated without the lead shields becoming radioactive. Lead forms both plumbeous salts, in which the metal is divalent, and plumbic salts, in which it is tetravalent; the plumbic salts are, however, difficult to prepare and are readily decomposed by water.

Lead stands immediately above hydrogen in the electrochemical potential series. Lead does not, however, generally dissolve in dilute acids because hydrogen is evolved on pure lead only at very high overpotential, and because of the common formation of insoluble surface coatings which inhibit further reaction. Lead is insoluble in sulphuric acid, due to the formation of insoluble lead sulphate coating, and is practically unaffected by Hydrofluoric acid. Nitric acid, because of its strong oxidizing power, dissolves lead readily, whereas lead dissolves sparingly in hydrochloric acid solutions. The relatively great susceptibility of lead to attack by acetic, citric, tartaric and other organic acids is largely due to the formation of very stable organo lead complexes.

Lead is not attacked by pure water in the absence of air. In the presence of atmospheric oxygen, lead becomes susceptible to attack by all acids, including very weak acids and water. The rapid tarnish of metallic lead in moist air and the injurious effects due to the increased plumbosolvency of soft water are well known phenomena.

When heated in air, metallic lead turns to monoxide Litharge, Pb O; further heating in air converts this to Pb_3O_4 at $430^{o}c$ and above $550^{o}c$ the latter phase dissociates into PbO and O.

1.1.2 Properties of Lead Compounds: (Inorganic)

Although lead has four electrons in its valence shell, only two ionize readily. The inorganic salts of lead (II), lead sulphide and oxides of lead are generally poorly soluble with the exception of nitrates and chlorates. Some of the main properties of lead compounds are given in Table 2.

1.1.3 Properties of Lead Compounds (Organic)

Under appropriate conditions of synthesis, stable compounds are formed in which lead is directly bound to a carbon atom. Tetraethyl lead and tetramethyl lead are well known organo compounds. They are of great importance owing to their extensive use as fuel additives. Both are colourless liquids and their volatility is lower than most gasoline components.

1.2 Natural Sources of Lead in the Environment

In nature, bivalent lead is commonly found as the sulphide, selenide and telluride, as well as in a large number of sulfo-salts where it acts as the cation. On the other hand, tetravalent lead is found as the oxide in highly oxidizing environments such as in hot arid areas. The lead contents of some rock-forming minerals is shown in Table 4. On account of its ionic radius (between 1.18 and 1.32 Å), Pb^{2+} can replace potassium (1.33 Å), strontium 1.12 - 1.27 Å), barium (1.34 - 1.43 Å) and even calcium (0.99 - 1.06 Å) and sodium (0.95 - 1.0 Å) in mineral lattices.

Among silicate minerals, potassium feldspars of pegmatite are notable accumulators of lead; micas usually contain less lead than potassium feldspars. The high lead content of base metal sulphides may be due to the entrapment of fine-grained galena in

Table 2 Some Properties of Lead Compounds (Inorganic)[2]

Name of compound	Formula	Molecular wt	Melting Point °C	Boiling Point °C	Solubility in cold water (g/litre)	Soluble in
Lead Acetate	$Pb(C_2H_3O_2)_2$	325.28	280	-	4.43	Hot water & Glycerine
Lead Azide	$Pb(N_3)_2$	291.23		explodes at 350	0.23	Acetic acid; hot water (0.9 g/l)
Lead Carbonate	$Pb\ CO_3$	267.20	315 (decompose)	-	0.0011	Acid; alkali: decomposes in hot water
Lead Chlorate	$Pb(ClO_3)_2$	374.09	230 (decompose)	-	Very soluble	Alcohol
Lead Chloride	$Pb\ Cl_2$	278.1	501	950	9.9	NH_4 salts; slightly in dilute HCl & NH_3; hot water (33.4 g/l)
Lead Nitrate	$Pb(NO_3)_2$	331.20	470 (decompose)	-	376.5	Alcohol, alkali, NH_3; hot water (1270 g/l)
Lead Sulphide	PbS	239.25	1114	-	0.00086	Acid

Table 3 — Properties of some organic compounds[2]

		Molecular wt	Boiling Point	Melting Point ^{o}C	Solubility in cold water	Soluble in
Tetraethyl lead	$Pb(C_2H_5)_4$	323.44	200^{o}C (decompose)	-136.80	Insoluble	Benzene Petroleum
Tetramethyl lead	$Pb(CH_3)_4$	267.3	110^{o}C	- 27.5	Insoluble	Alcohol Ether

Table 4

Lead Concentrations in common rock-forming minerals[3]

Mineral name	No. of analysis	Mean (ppm)	Range (ppm)
Potassium feldspar (pegmatitic)	219	98	0.9 - 10,000
Potassium feldspar (granitic)	419	53	3 - 700
Amphiboles	85	15	1 - 70
Biotites	259	21	4 - 95
Muscovites	32	26	5 - 77
Olivines	15	1.9	0.2 - 8.5
Pyroxenes	20	5.9	0.3 - 20
Quartz	15	1.0	0 - 5
Pyrites, sedimentary	297	62	2 - 1000
Pyrites, metamorphic	20	56	4 - 500
Apatite	58	102	5 - 2000
Halite	109	0.18	0 - 1.7
Sylvite	27	0.56	0.04 - 2.5
Carnallite	6	0.06	0.02 - 0.16

these mineral phases. The high concentration of lead in iron and manganese oxides reflect the fact that these compounds have a very strong affinity for lead.

1.2.1 Lead in Rocks

Lead is the most abundant of the heavy metals in the earth's crust. An important factor contributing to its relatively high abundance is that its three major isotopes, ^{206}Pb, ^{207}Pb, and ^{208}Pb, are the end products of the decay of a considerable number of the radioactive nuclides produced by neutron capture processes. Of overwhelming importance in this regard are the decay of uranium and thorium; these elements are highly enriched in the earth's crust and their decay throughout geological time has produced about one-third of the lead in the crust today. The average crustal abundance of lead is found to be 16 ppm on the basis of various reports.

The most important sources of lead are igneous and metamorphic rocks. The lead content of these rocks ranges between 1-23 ppm. The lead content of sedimentary rocks is of the same order of magnitude (Table 5).

1.2.2 Lead in Soils

Lead is present in all soils which range from less than 1.0 ppm in uncontaminated soils to well over 10% in ore bodies. The concentrations encountered in normal uncontaminated soils (Table 6) are similar to concentrations found in rocks with an average range of 2 - 200 ppm, and mean value for soil as 16 ppm. Lead in soils may be derived from natural or anthropogenic sources. The natural sources include weathering of rocks and are deposits,

Table 5

Lead Concentrations in Rocks[3]

Rock Type	No. of samples	Mean (ppm)	Range (ppm)
Igneous Rocks:-			
Peridotites	17	0.2	0.01 - 0.5
Gabbro	20	1.9	0.4 - 6.4
Andesites	166	8.3	0.6 - 36
Syenite	78	13.9	4.7 - 50
Rhyolites	130	21.5	3 - 50
Granites	1460	22.7	2 - 200
Metamorphic Rocks:-			
Eclogites	29	1.5	0.1 - 5.5
Marble	12	3.9	0.9 - 7
Hornfels	44	17.9	6 - 25
Slates	34	21	7 - 46
Sedimentary Rocks:-			
Sandstone	724	9.8	1 - 50
Shales (bituminous)	1130	27.4	1 - 100
Shales (kaolinitic)	18	49	30 - 100
Limestone dolomites	950	6.7	1 - 100

volcanoes, fires and blowing dust.

Lead primarily occurs in potassium feldspars and micas of igneous and metamorphic rocks, with the exception of biotite, these minerals are farily resistant to weathering. Furthermore, the low concentration of lead in surface waters is evidence that lead is not readily solubilized during chemical weatherings, owing to fluvial, glacial and/or eolian action, and lead concentration can show up at considerable distances from the source ore beds. Alloway and Davies[4] and Davies and Lewin[5] have reported a classic example from Devon and Cornwall of flood plains which are highly

Table 6

Lead Content of some Uncontaminated Soils

Soil location	Average Pb (ppm)	Reference No:-
Russia	12	6
New Zealand	16	7,8
Scotland	30	9
Wales	42	4
Egypt	21	10
Nigeria	18	11

contaminated (lead levels 116 - 1200 ppm) by waterborne lead. Similarly, pollution in lead mining in areas of Derbyshire (soil lead levels ranging between 140 - 1575 ppm) have been reported.[12,13]

1.2.3 Air

The atmospheric lead cycle can be considered to consist of three phases: the liberation of lead at source, the dispersal of the lead in the atmosphere and deposition of airborne lead in

different ecosystems. The global cycling of lead via the atmosphere has also been receiving considerable attention since significant accumulation of anthropogenic lead were discovered in very remote areas of the world.[14,15] Patterson[16] estimated from geochemical data that the concentration of lead in air of natural origin is about 0.0006 $\mu g/m^3$. The atmospheric concentration of lead measured at points most remote from civilisation is of the order of 0.0001 - 0.001 $\mu g/m^3$. If Patterson's estimates are correct, then even the air over uninhabited, remote, continental areas may be contaminated by human activities.

Worldwide emission of lead from natural sources is estimated to be 18,600 tons per year. Contributions from windblown dusts constitute about 85% of the natural lead emissions. About 10% of airborne lead from natural sources is from plant exudates and other natural hydrocarbons.[17] The remaining 3% comes from forest fires, volcanogenic and meteoric particles. The contributions of lead from radioactive decay and sea salt sprays are relatively insignificant. The release of lead from natural sources is small (about 4%) compared to the technological emission. Of the 4.4×10^5 tons released by anthropogenic lead emissions for 1974/75[17], about 61% comes from combustion of leaded gasoline, the other major contributors of airborne lead include production from base metals (23%), mining and smelting of lead (8%) and non-automotive burning of fossil fuels (5%).

1.2.4 Water

Analysis of ground water have revealed lead concentrations ranging from 1 - 60 $\mu g/l$. Edmunds,[18] reported that ground waters from carboniferous limestone from Derbyshire contained

1 - 16 µg/l. The lead content of various carbonate rich mineral waters from Czechoslovakia averaged 22 to 66 µg/l[19] and those from sulphate rich mineral waters of Germany ranged from 1 - 67 µg/l.[20] Goleva et al.[21] reported that anomalous lead contents in natural waters are related to oxidation of lead bearing suphide deposits or leaching from rocks by chloride and bicarbonate waters. Maximum lead contents of 3000 to 5000 µg/l were found in mine waters of deep seated thermal fluids.

Lead compounds have entered the oceans over the last few hundred thousand years at an average rate of 1.7×10^{10} g/yr.[22] The total flow of rivers into the ocean is about 3.7×10^{16} l/yr.[23] Therefore, an approximate world average natural lead level in surface water is 0.5 µg/l. However, this natural level would vary greatly with location, depending on the geology of the drainage areas as well as the activities of man. Patterson (1965) estimated that the lead content of surface waters increased from a prehistoric level of 0.5 µg/l to 5 µg/l in modern times. The estimated global mean lead content in lakes and rivers is 1 - 10 µg/l.[24] High concentrations of lead in surface waters usually occur near sources of contamination. Industrial discharge and rainfall are other major contributors of lead to river waters.

The concentrations of lead in sea water have been found to be lower than in freshwaters. Lead levels in the sea water in the range of 0.08 - 0.4 µg/l have been reported.[25] In deep sea water reported levels are even lower, i.e. 0.03 to 0.04 µg/l.[26]

Large quantities of lead aerosols in the atmosphere are eventually returned to earth by fallout, impaction and washout. Therefore rainwater may contain a significant amount of lead as a

result of airborne lead being washed out in the precipitation process. Ettinger[27] reported that rainwater samples collected at Ohio showed 3 out of 23 samples to contain an excess of 50 µg/l. Peirson et al.[28] obtained an average lead content of 39 µg/l for rain samples collected at Lake Windermere in 1971.

1.2.5 Plants

Lead occurs naturally in all plants. Extremely variable concentrations of lead in plants have been reported. Warren and Delavault[29] have concluded that the normal concentrations of lead in leaves and twigs of woody plant is 2.5 mg/kg on a dry weight basis. For vegetables and cereals they estimated normal concentration to be 0.1 - 1.0 mg/kg dry weight. Mitchell[30] found that the usual concentration of lead in pasture grass was 1.0 mg/Kg dry weight.

1.2.6 Environmental Contamination from Natural Sources

The contribution of natural sources of lead to lead concentrations in the environment is small. As regards exposure of man, these sources are negligible except in anomalous local contamination environment. Through various breakdown processes, rocks yield lead which is transferred to the biosphere and the atmosphere and ultimately back to earth's crust in the form of sedimentary rocks. Sources contributing to airborne lead are silicate dusts, volcanic halogen aerosols, forest fires, sea salt aerosols, and lead derived from the decay of radium.

1.3 Production, Uses and Distribution of Lead in the Environment

1.3.1 Lead Mining

Lead is produced from ores and recycled lead products. Lead occurs in a variety of minerals, the most important of which are

galena (PbS), Cerrusite ($PbCO_3$) and Anglesite ($PbSO_4$). Galena is by far the most important source of primary lead. It occurs mostly in deposits associated with other minerals, particularly those containing zinc. Mixed lead and zinc ores account for about 70% of the total primary lead supplies. Ores containing mainly lead account for about 20% and the remaining 10% is obtained as a by-product from other deposits. Silver is the most important of the other metals frequently present in lead deposits but copper may also be present in high concentration. Other minor constituents of lead ores are gold, bismuth, antimony, arsenic, cadmium, gallium, thallium, indium, germanium and tellurium. The lead content of ores is comparatively low (3 - 8%), but even ores with lower lead contents may be commercially valuable.

The level of world mine production of lead has increased in recent years, from 2.6 million tonnes in 1965 to about 3.6 million tonnes in 1975. The most important lead mining countries are Australia (10% of the total world output), Bulgaria (3%), Canada (9.6%), China (3.8%), Mexico (4.5%), Peru (5.5%), U.S.A. (16%), U.S.S.R. (14.5%) and Yugoslavia (3.5%). In addition there are about 50 countries producing only small amounts each, making together only some 12% of the world production.

1.3.2 Smelting and Refining

Smelting and refining is classified as either primary or secondary, the former producing refined lead from concentrates (primary lead) and the latter the recovery of lead from scrap (secondary lead). Secondary lead accounts for about half the consumption in the United States of America and it has been estimated that about 35% of the total world supply comes from

secondary sources.

1.3.3 Consumption and Uses of Lead and its Compounds

The estimated total world consumption of lead in 1977 was about 4.4 million tonnes. There has been a steady increase to modern day consumer uses of lead over the last thirty years (Table 7), even when there has been a decline in use of refined lead in the United Kingdom.

Table 7

World Refined Consumption of Lead[31,32] (thousand metric tonnes)

	U.K.	Europe	U.S.A.	World Total
1940	348	747	563	1653
1950	236	640	803	1856
1960	286	1061	647	2633
1970	262	1358	826	3805
1975	238	1169	811	3878
1977	241	1380	988	4436

The manufacture of electric storage batteries is responsible for the largest consumption of lead. This industry uses both metallic lead in the form of lead-antimony alloy, and lead oxides in about equal proportions. The metallic lead is in the grids and lugs, while the oxides, litharge (PbO), red lead (Pb_3O_4) and grey oxide (PbO_2) are used in active material that is pasted on the plates. The battery industry also constitutes the major source of lead for secondary lead production. It has been estimated that

Table 8

Uses of Lead in U.K.[32] (Thousand metric tonnes)

	1951	1961	1971	1977
Batteries	62	68	101	68
Alkyl leads	5	25	44	55
Sheet and pipe	72	73	54	47
Cables	92	97	52	31
Oxide and other compounds	34	23	31	31
Alloys	15	21	17	13
Solder	12	16	14	14
Shot	6	6	6	8
White lead	21	8	3	2
Collapsible tube	6	4	2	1
Rolled and extruded products	-	7	2	1
Miscellaneous	17	20	20	19
Total	341	369	346	290

up to 80% of the lead in storage batteries is recovered at secondary smelters.

Alkyl lead compounds have been in use as anti-knock additives for almost 50 years. Use of tetraethyl lead and tetramethyl lead increased steadily up to 1973 (380,000 tonnes). The gradual decrease in consumption of about 301,000 tonnes in 1975 was almost entirely attributable to a decrease in the use of lead for gasoline additives in U.S.A. The use of refined lead for the manufacture of alkyl leads has increased (Table 8) considerably in the U.K. since 1951. The world wide decrease in the use of lead for fuel additives is likely to continue as more cars fitted with catalysts (presumably mostly in U.S.A.) requiring lead free gasoline will come into use. The maximum permissible level in the Federal Republic of Germany has been 0.15 g of lead per litre of petrol since January 1976 and Japan has been 0.31 g of lead per litre since 1971. Some European countries introduced limits of 0.4 g of lead per litre but most European countries have deferred their decision because of the economic implications of lowering the lead content. In the United Kingdom, there has been a phased reduction of the lead content of petrol from the 1971 value of 0.84 g per litre to the present day 0.46 g per litre. It is further proposed to reduce this level to 0.15 g/litre by Jaunary 1986.

The elimination or the reduction of lead additives will lead to lower octane gasoline, or necessitate the maintenance of octane ratings by some substitute, or substitutes, for lead. Lower octane gasoline requires lower compression ratios, which signify a loss of engine performance and an increase in gasoline

combustion, this is an undesirable consequence against the background of energy crisis. A loss in performance would particularly effect the relatively small European cars. The means to increase the octane ratings by other additives will thus assume major importance. The easiest and least costly way would be that of increasing the content of aromatic hydrocarbons. Unfortunately, the content of aromatic hydrocarbons in European fuel is already higher, for instance, than that in the U.S.A.

The implication connected with an increased content of aromatic hydrocarbons in gasoline is probably even more obscure than the continued use of lead. Berlin, Gage and Johnson[33] have reviewed the likely consequences of such an increase. An increase of potentially carcinogenic polynuclear aromatic hydrocarbons in ambient air would occur. Probably, an increase of toxic products, such as eye irritating ones, would take place as a result of photochemical reactivity of a number of hydrocarbons. Moreover a subsequent increase in the benzene content would introduce an occupational risk for all those involved in the handling of gasoline. Although the emission of hydrocarbons can partly be controlled by the use of catalytic exhaust treatment devices, the lead additives in gasoline cause impairment of the effectiveness of such equipments; thus, further aspects favouring a reduction of lead in gasoline must be borne in mind.[34] Regardless of the solution that is chosen, the reduction of lead additives will be associated with high costs. Other uses of lead in various industries in the United Kingdom is given in Table 8. The relative importance of the cable industry as a lead consumer has declined considerably, mainly owing to the introduction of plastic sheathing and insulation. However, the

amount of lead used is notable. Alloys used for cable sheathing contain small amounts of many other elements including cadmium, tellurium, copper, antimony and arsenic.

In the chemical industry, although a wide range of lead pigments are still produced they are increasingly being substituted by other less toxic pigments. Red lead is used in the painting of structural steel work and lead chromate is often used as a yellow pigment. Lead arsenate was, at one time, an important insecticide but is now little used. The petroleum industry also uses a small amount of litharge dissolved in sodium hydroxide solution to remove sulphur compounds in the refining of petroleum.

The building and construction industries use lead sheet for roofing and other flashings, wall cladding and sound insulation. Lead also forms alloys readily and is used in solder, bearing metals, brasses, type metal, collapsible tubes and for radiation shielding. There are many minor uses of lead compounds but these account for only a small proportion of total lead consumption.

1.3.4 Environmental Pollution from Production and Consumption of Lead

1.3.4.1 Environmental Pollution from Production

Mining, smelting and refining as well as manufacturing of lead containing compounds and goods can give rise to lead emission. Smelters of lead ores are well known to create pollution problems in local areas. The zone of air pollution in one large smelter in the U.S.A. extended to approximately 5 km from the smelter while soil contamination extended as far as 10 km.[35] The influence on the surrounding air and soil depends on a large

extent on the height of the stock, the trapping devices in the stacks, the topography and the local features. Secondary smelters producing lead from scrap are comparatively small, numerous and freqeuntly situated close to human environment. Pollution in the surroundings of such smelters can be severe enough to produce a measurable increase in the intake of lead by people living nearby.

1.3.4.2 Environmental Pollution from Industrial uses of Lead

The consumption of alkyl lead additives in motor fuels account for the major part of all inorganic lead emissions. The consumption of lead for the manufacture of alkyl leads was estimated at 380,000 tonnes in 1973 and 300,000 tonnes in 1975. Of this amount over 70% enters the environment immediately after combustion.[36,37]

Definitive data on the inorganic forms of lead in the atmosphere are lacking, except for the identification of several lead compounds emitted in the exhausts of automobiles using leaded gasoline as fuel. The chief lead emission products are lead bromochloride and the alpha and beta forms of ammonium chloride and lead bromochloride and minor quantities of lead sulphate. The chemical and physical characteristics of these unstable compounds are largely unknown.

The most urgent consideration in alkyl lead pollution abatement is the elimination or reduction of lead emission from the exhaust pipe. In the longer term, engines of lower octane requirement and therefore of lower efficiency, could be accepted.[38] Habibi et al.[39] suggest the control of exhaust particle lead emissions by utilization of a cyclone trap system on the exhaust system of the automobile. Such a system could greatly reduce the

amount of lead released into the atmosphere, but much research needs to be done in developing such an exhaust device.

The manufacture of storage batteries emitted smaller amounts, for example 480 tonnes in a year in U.S.A., and emissions were still smaller in the production of lead oxides, lead pigments, type metal and solder etc. However, contamination of domestic water supplies, foods and beverages resulting from the use of lead pipes, PVC pipes and from cans with lead containing solders may under circumstances be hazardous to human health.[40]

1.4 The Circulation of Lead in the Environment

The transport and distribution of lead from stationary or mobile sources into the environmental media is mainly through the atmosphere. The mass transfer of lead from air to other media is as yet poorly defined and the various mechanisms involved in the removal of lead from air are not fully understood. Although some data indicate that an important proportion of lead may be removed through sedimentation,[41] the most efficient clearing mechanism is probably rain.[42]

Lead is rapidly removed from water when it passes through soil and bottom sediments. This is due to the high capacity of organic matters to bind lead firmly. Because of this clearing mechanism, lead concentrations in both natural waters and water supplies are generally low.

The lead emitted by automobiles, as different sized particles, is carried away by air transport and is probably widely dispersed and diluted since the atmospheric retention time of small particles is probably fairly long. In spite of the great dilution of

airborne lead that occurs during transport from centres of human activity, there is evidence indicating that a long term global accumulation of lead has occurred. Studies in Greenland showed that ice formed in 1750 had lead concentrations 25 times greater than ice estimated to have been formed in about 800 B.C.[43] Similar studies on Polish glaciers by Jaworaski et al.[44] have shown approximately 16 fold increase in lead concentrations over the past 100 years. Studies on Swedish mosses have also shown four fold increase over a period from 1860 to 1968.[45]

The transfer of air lead to the biota may be direct or indirect. For plants, the fallout contribution may be direct via the above ground parts or it may be indirect by way of the soil. The total concentration of lead in soil does not correlate well with the concentration in the plants but a correlation does exist when adjustment is made for the degree to which the soil lead can be brought into aqueous solution of ammonium lactate and acetic acid.[46] Transfer of lead from plants to animals is not well defined. However the concentration in most are similar to those found in vegetables and grains. Thus, there is no evidence of biological accumulation proceedings from plants to animals.

Little information exists with regards to the biotransformation of lead by micro organisms in the environment. Wong et al.[47] have reported that micro organisms in lake sediments can transfer certain inorganic and organic lead compounds into volatile tetramethyl lead. However, the mechanism of this transformation is not clear.[48]

1.5 Environmental Levels and Exposures

The general population is exposed to lead by ingestion of food and water and inhalation. In addition children are exposed by eating non-food items and those working in the lead industries suffer exposure over and above their exposure as members of the general population.

1.5.1 Food

The contribution of food to man's exposure to lead has been under study for many years, beginning with the studies of Kehoe et al.[49] who found lead in every item of food in both industrial and primitive societies. The concentration of lead in various items of food is highly variable. For example, Schroeder et al.[50] found that the range was 0 - 1.5 mg/kg for condiments, 0.2 - 2.5 mg/kg for fish and seafood, 0 - 0.37 mg/kg for meat and eggs, 0 - 1.39 mg/kg for grains and 0 - 1.3 mg/kg for vegetables. Average lead levels in wines could range from 60 to 255 µg/l,[51] and is likely to be a substantial source of lead in some cases. The concentration of lead in milk is a matter of special concern because milk is a major dietary constituent for infants. Human breast milk has been reported to contain 12 µg/l.[52] Cow's milk has been reported to have similar concentration.[53] The concentration of lead in processed cow's milk is higher than in human milk or in milk obtained directly from cows.[54]

Although plants do not take lead up readily, fruits and vegetables grown in areas exposed to smelters emissions may be appreciably contaminated. Kerin[55] determined lead in the total diet of peasants near a smelter and found that daily ingestion of lead with food was 670 - 2640 µg. This is considerably higher

when compared with the average dietary lead intake of a normal male adult (100-200 µg/day).

1.5.2 Air

The highest concentration of lead in air is found in dense population centres. As one moves away from the centre of the city, the concentration falls progressively. The non-urban sites show less than 0.5 $\mu g/m^3$, while the urban sites have values ranging from 5 - 10 $\mu g/m^3$.[56]

People who live in close proximity to dense automobile traffic are exposed to appreciably higher concentrations than others. The respiratory lead from air depends on total lead concentration, particle size distribution, particle shape, chemical composition, physiochemical properties and respiratory volume.

The particle size distribution of lead in ambient air has been studied by a number of investigators. Robinson and Ludwig[57] reported a mass median equivalent diameter of 0.25 µm, with 25% of the particles smaller than 0.16 µm and 25% larger than 0.45 µm. Lee *et al.*[58] have reported mass median equivalent diameters of 0.42 - 0.69 µm. The chemical composition of airborne lead particulates has been studied by Terhaar and Bayard[59] with an electron microprobe analyser. Data from Terhaar and Bayard indicates that car lead exhaust is initially composed of lead halides that are converted to oxides, sulphates and carbonates with aging. Alkyl lead vapours occur in ambient air because some of the alkyl lead in gasoline escapes combustion. Normally, in populated cities the levels of organic lead are 10% of the total lead.[60]

The air in the vicinity of lead smelters may be appreciably polluted and thus can effect the general population. Landrigan et al.[35] have studied the environmental impact of large ore smelter near El Paso (Texas). The annual mean concentration in 1971 was approximately 80 $\mu g/m^3$ in the immediate vicinity of the smelter and fell off rapidly, attaining a near background level of 1 $\mu g/m^3$ at about 5 km.

1.5.3 Water

Man's exposure to lead through water is generally low in comparison with exposure through air and food. In one American study,[61] only 41 out of 2595 samples of tap water contained more than 50 µg/litre and 25% contained no measurable amounts of lead.

Under some circumstances, the concentration of lead in drinking water can become extremely high.[62] Goldberg[63] describes a case in rural Scotland, where four people developed clinical lead poisoning and other showed biochemical evidence of grossly elevated lead exposure through drinking water. The concentration of lead in the domestic water supply was 2 - 3 mg/litre. This was due to water storage in lead tanks. In another study from Scotland, Beattie et al.[64] showed that lead pipes in plumbing of homes can also result in high concentrations of lead in soft water.

Natural surface waters have been reported to contain less than 0.1 mg/l.[65] In unpopulated areas the concentrations are of the order of 1 µg/litre or less.[66]

1.5.4 Miscellaneous Sources

The intake of lead in food, air and water is a major concern as regards the general population. Another frequent exposure source, smoking, probably makes a small contribution to the lead

burden. Szadkowski et al.[67] reported 0.483 ± 0.267 µg of lead per cigarette in the total smoke for eight brands of cigarettes. Menden et al.[68] have reported that direct inhalation of intake of lead from smoking 20 cigarettes a day would be about 1 - 5 µg.

The presence of high concentrations of lead in illicitly distilled whisky occurs commonly in the U.S.A. and causes poisoning in adults. The concentration of lead in the final product frequently exceeds 10 mg/litre.

Another source of poisoning is improperly glazed earthenware vessels. Improper glazing results in the leaching of lead into the vessel, particularly when the contents are acidic. In a test of leaching of lead from commercial and handicraft pottery, Klein et al.[69] found that 4% acetic acid allowed to stand in warm temperature in the vessels for 18 hours often acquired concentrations of lead in excess of 100 mg/litre. In more than half the cases, the concentration of lead exceeded 7 mg/litre. Another source of lead poisoning in the general population is the use of discarded storage battery casing for fuel.[70] Because of the wide variety of application of lead, additional potential hazards are still being identified.

1.6 Health effects of lead

Lead has been known for centuries to be a cumulative metabolic poison. Although occasional episodes of classical lead poisoning still occur, particularly in young children, acute exposure is becoming a diminishing problem. Of greater concern is the possibility that continuous exposure to lower levels of lead, as a result of widespread environmental contamination, may result in adverse health effects. Although other systems may also be

affected it has been well documented that lead impairs the renal, hemopoietic and nervous system.[71]

1.6.1 Effects on the Hemopoietic System

The hematological effects of lead are related to

(i) the derangement of hemoglobin (Hb) synthesis,

(ii) the shortened life span of circulating erythrocytes,

(iii) the secondary stimulation of erythropoiesis.

These effects may result in anemia.[72]

The evidence for the inhibition of heme synthesis by lead dates back to 1850 and steps involved in derangement have since been examined in detail.[73,74] Some of the intermediates in heme are, subsequently: δ aminolevulinic acid (ALA), porphobilinogen (PBG), uroporphyrinogen III, coproporphyrinogen III, protoporphyrinogen IX (PPG) and protoporphyrin IX (PP). The final step is insertion of Fe^{2+}.

It is now farily well established that lead can effect many steps during the biosynthesis of heme although ALA dehydrase (ALAD) and heme synthetase seem to be the enzymes most sensitive to the action of lead. The immediate results of the lead interference in heme synthesis is the increased excretion of a variety of intermediate compounds. Higher lead concentrations may effect the degree of maturation of red cells, the globin synthesis and the morphology and stability of cells. In general terms lead inhibits the SH functional group less readily than mercury or cadmium. The actual mechanism by which the effects of lead on erythrocytes come about are complex and include inhibition of the hemoglobin synthesis, change in size and shape of red blood cells, abnormal production and osmotic resistance of erythrocytes as well as

crenation and fragility of erythrocytes leading ultimately to hemolysis (Figure 1).

1.6.2 Effect on the Nervous System

The effect of lead on the nervous system is both structural and functional, involving the cerebellum as well as the spinal cord and motor and sensory nerves leading to the specific areas of the body. The Central Nervous System (C.N.S.) effects may thus lead to the general deterioration of intellectual functions, sensory functions, neuromuscular functions and psychological functions.

1.6.2.1 Encephalopathy

Acute encephalopathy is one of the most serious consequences of plumbism. The classic symptoms of lead encephalopathy include sporadic vomiting, ataxia, apathy, coma and convulsions. The morphological changes commonly noted in the brain include cerebral edema, proliferation and swelling of endothelial cells accompanied by dilation of capillaries and arterioles, proliferation of glial cells and focal necrosis as well as neural degeneration.

The onset and course of acute lead encephalopathy are always unpredictable, making the dose effect and dose response relationship difficult to assess. It is usually associated with blood lead (PbB) levels in excess of 190 µg/100 ml, although levels ranging between 100 to 1310 µg/100 ml have been reported in children with lead encephalopathy.[76] Children who have already experienced lead encephalopathy and are subsequently re-exposed to lead, will almost certainly develop CNS damage permanently.

1.6.1.2 Peripheral Neuropathy

Peripheral neuropathy has been considered to be a rare

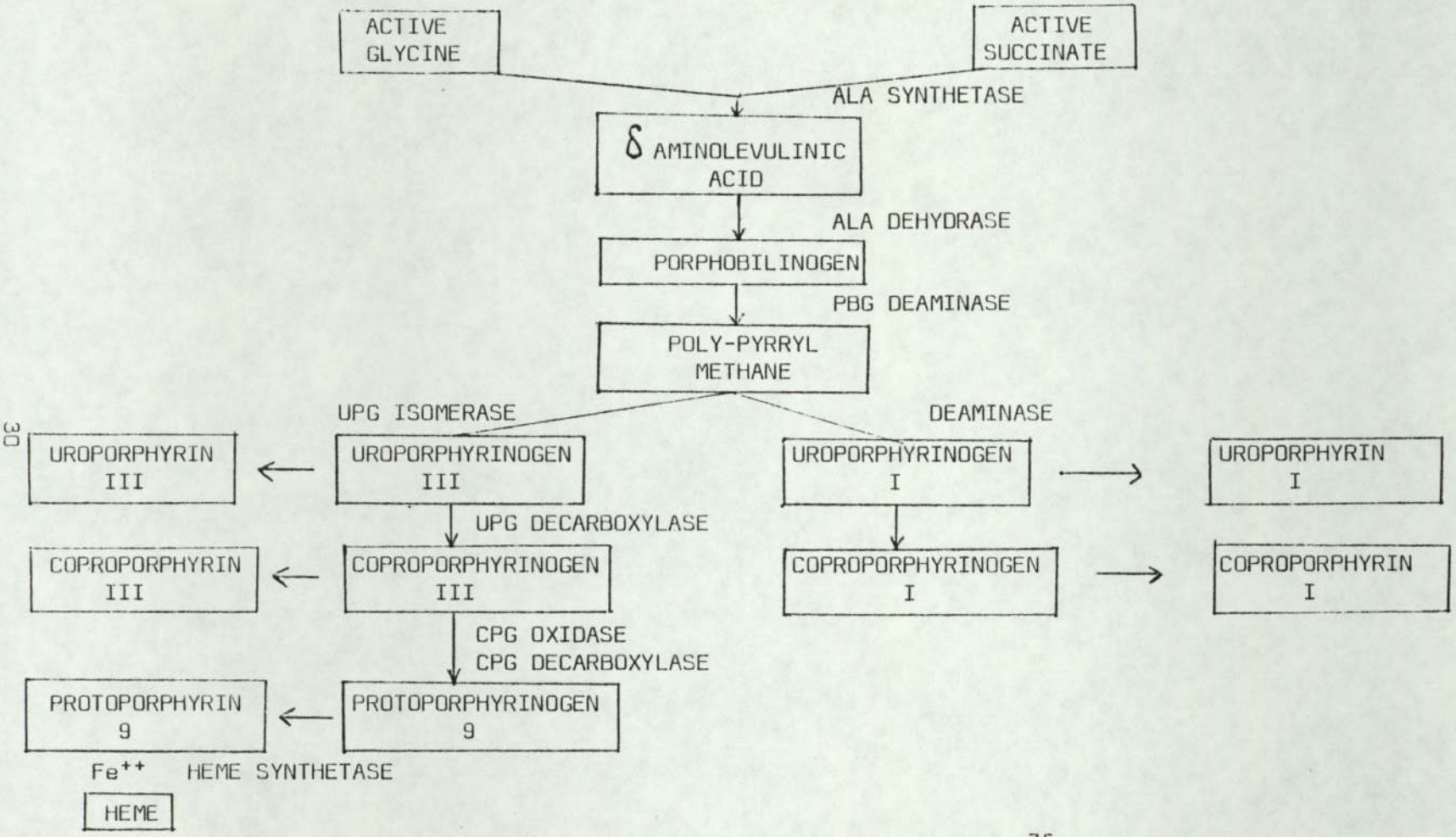


Figure 1. Schematic outline of heme synthesis[75]

occurrence in children with lead intoxication. However, Feldman et al.[77] suggested that childhood neuropathy may be overlooked and overshadowed by the clinical symptoms of encephalopathy. These authors observed slightly reduced motor nerve conduction velocities in children with a known history of plumbism.

1.6.2.3 Neurological and Behavioural Toxicity of Chronic Lead Exposure in Children

A growing concern is the possibility that chronic asymptometic lead exposure may cause minimal brain dysfunction, behaviour problems, and neurological impairment in children exposed to lead in utero and during early childhood.[78] Wiener[79] stated that there were methodological shortcomings in all of the studies and concluded: "Those reports which claimed positive findings had either used too few cases from which to generalize or had not provided for controls of relevant variables such as social class, pica or premorbid status".

David et al.[80] studied 589 children from inner city areas of New York. 579 blood lead and 370 penicillamine provoked urine samples were analysed. The population studied was free of manifest psychiatric and learning difficulties and in which lead levels are well within the range presently considered normal. Not one of the lead levels found in this population was in the range considered synonomous with classical lead poisoning(>60 µg/100 ml), and further only 15% of the blood levels that were found even fell into that range which has recently come to be considered suspicious (25 - 55 µg/100 ml).

Association between lead exposure and hyperactivity in children has been suggested by these authors. Children, whose

hyperactivity had no known cause, had higher blood lead levels and body burdens of lead than normal children or children whose hyperactivity had a 'known cause'. These authors further showed that there were improvements in mental functioning on reduction of blood lead levels by treatment with penicillamine. Treatments over a period of two months in behavioural improvement in 60 - 70% of the children treated, and there were even improvements in measured IQ, which ranged from 90 - 97.

However, since the numbers in the 'known cause' group were very small, it cannot be ruled out that lead absorption was secondary to hyperactivity, particularly since it has been shown that disturbed children tend to exhibit an increased incidence of pica.[81,82] Other studies[83 - 86] suggesting a causative association of lead exposure and mental impairment have been equally inconclusive. All these studies have dealt primarily with urban children whose exposure to lead may come from many sources.

Attention has also been focussed on the effects of chronic lead exposure on children living in the vicinity of ore smelters.[87] Landrigan et al.[35] concluded from their study that at blood lead levels of 40 - 80 µg/100 ml subtle impairment of nonverbal cognitive and perceptual motor skills occurred. However, their controls were poorly matched with respect to age, history of pica etc; the etiological role of lead cannot clearly be established.

Another study by Lansdown et al.[88] found no association between blood lead and intelligence and activity levels of school children living near a smelter. These authors concluded that social factors were more important than physical exposure to lead in determining mental development. However their social factors

were not quantified and very subjective assessments of behaviour were used thus rendering their conclusions dubious.

Needleman et al.[89] used tooth lead levels as a measure of learning abilities in school children from Boston. Their study is based on the relationship between dentine lead levels and teachers' rating on 2,146 children. The dentine lead levels were classified into six groups from low to high, so that class 1 was under 5 ppm, class 2 between 5 - 8 ppm and class 6 over 27 ppm. All the children studied were of normal birth weight and never had symptoms severe enought to cause the mother to bring them to the doctor for treatment for lead poisoning. Needleman et al. found that these children differ on a number of skills important to their learning, and in classroom behaviour in a way which relates to their tooth lead levels. These lead related differences are found primarily in the areas of verbal and linguistic processing, attention and classroom behaviour. However the studies by Needleman et al. have been criticised for lack of proper controls.[90]

Thus the data presently available are still inadequate to conclude that subtle psychological, emotional and neurological sequelae occur in children as a result of lead exposure at levels below those causing clinical symptoms.

1.6.3 Other Neurological Effects

Plumbism may also impair the visual and auditory system, lead effects the intrinsic muscle and oculo-motor nerves of the visual system. Changes in the character of the red blood cells and supporting fluids may lead to changes in the intraocular tension. Such changes may lead to mydriasis or visual paralysis.[91]. Warren[92] has speculated on a link between lead exposure and several chronic

neurological diseases. Cone et al.[93] reported that cerebrospinal fluid of multiple sclerosis patients contained elevated lead levels.

1.6.4 Effects on Other Organ Systems

1.6.4.1 Effects on Chromosomes

Contradictory reports have been published in the last few years with respect to a suggested increased occurrence of chromosome aberrations in workers occupationally exposed to lead. No evidence of increased chromosome aberration in lead manufacturing workers, in policemen with increased blood lead levels,[94] or in male workers exposed to lead oxide fumes in a ship breaking yard was found.[95] However, an increase in chromosome aberrations in people occupationally exposed to lead has been reported by Secchl et al.[96] Schwanitz et al.[97] and Deknudt et al.[98] Unfortunately most of these studies are based on a few subjects and thus the biological significance is unknown.

1.6.4.2 Effects of Lead on Reproduction

Lead has been known to effect women during pregnancy for more than a century. Teratogenesis has been reported and documented by animal experimentation. In addition to lead's direct adverse effect on the course of pregnancy, it has possibly an indirect effect by its toxic action on the male germ cell.

1.6.4.2.1 Early Studies

During the late nineteenth and early twentieth centuries, women in pottery and white lead industries felt that lead was an abortifacient. In 1860, Constantin Paul[99] published figures indicating a profound effect of paternal plumbism on sterility and on the viability of the offspring. Reid[100] gathered statistics on women in English potteries and white lead works, reporting women

in lead work, as compared to ones not employed in this work, were more likely to be sterile; and if they became pregnant, to miscarry. If the pregnancy went to term, it was more likely to end in stillbirth; and if the child was born living, death was more likely to come in the first year of life.[101]

Pindborg[102] reviewed 25 well documented cases of mild to moderately severe lead poisoning in Danish women who had ingested lead oxide as an abortifacient. Sixty percent of the pregnancies in the first trimester ended in abortion. Four pregnancies of five or more months duration resulted in one abortion and three normal infants.

Rennert[103] in 1881 found a high prevalence of convulsions and a peculiar form of macrocephaly in the German village of Almerode where pottery glazing was the home industry. Out of 79 children born, where both parents were leaded, 7 were stillbirths. In 54 cases macrocephaly and in 39 cases convulsions were noted.

In 1908, Chyzzer[104] published an account of an Hungarian village engaged in home pottery glazing where the same peculiar form of macrocephaly prevailed. This was further confirmed by Oliver[105] in 1914.

Torelli[106] in 1930 studied the influence of chronic lead poisoning on offspring of parents in the printing trade in Milan, Italy. He found the abortion rate for Milan in general to be 4 to 4.5%, but among the wives of printers the rate was 14% and among women printers 24%. The average death rate in 1930 in Italy for children during the first year was 150 per 1000 births, but for this group it was 320 per 1000.

Teleky analysed German female workers in the printing industry, and stated that spontaneous abortions were three times as common among those exposed to lead as those not exposed.[107]

Aldridge in England reporting on 71 females working with lead after marriage, found that 11% of pregnancies ended in miscarriage. The neonatal mortality was about 40%.[108]

Tardieu[109] reported that 608 out of 1000 pregnancies in France in lead workers ended in abortion.

Legge[110] in summarizing the reports of eleven English factories, found that of 212 pregnancies in 77 females working with lead, only 61 children were produced. Fifteen had never become pregnant; there were 21 stillbirths, miscarriages occurred ninety times, and of 101 children born, 40 died in their first year. Legge concluded that lead affected pregnancy directly through the females exposure to lead. Legge further stated that there was little evidence to suggest any adverse effect on pregnancy or the health of the offspring if only the male was exposed to lead.

However, in 1905, Rudeaux[111] analysed 442 pregnancies in women married to lead workers, 66 ended in abortion and 241 in premature briths. Also in 1905, Deneufbourg noted 23 abortions and stillbirths among 134 pregnant females exposed to lead. He found 26% abortion and stillbirth rate in the wives of male lead workers.

In another study from Japan, Koinuma[112] compared the marital life records of workmen exposed to lead in storage battery plants with the records of those working in non-leaded occupations. The sterile marriages constituted 24.7% of the lead group and only

14.8% for the non-lead group. The percentage of pregnancies ending prematurely or in stillbirth was 8.2 for the lead group and 0.2 for the control group.

In 1911,[113] Oliver published statistics on the effect of lead on pregnancy:

	Abortion and stillbirth (per 100 females)	Neonatal deaths (per 100 females)
Housewives	43.2	150
Female workers (millwork)	47.6	214
Females exposed to lead premaritally	86.0	157
Females exposed to lead after marriage	133.5	271

In another report from Japan, Nogaki[114] made a detailed study of the pregnancies of 104 Japanese women before and after beginning lead work showed an increase in miscarriages to 84.2/1000 pregnancies from a prelead period of 45.6/1000. The miscarriage rate for 75 comparable employees not exposed to lead was 59.1 per 1000 pregnancies.

Thus the earlier studies did indicate that lead had a damaging effect on fertility, the course of pregnancy and the development of the foetus. However, none of these studies have any analytical lead data to support their views certainly, the lead exposures were uncontrolled and may have been very high.

1.6.4.2.2 Recent Studies

More recently, Wilson,[115] in Scotland, reported the effects of effects of abnormal lead content of water supplies on maternity patients. Out of 75 pregnancies studied, five pregnancies were abnormal. The details of this data are as follows:

i) Intrauterine death of foetus at 33 weeks. Maternal blood lead levels were 55, 34 and 72 µg/100 ml at weekly intervals after foetal death, and 51 µg/100 ml two months later.

ii) Congenital defects (nystagmus, albinism and haemangioma). Maternal blood lead was 31 µg/100 ml. The mother had been sterile for six years previous to this pregnancy.

iii) Congenital defects (multiple haemangiomate). No analytical data given.

iv) Two abnormal pregnancies occurred in a woman with four previous miscarriages and two surviving children. The blood lead levels were 53 µg/100 ml.

Palmisano et al.[116] have reported a case of a ten week old infant with evidence of neurological defects, intrauterine growth retardation and postnatal failure to thrive which was the conceptus of a 33 year old woman with a history of long term ingestion of 'moonshine' whisky. After challenge doses of calcium EDTA, the infant and the mother each excreted an abnormally large amount of lead in urine. A recent survey based on the chemical analysis of untaxed 'moonshine' whisky samples disclosed that 53% contained concentrations of lead above 1000 µg/litre.

Thus, so far, there were no detailed studies based on analytical tissue lead, on the effects of lead on reproduction in human beings.

1.6.4.3 Effects on the Immune System

Several studies in animal models have suggested that lead may interfere with various aspects of the immune response. Lead has been reported to result in an increased susceptibility to infection in mice and rats.[117,118] A difficulty in determining

the effects of lead on the immune system of children, is that urban, lead exposed children are also likely to be at high risk for contracting infectious diseases. Reigart and Graber[119] found that 12 children with blood lead levels of 41 - 51 µg/100 ml did not differ from seven children with blood lead levels of 7 - 13 µg/100 ml with respect to complement levels, immunoglobulins, or anamnestic response to tetanus toxoid antigen. There do not appear to be any systematic epidemiological investigations on the effects of elevated lead levels on the incidence of infectious diseases in man.[120]

1.6.4.4 Effects on the Endocrine System

Excessive exposure to lead has been associated with an impairment of endocrine function in both man and experimental animals. The uptake of iodine and its conversion to protein bound iodine was retarded in lead intoxicated rats. Iodine uptake was also decreased in lead poisoned patients.[121] High lead levels resulted in a decreased secretion of pituitary hormones[122] and an impairment of adrenal gland functioning[123] in lead poisoned patients.

1.6.4.5 Toxicity of Alkyl lead Compounds

The risks associated with the use of tetrethyl lead (TEL) became a cause of concern soon after its introduction in automotive fuels, and following a number of deaths and non-lethal intoxications of those occupationally exposed. Although hazard to the public at large is probably small there is little doubt that leaded gasoline can be dangerous and even fatal under certain circumstances. Considerable morbidity and occasional fatalities have been reported in men with long term exposure to leaded gasoline[124] and

workers using leaded gasoline in the manufacture of inexpensive rubber shoes.[125]

In general, the symptoms of organic lead poisoning differ from those of inorganic lead. The main symptoms are: disturbance in sleep pattern, hallucinations, nausea, anorexia, vomiting, vertigo and headache, muscular weakness, weight loss, tremor, diarrhoea, abdominal pain, hyperexcitability and mania. Psychological and neurological symptoms which develop late during inorganic lead poisoning are manifested early during organic lead poisoning. It is also noteworthy that significant differences in toxicity have been observed between tetraethyl lead (TEL) and tetremethyl lead (TML), with TML being about seven times more toxic to experimental animals.[126]

The tissue distribution of organ lead compounds also differ from that for inorganic lead. Because of their solubility in liquids, alkyl leads are preferentially accumulated in the CNS leading often to nervous system impairment without hemotological, renal or gastrointestinal effects.[127] The concentrations of lead in the brains of people poisoned by TEL poisoning have been found to be higher than those in brains of inorganic lead poisoned people.[128] The toxic metabolite associated with TEL poisoning is believed to be the tri-alkyl-lead ion produced mainly in the liver.[129] The major symptoms which indicate inorganic lead poisoning based on target organ syndrome is summarized in Table 9, where group A signs are considered major manifestations whereas group B represents more generalised, less specific signs and symptoms.[130]

Table 9

System	Symptoms	
	Group A	Group B
General appearance	Marked pallor and profound anaemia	Pallor, Anaemia and drawn expression
Digestive system	Colic, Obstinate constipation	Loss of appetite or repungnance to food Vomiting on eating solid food Gastric disturbance
Muscular system	Muscular incoordination	Loss of strength and tiring easily
Nervous system	Peripheral motor paralysis of certain extensor muscles, wrist and ankle drop	Headache, Mental lethargy, Tremor, Dizziness, Convulsions, Encephalopathic condition
Vascular system	Blood basophilic degeneration with diminished haemoglobin	Arteriosclerosis Hypertension
Special organs	Gums: lead line Stool and urine: lead miscarriages: repeated	Eyes: impairment of vision Muscular incoordination Joints: various pains Blood: diminished haemoglobin

1.6.6 Measurements of Subclinical lead Health Effects

The symptoms of acute lead poisoning have now become quite rare and are confined largely to two population groups: lead workers and young children in old houses with lead paint. Nowadays, the number of people exposed to low but potentially dangerous levels of lead insult from industrial pollution and automotive exhausts is larger than ever and includes many children who are

particularly susceptible.[131-133] This has become a problem of major concern in many industrialized nations and considerable effort is now being made to assess the subclinical effects of lead. Besides the evidence of lead exposure, diagnosis of such potential health hazards depend mainly upon the assay for the lead concentrations in body fluids, deviant enzyme activities and the presence of abnormal concentrations of some metabolic intermediates. Table 10 shows the various biological indices of clinical and subclinical lead effects.

Table 10

Inorganic Lead: Biological Indices of Exposure

I. Interference with heme synthesis	II. Lead absorption (deposition) Excretion Index	III. Other Types of Indices
δ-Aminolevulinic Acid (ALA)	Blood lead	Electromyography
Coproporphyrin	Urinary lead	Serum protein
Uroporphyrin	Hair lead	Renal tubular function
Porphobilinogen	Nail lead	Endocrine function
Hemopoietic Index	Bone lead	Neurobehavioural function
Blood ALA		
Erythrocyte ALA – Dehydratase activity		
Erythrocyte non-heme iron		
Bone marrow sideblasts		
Reticulocytes		
Punctate basophilia		
Erythrocyte life span		
Hemoglobin hematocrit		

1.6.6.1 Whole Blood and Urine

Whole blood lead (PbB) concentrations have been the most widely used test to monitor for a potential lead hazard. However, its diagnostic value at the borderline of hazardous conditions is

quite limited. It has been found that PbB is more a measure of very recent exposure[134] than of total body burden, and most lead in the body is within the hard and soft tissues.[135]

If there were no lead pollution, the natural PbB level has been estimated by Patterson[16] from geochemical arguments to be only 0.25 µg/100 ml. However, the present day normal range in unexposed adult population is between 18 - 22 µg/100 ml.[136]

The PbB concentration is very dependent upon the recent and current atmospheric lead exposure (Table 11). The data show that PbB level increases in proportion to involvement with conditions of high traffic density.[137] The higher concentration of PbB in urban residents compared to rural residents is the consequence of traffic density effects.

Table 11

Blood Lead in Selected Groups of Man

	No. of subjects	Pb µg/100 ml
Suburban non-smoker, Philadelphia	9	11.0
Residents of rural California County	16	12.0
Commuter non-smokers, Philadelphia	10	13.0
Suburban smokers, Philadelphia	14	15.0
Policemen, Los Angeles	155	21.0
Firemen, Cincinnati	123	25.0
Policemen, smokers, Philadelphia	83	26.0
Service station attendants, Cincinnati	130	28.0
Traffic policemen, Cincinnati	40	30
Car drivers, Cincinnati	14	31
Garage mechanics, Cincinnati	152	38

1.6.6.2 Erythrocyte

At least 90% of the lead in blood is generally associated with erythrocytes.[138] The lead of erythrocytes occurs both within the cells and in association with the membrane in a ratio of 9 : 1.[139] Within the cell it is bound to haemoglobin, to a colourless component with molecular weight of about 10,000 and to other lower molecular weight components.[140]

1.6.6.3 Serum and Plasma

Accurate measurements of the plasma (PbP) and serum lead (PbS) have been more difficult because of the usual methodologic difficulties and low concentrations. Robinson et al.[141] reported PbP for 82 children with no previous history of pica or lead poisoning. In 53 lead was not detected, in 22 it was <9 µg/100 ml and in 7 it was >9 µg/100 ml. Similarly, Rosen et al.[142] determined plasma lead concentrations for 105 children and found that the plasma lead concentration was between 1 - 7 µg/100 ml, with a mean concnetration of 3.3 µg/100 ml. Serum lead levels are also very low in occupationally unexposed populations.

1.6.6.4 Enzymes and Accumulation of Metabolic Intermediates

The usual compounds and media that have been assayed for an effect of lead absorption are: δ-aminolavulinic acid dehydrase (ALAD, present in erythrocytes and in other tissues), δ aminolevulinic acid (ALA in urine); coproporphyrin III (CP, excreted in urine) and protoporphyrin IX (present in erythrocytes and usually assayed in whole blood).

1.7 Measurements of lead in placentae, foetal tissues and blood

To study the extent of prenatal lead exposure and to determine the role of lead as a teratogenic agent, accurate

measurements of lead in placentae, foetal tissues and blood are a pre-requisite. The details of the methods developed for this purpose are discussed in the next chapter.

2. METHODS AND MATERIALS

2.1 Methods of Lead Analysis and suitability for biological materials

There are a number of methods which may be used for lead analysis. The selection of the method for lead analysis mostly depends on the nature of the sample to be analysed, nature of the laboratory equipment available and last, but not the least, the expertise of the analyst. Some of the most commonly applied methods are discussed below:-

2.1.1 Colorimetric Analysis

Colorimetric methods have been widely used in the past and are still used today on account of their low cost, sensitivity and simplicity. One of the most successful extracting agents for lead is dithizone (1,5-diphenylthiocarbazone). Lead (II) dithizonate is a red compound, insoluble in water but soluble in most organic solvents. When extracted with carbon tetrachloride, the lead dithiozonate strongly absorbs at 530 nm. Under optimal conditions, 0.1 ppm Pb in a sample can be determined. The most important disadvantage of the dithizone procedure is its sensitivity to interference substances, especially to the formation of oxidation products. Selectivity for lead is obtained by adjusting the pH value to 8.5 to 9.5 and by the addition of potassium cyanate and ammonium citrate. Both wet digestion and dry ashing have been used prior to dithizone colorimetry. Carbon tetrachloride is the usual extraction solvent but chloroform has also been used. The higher pH values necessary for dithizone extraction may result in losses of lead by absorption. On the whole the dithizone method is still a very useful method in hands of well trained analysts, particularly in locations where

more modern analytical instruments are not available, although the method is less reliable at levels below 1 ppm.[143,144]

2.1.2 Voltammetry

Voltammetry is based on measurements on current voltage curves independent of the electrode used, polarography being voltammetry using a mercury electrode. Conventional direct current polarography has been used for various biological materials.[145,146] These techniques have the advantage that no preconcentration of lead is necessary.

Anodic stripping voltammetry (ASV), or inverse polarography is based on the use of electrolytic cell in which the working electrode is made cathodic, so that all trace metals in the sample are deposited. The deposited metals arrive at the electrode surface at rates determined by their individual concentrations, the diffusion properties of the electrolyte solution and the area of the electrolyte used and the deposition time is carefully measured. The working electrode anodic is then made by reversing the potential; as the anodic potential is increased, each metal is separately removed at its characteristic voltage. As the metals are stripped, the resulting current in the cell is measured and is proportional to the concentration of the metal. ASV is more sensitive than the classical form of polarography.

The results obtained by anodic stripping voltammetry have been compared with those from atomic absorption spectrometry. In most cases the two are comparable.[147]

2.1.3 Activation Analysis

2.1.3.1 Neutron Activation Analysis

Thermal neutron activation analysis has not been widely used for the determination of lead because of the unfavourable properties of the nuclide formed. Neutron activation analysis of the short-lived lead nuclide (^{207}Pb) by pulse and steady state irradiation has been studied.[148] The method has been used for paint analysis with a detection limit of 1%.[149]

2.1.3.2 Gamma Photon Activation

Gamma photon activation analysis is the most practical activation method for the determination of lead in biological materials. The most sensitive technique is based on the (α, n) degradation of ^{204}Pb to ^{203}Pb by irradiation of the sample in a beam of high energy from an electron linear accelerator or betatron in which electrons of energy 20 - 45 MeV strike a gold-plated tungsten target. The properties of ^{203}Pb simplify radiochemical separation from the matrix, and the analysis can be carried out non-destructively. The limit of detection of this method being about 0.1 µg of lead.[150]

2.1.4 Spark Source Mass Spectrometry

This method involves the production of ions in an ashed sample placed between electrodes by high voltage excitation. Non-conducting samples are mixed with graphite and/or silver. The resulting charged nuclei are introduced into a mass spectrometer. Branquinho[151] and Stary[152] have described the methods for the use of ^{212}Pb to measure overall recovery in trace lead analysis. The method has been used for biological materials.[153] One of the important disadvantages of this method is that the determination

of levels less than 10 µg Pb are not reliable.

2.1.5 Mass Spectrometry

Direct insertion of diethyl dithiocarbamate chelates into a mass spectrometer can be used for the determination of a number of metals. The sensitivity of the technique is limited by the instrumental blank but amounts of less than a nanogram of each element can be detected.[154]

2.1.6 X-ray Fluorescence Spectrometry

Goulding[155] has studied the use of a non-dispersive X-ray spectrometer with an isotopic source and semi-conductor detector for the measurement of trace elements in biological materials. The accuracy for at 1 - 10 µg Pb is from 10% to 1% respectively. The method has been used for determination of lead in minerals with a detection limit of 0.1 ppm.[156] The technique is not suitable for biological materials.

2.1.7 Solid-state Luminescence Spectrometry

Trace amounts of lead can be determined in plant material after drying and wet ashing followed by co-precipitation of lead with Calcium Oxalate and ignition at 850 - 900°C. The solid-state luminescence of Calcium Oxide lead phospher at an excitation wavelength at 350 nm and an emission wavelength at 530 nm can be used.[157] Not many studies have been carried out on biological materials by this technique.

2.1.8 Proton-induced X-ray Emission Spectrometry

Heavy charged particles can be used as an excitation source free from interference. Johnsson[158] has reviewed the techniques and its possibilities. Walter[159] used a 3 MeV beam of protons to excite X-ray emission from a wide range of biological samples with an Si(Li) detector. Linear response was obtained with

several elements in the range of 5 ng to 2 µg.

2.1.9 Electron Spectroscopy for Chemical Analysis (ESCA)

ESCA involves the measurement of the kinetic energy of electrons emitted following the monochromatic X-rays. Hercules[160] applied ESCA to glass fibre discs impregnated with dithiocarbamate, on which trace metals were collected; the limit of detection was 10 ppm. This technique has not been applied to biological materials.

2.1.10 Gas-liquid Chromatography

This technique has not been applied for biological materials, although the chelates with hexafluoro monothio acetylacetone can be detected at low concentrations and may be characterised by mass spectrometry.

2.1.11 Potentiometry

Berzina[161] has studied the feasibility of potentiometric determination of lead by titration with 4-methyl and 5-bromo-8-mercaptoquinoline. The method is not sensitive for biological materials.

2.1.12 Ion Selective Electrodes

The use of solid state lead (II) electrode activated with lead sulphide and silver sulphide has been reported.
This method has not been applied for biological materials.

2.1.13 Emission Spectroscopy

Emission spectroscopy has been used in a number of laboratories for the determination of lead and other trace elements in biological materials. The sample itself or the ash is usually mixed with graphite and placed in a graphite electrode and excited

using a D.C. arc. A combustion flame provides a remarkably simple means for converting metal elements into free atoms. The free atoms so formed can be detected and determined quantitively by emission, atomic absorption or fluorescence spectroscopy. The method has been used by Bryuikhanov[162] for lead in blood, Busev[163] after ashing and extraction with hexamethylene ammonium hexamethylene dithiocarbamate in biological materials and Galinskaye[164] in biological material after wet destruction and ashing. Emission spectroscopy has the advantage of being rapid, sensitive and specific and potentially capable of giving data for a number of elements in the same sample. One of the most significant disadvantages is the matrix effect. Niedermeir[165] found that as little as 10 ppm of Fe, Mg, Ca, K or P had a marked influence on spectral response of many trace metals including lead. Holcombe[166] reports absolute detection limit for lead as 15 ng. Emission spectroscopy has not been studied very thoroughly for different biological matrixes, although it has great potential because of its multielemental determinations capacity.

2.1.14 Atomic Spectroscopy

Atomic spectrometry can be broadly divided into two main techniques: atomic absorption (AAS) and atomic fluorescence spectroscopy (AFS). Both these techniques can be subdivided into flame and flameless techniques. The many advantages of atomic spectroscopy have led to its use in numerous procedures for the determination of lead in biological materials.

2.1.14.1 Atomic Absorption Spectroscopy (AAS)

During the past decade, AAS has been widely demonstrated to be a sensitive and selective technique for trace analysis of lead

in various biological materials. It has recently become clear that the determination of lead, at levels of <1 ppm, proved a more complex problem than was first anticipated. Calcium ions, for example, are one source of the interference in flame and flameless atomic absorption spectroscopy. Although different methods of sample pretreatment may be used, the measuring technique is similar in all cases. Most authors prefer to measure the absorption at 283.3 nm rather than 217.0 nm, which is the more sensitive resonance line of lead. Flame atomic absorption spectroscopy has been used by many authors for the determination of lead in biological materials.[167,168] Flame atomization systems exhibit some disadvantages, such as flame background absorption and emission at the wavelength of the resonance line of the element of interest, the relatively large volume needed with a conventional nebulizer and the difficulty of introducing solid samples into a flame. These and other aspects have led to the development of flameless equipment modification of atomic absorption. Amos[169] in a review of flameless atomization in AAS, describes various designs of filament and furnace atomizers then in use. The absolute sensitivities and absolute detection limits for the flameless methods are in the picogram range. This field has offered opportunity for the development of rapid methods for the determination of trace metals in the biological materials. Compared with conventional flame techniques, flameless atomization makes it possible to achieve absolute detection limits which are several orders of magnitude better. Sample sizes of the orders of microlitres may thus be adequate to give an analytical signal which can be measured. It is also possible to ash organic matter

before analysis, thus simplifying the pretreatment stage. There are some disadvantages with flameless methods, spectral and chemical interferences being the most important of these. Chemical interferences occur when the presence of extraneous ions causes incomplete dissociation of the metal compound present; the ground state atom population is affected with consequent chemical interference. Decrease in peak heights are observed for lead when measured in the presence of a 100 fold excess of Mg, Na, K, Ca and Zn, a circumstance which may happen frequently in various biological materials.[170]

2.1.14.2 Atomic Fluorescence Spectroscopy (AFS)

Atomic fluorescence spectroscopy affords higher sensitivity that AAS for some elements. Considerable effort has been devoted to the development of improved cathode lamps, electrodeless discharge tube sources, and selectively modulated and pulse operated sources. Neither atomic fluorescence spectroscopy in the flame mode or flameless atomic fluorescence spectroscopy has been used for the determination of lead in biological materials, although the methods seem promising.[171]

2.1.15 General Conclusions regarding Problems of Lead Analysis

Collaborative studies in which the same sample is examined by one method by a number of analysts have shown that the reliability of lead analysis, at levels < 1 ppm, leaves much to be desired. The results of intercomparison studies in which several methods are applied to one sample by several analysts are also disappointing. The sensitivities, the limits of detection and the accuracy of the methods normally used for quantification, such as flame, flameless AAS and ASV, do not

appear to be the main sources of difficulties. The problems appear to be the nature of matrix effects, a lack of understanding of the extent, the origin of, and the methods of avoiding laboratory contamination. Due attention to the evaluation of blank valves is particularly important. As most of the work reported in further chapters has been carried out by flameless graphite furnace atomic absorption spectroscopy, section 2.2 deals with the problems associated with carbon furnace analysis.

2.2 Graphite Furnace AAS

2.2.1 Historical Development

The development of electrothermal atomizers can be traced back to works of King in 1905 and 1908.[172,173] King was interested in the observation of emission spectra of elements without the complicating factors of electrical conduction, occurring in the atomic vapour produced by an arc or spark, and unknown chemical reactions, occurring in the atomic vapour produced by a flame. His aim was to obtain emission spectra produced, as nearly as possible, solely by the effect of heat.

King first used an arc-heated furnace. The apparatus consisted of a carbon rod of 16 mm diameter with a 5 mm diameter hole drilled through. This tube furnace was held inside a carbon block but insulated from it by two tubes of asbestos placed at each end. A hole was drilled out of a centre of the carbon block, at the base to allow a carbon electrode to enter. Copper clamps fitted to one end of the horizontal tube furnace and the vertical carbon electrode enabled the apparatus to be connected to a power supply. Samples were placed at the centre of the furnace and

electrode was raised until it formed an arc (up to 30A at 220V) with the furnace wall. Using this design, temperatures of 2200°C were obtained.

King continued to use his electrically heated furnace to investigate the emission spectra of many elements. No use was made of the principle of electrothermal atomization for quantitative analytical measurements, until 1956, when two groups of workers in the U.S.S.R. used an electrothermal vaporisation technique to separate and preconcentrate trace elements onto a graphite electrode prior to emission spectrochemical analysis.[174] However, their work remained very preliminary, until in 1959, L'vov[175] began to publish his classic work on the application of electrothermal furnace atomizers for quantitative atomic absorption analysis. The graphite furnace used in his early work, was similar in principle to the arc atomizer of King.[172] The apparatus consisted of a graphite furnace, 100 mm in length, of 10 mm external diameter and 3 mm internal diameter, lined with tantalum foil, to decrease the loss of atomic vapour by diffusion through the porous carbon, a carbon electrode, for sample introduction and an auxiliary electrode for arcing (dc) to the sample electrode. This furnace was contained under an aluminium cover containing two quartz windows for the atomic absorption measurements, and one window for observation. Analysis was carried out by placing samples, 0.1 mg, on the tip of the sample electrode, which was then evacuated and filled with argon. The carbon furnace was heated to the desired temperature, using up to 10 kw power, before a sample electrode was moved into the opening in the furnace wall. As the sample entered the furnace

a d.c. arc was automatically switched on for 3 - 4 seconds between the tip of the sample electrode and the auxiliary electrode, causing the sample electrode tip to heat and sample vaporisation and atomization to occur. L'vov's apparatus, while giving the best absolute sensitivities for atomic absorption analysis, has only been used in U.S.S.R. for routine analysis.

In 1967, Massman[176] produced his much simplified and compact versions of the King furnace to enable both atomic absorption and atomic fluorescence measurements to be made. The graphite furnace for atomic absorption analysis, consisted of a graphite tube, 55 mm in length, of 6.5 mm internal diameter and 8.0 mm external diameter; a hole, diameter 2 mm, was drilled through the wall at the centre of the tube for the introduction of liquid samples, 5 - 200 ul. The graphite tube operated in an atmosphere of argon, was supported between two water-cooled steel cones which were electrically insulated from each other.

Based on the work of Massman, Perkin-Elmer[177] introduced a heated graphite atomizer (HGA) in 1970. The HGA 70 consists of a graphite cylinder, 51 mm long by 8.6 mm internal diameter, supported at each end by graphite cones, which are held in the water cooled metal housing of the atomizer unit. The graphite cones act as optical apertures by limiting the amount of light, from the heated tube, reaching the photo multiplier. The graphite tubes can be heated to temperatures of 2500^{o}C within 5 seconds. The graphite tube is protected from atmospheric oxidation by a stream of argon or nitrogen flowing round and through the tube. The outside temperature of the atomizer is maintained below 60^{o}C by the flow of water through the metal housing. The whole atomizer

is positioned in the atomic absorption spectrometer in place of the normal burner assembly, so that light beam from the hollow cathode (or EDL) lamp passes through the centre of the graphite tube. Liquid samples (100 µl maximum) are introduced into the furnace through the centre hole in the graphite tube while solid samples are introduced through the open ends of the graphite tube.

The design of HGA system has been modified several times since its introduction, with the production of HGA 74. The atomizer, HGA 74 which has been used in our studies, is smaller with another important feature being the reduction of graphite tube itself. The tube size in the HGA 74 was reduced to 28 mm length and 6.5 mm diameter, and internal ends of the tube were grooved to retain sample solution so that they could be used equally well with aqueous or organic solvents. The reduced size of the graphite tube enabled the high power requirements of the HGA 70 to be reduced considerably (10V and 300A maximum) and the highest temperature attainable to be increased to 2700^{o}C. The inert flow gas system was changed, with the internal flow of gas through the graphite tube passing from the two cold ends to the hot central region; this modification has required that quartz windows be fitted to either end of the furnace, to give a semi-enclosed configuration. This new flow pattern greatly reduced the problem of vapour condensation at the cool ends of the graphite tube that was so pronounced with the HGA 70 design.

2.2.2 Advantages of Graphite Furnace AAS over the Flame Atomization

There are three main advantages claimed for the graphite furnace AAS.

2.2.2.1 Increased Sensitivity

The theoretical improvement in sensitivity which should be attainable with electrothermal atomizers can be easily calculated. Assuming, for flame atomization, that sample spray rate is 6 ml per minute, that 10% of the sprayed sample reaches the flame, and that the residence of time of atoms in the analytical zone of the flame is 10^{-4} seconds, then the volume of sample present in the analytical zone at any time is 10^{-6} ml. Assuming, for electrothermal atomization, that the production of atoms occurs as efficiently as in the flame and that the total sample is atomized before significant loss of the atomic population occurs from the atomizer, then for a 100 µl sample volume the improvement in sensitivity should be 100,000 times.[178] However, in practical terms, the levels of improvement are well below the theoretical value calculated by L'vov. For example improvement in sensitivity for lead is 1000 times only. There are possibly two reasons for this observation: either the atomization process is considerably less efficient in electrothermal atomizers than in flames, or the production of atoms in the atomizer is slow compared to their rate of removal.

2.2.2.2 In situ Sample Treatment

Selective volatilization and matrix modifications within the atomizer, together with the ability to analyse directly viscous liquids, are important advantages, and the techniques have been used frequently for organic matrices and occasionally for inorganic matrices.

2.2.2.3 Small Sample Size

The facility to analyse small quantities of sample is an

advantage only if limited sample quantities are available, and it is not an inherent advantage of the analytical technique. However, for many analytical problems, particularly in environmental and clinical applications it is an essential advantage of the technique.

Instrument time per determination is greater for electrothermal atomization than with flame measurements although total analysis time can often be shorter owing to the use of a simpler analytical method.

2.3 The Destruction of Organic Matter and the sample preparation

One of the most crucial steps in trace metal analysis is the separation of the metal from the large mass or organic material with which it is associated. The ubiquity of lead enhances the difficulties. Contamination can be expected at all stages of the analysis, including sampling, sample preparation and measurement. With the exception of such techniques as X-ray fluorescence, spark source mass spectrometry, emission spectrographic analysis, gamma photon activation analysis, all the methods for the determination of lead are basically destructive. Problems associated with methods for the destruction of organic matter prior to metal analysis have been discussed by Gorsuch[179] and Tolg.[180] These deal in detail with the problems of dry and wet combustion, oxygen flask combustion and oxygen bomb and low temperature radio-frequency activated oxygen techniques. The destruction of organic matter is normally accomplished by one of two techniques, wet oxidation (i.e. wet ashing, wet decomposition, wet destruction) and dry ashing. The main problem in wet oxidation is the possible contamination of the sample by the reagents, which are often

required in large quantities. Other disadvantages include the close and constant attention required and the difficulties involved with the use of perchloric acid. The main problem in dry ashing is the possibility of volatilization of the metal to be analysed. Other drawbacks may be absorption on unburned carbon and insoluble silicates, reaction with, and subsequent retention, on the vessels used for ashing and the fact that the methods are time consuming ($\simeq$ 12 - 40 hours).

2.3.1 Wet Decomposition

For destruction of organic material, the samples are digested (sulphuric and perchloric acids, sodium hydroxide, tetramethyl ammonium hydroxide and enzymes), and oxidized (hydrogen peroxide, sulphuric, nitric, hydrochloric and perchloric acids), the various reagents being used alone or in combination with various conditions. Halogen containing agents enhance the chances of volatilization of lead due to formation of lead halides. The combination of nitric and perchloric acids has been used by Marumo et al.[181] for biological material digestions. The combination of nitric and sulphuric acids has been used for biological materials by Thomas et al.[182] Sometimes an additional oxidant, such as hydrogen peroxide, has also been used.[183] The combination of nitric sulphuric and perchloric acids has often been used for various biological materials including meat and animal products.[184] One of the difficulties using sulphuric acid is the co-precipitation of lead sulphate with calcium which is present in high quantities in most biological materials. Other combinations used for destroying organic matter are nitric, hydrochloric and perchloric acid for biological materials, and sulphuric and hydrogen peroxide for

biological materials.[185] The use of hydrogen peroxide alone, under pressure has also been described for destruction of biological material[186] and of nitric acid under pressure for biological materials.[187] It is evident, thus, that the choice of the method, for destruction of organic matter, does depend largely on the nature of the material to be analysed.

2.3.2 Dry Ashing

The most serious problem encountered in using the dry ashing method is the possibility of volatilization of lead and the absorption on unburned carbonaceous matter and on the surface of the crucible. There are a number of procedures available using platinum crucibles, silica crucibles and pyrex beakers at various temperatures from 200 to 600^{o}C with and without ashing aids. Most of the authors agree that the ashing temperatures exceeding 500^{o}C should not be used. For biological materials a number of reports have been published.[188-190] Dutilh[191] concluded from recovery studies with radio labelled materials that at least 40% loss of lead at 500^{o}C can be expected for milk. Webber[192] also found lead losses when ashing plant materials at 400 - 500^{o}C. The use of ashing temperature higher than 500^{o}C is infrequent.

2.3.3 Comparison of Wet and Dry Ashing

There has been little work on the comparison of both these techniques. The main problem associated with wet ashing is that of contamination from reagents and long procedures, whereas the main difficulty with dry ashing technique is the volatilization of lead at temperatures which are essential for complete ashing. Thus it is difficult to recommend a simple destruction method for general application because of differing requirements, depending upon the nature of the material to be analysed.

2.3.4 Concentration of the Samples to be Analysed

Preconcentration or other separation of lead may be necessary because of very low levels of lead present in biological materials. This may be done by co-precipitation, ion exchange, electrodeposition or chelation.

2.3.4.1 Co-precipitation

The first order isomorphism between the sulphate of lead and strontium observed by Goldschmidt in 1934 has been used by Rosenquist[193] to recover traces of lead by co-precipitation with an excess of strontium sulphate. The technique has been further developed by Flann[188] and has also been used as a preconcentration step for atomic absorption spectroscopy. Hoover et al.[194] have used this technique for atomic absorption spectroscopy for plant and animal product lead analysis.

2.3.4.2 Ion Exchange

The use of an ion exchange material for the preconcentration and separation of lead has been described by many authors.[195-197] This technique has been mainly applied for water analysis[198] and only infrequently for biological material.

2.3.4.3 Electrodeposition

Certain heavy metals can be determined in brines by decomposition on mercury drop electrode followed by volatilization of the mercury in a graphite furnace and subsequent determination by flameless atomic absorption spectrophotometry.[199] This technique has been most useful for dilute solutions and does not appear to have been applied for biological materials.

2.3.4.4 Chelation

The method of separation or preconcentration most frequently used is chelation. This course of conversion of metal ions into

a stable, neutral, extractable complex with a chelating agent, followed by solvent extraction. A number of chelating agents have been used, including Cupferron,[200] 8-hydroxy quinoline,[201,202] thenoyltrifluoracetone,[200] hexafluoromonothioacetyl acetone,[203] ammonium tetramethylene dithiocarbamate[204] and iodine.[205] Diethylammonium diethyldithrocarbamate has been suggested by Roschnik[206] and Snodin,[207] particularly for the determination of lead in biological materials. However, the most widely used reagents are dithizone(diphenylithiocarbazone), ammoniumpyrrolidine dithiocarbamate and sodium diethyldithiocarbamate.

2.3.4.4.1 Dithizone

Dithizone, discovered by E. Fischer in 1925, has been one of the most successful agents for lead. Conditions can be chosen in such a way that even selective extraction is possible. The absorbency of the extract can be measured or the lead can be extracted by shaking with a small volume of dilute nitric or hydrochloric acid which is then examined further. Dithizone is also very sensitive to oxidation. In spite of this drawback, dithizone is often used, and in the hands of experienced analysts, the method is reliable.[208,209]

2.3.4.4.2 Ammoniumpyrrolidine Dithiocarbamate (APDC)

Ammoniumpyrrolidine dithiocarbamate is the main chelating agent used for the preconcentration of lead, mostly in combination with methyl-iso-butyl ketone (4-methylpentane-2-one,MIBK). This method has been used for biological materials[210] and for human milk. Many of the methods require for the adjustment of pH from 2.8 to 3.5 prior to addition of APDC. Marumo[181] also experienced interference by iron; this can be avoided by pre-extraction with

Cupferron and MIBK. The absorbency of the obtained extract can be measured by atomic absorptiometry spectrometry. One of the main disadvantages of APDC is its low solubility in water.[211]

2.3.4.4.4 Sodium Diethyldithiocarbamate

Sodium diethyldithiocarbamate tends now to be preferred to dithizone, since it is less susceptible to oxidation and acid decomposition, whilst its complexes are more soluble in organic solvents. In spite of the fact that preconcentration of heavy metals is usually necessary, there are relatively few publications dealing with detailed data on the effect of pH on the extraction. Scharfe[212] determined the stability contents of several metal dithiocarbamate complexes and came to the conclusion that at pH 1.0 pyrrolidine dithiocarbamate is more stable than diethyl dithiocarbamate.

2.3.4.5 The Usefulness of Preconcentration Techniques

There is no single universally accepted procedure for the preconcentration or separation of traces of lead from interfering substances also present in the digest or ash. The use of ammoniumpyrollidine dithiocarbamate is widely accepted in combination with methyl-iso-butyl-ketone. The aqueous phase should be at about pH 2.5 for the extraction and the time for extraction should be limited to less than one minute. Sodium diethyldithiocarbamate is available in purer form than APDC and has some advantages. Again, the pH of the aqueous phase to be extracted and time of extraction are important and should be established by recovery experiments for each type of sample. Dithizone can also be used, by using pH 8.5 to 10.0 and with suitable masking agents, the preconcentration step can be made specific for lead when estimated spectrophotometrically.

2.4 Determination of Lead by Soluene Solubilization and Graphite Furnace AAS

The destruction of organic matter in human tissues is a pre-requisite for any trace metal analysis by graphite furnace atomic absorption spectroscopy. The two common methods used for this purpose are dry ashing and wet oxidation with various acids. Considering the disadvantages with these methods, discussed in section 2.3, it was decided to investigate the use of Soluene 350. Soluene 350, which is available from Packard Instruments Ltd., is a quaternary ammonium hydroxide tissue solubilizer specifically formulated for the use with toluene and xylene based scintillation counting solutions.

Jackson et al[213] reported the use of soluene 100 for the determination of Zn, Cu, Fe and Mn in animal tissues by flame atomic absorption spectroscopy. The authors further suggested that with the use of soluene 100, one can prepare tissue samples for atomic absorption quickly and with minimal handling thus reducing sample loss or possible contamination from excessive sample preparation. They further reported that soluene enhances the sensitivity of the metals investigated.

In another study, Kaplan et al.[214] have reported the use of an unspecified quaternary ammonium hydroxide solution for flame determination of Ca, Ni and Zn in rat lungs. Kaplan et al. have also reported the usefulness of quaternary ammonium hydroxide because of its great degree of sensitivity and a minimal amount of sample handling.

Murthy et al.[215] used aqueous tetramethyl ammonium hydroxide (TMAH) solutions for atomic absorption determination of zinc,

copper, cadmium and lead in rat liver, kidney and hair samples. The analysis was carried out on a Perkin-Elmer model 303 AAS on flame mode. The recovery of metals added to TMAH digested samples ranged between 82 to 101% for Pb and 86 to 103% for Cd. These authors then conclude that the TMAH digestion method allows faster and safer processing and handling of samples in comparison to acid digestion procedures.

Gross and Parkinson[216] have also used an unspecified quaternary ammonium hydroxide for flameless determination of Cu, Pb, Mn and Zn in human tissues.

Bond et al.[217] have also reported the use of Soluene 100 for analysis of lithium, magnesium and sodium in rat tissues by atomic absorption spectroscopy.

2.4.1 Preparation of Samples

The preserved placental samples were thawed and excess fluid removed by absorbant paper and then weighed in 0.5 to 1 g portions. The weighed samples were transferred to numbered 10 ml test tubes.

Five ml of soluene was then added to each sample, and the test tubes were placed in a constant water bath at 55°C. The fresh placental tissues were completely dissolved in approximately four hours whereas the preserved (-20°C) placental samples took 4 - 5 days. The dissolved samples were made up to 10 ml with toluene. These samples were then used directly for lead determination by flameless atomic absorption spectroscopy.

In a similar way, still birth kidney and liver samples were dissolved in soluene for flameless lead analysis. Human milk and amniotic fluid samples were also dissolved in soluene for lead analysis. There was no detectable lead in human milk or amniotic fluid samples.

The following observations were also made during sample preparation procedure:-

(i) At lower temperatures the solubilization process of soluene was considerably reduced. Soluene did not dissolve placental samples at room temperature.

(ii) Under the specified conditions decidua vera and umbilical cord samples dissolved in approximately 2 hours.

(iii) Up to 0.4 ml of water could be dissolved in 1 ml of soluene. This property was of great significance as this meant that stock standard solutions could be prepared in soluene.

(iv) Still birth bone samples did not dissolve in soluene even when kept at 55^{o}C for prolonged periods (up to 48 hours).

2.4.2 Experimental

2.4.2.1 Instrumental Conditions

The samples thus prepared (i.e. human placenta, still birth liver and kidney, amniotic fluid and human milk) were analysed on a Perkin Model 360 atomic absorption spectrophotometer fitted with a deuterium background corrector and an H.G.A. 74 graphite furnace. The results were recorded on a Perkin-Elmer model 056 strip chart recroder. The following instrumental conditions were used:-

Wavelength	283.3 nm
Spectral slit width	0.7 nm
Gas flow	Argon with miniflow/gas stop setting
Sample size	20 µl
Chart recorder	2 or 5 mV full scale
Source	E.D.L (Electrodeless discharge lamp, Perkin-Elmer).

Drying, charring and atomization temperatures were experimented for optimum peak performance with standards prepared in soluene and toluene (1 : 1). The following time and temperature settings were found to be essential for graphite furnace lead analysis in soluene matrix:-

	Temperature (^{o}C)	Time (seconds)
Drying	60	60
Charring	450	30
Atomization	2100	10

Because of the viscosity of soluene prepared samples, a longer drying time, i.e. 60 seconds, was very critical. Negative peaks were observed (i.e. matrix effect) when drying time was less than 60 seconds.

No absorbance for lead was observed when soluene or soluene : toluene samples were analysed. This suggests that at charring temperature of 450^{o}C complete decomposition of soluene occurs and as the decomposition proceeds readily the matrix effect at atomication temperature becomes insignificant.

2.4.2.2 Glassware

To avoid possible contamination, all glassware was washed once with dilute nitric acid, twice with tap water, twice with deionized water and finally rinsed with toluene and dried in an oven before use. All the glassware was kept covered and properly stored to avoid any atmospheric lead contamination.

2.4.2.3 Standard Solutions

A stock solution of 100 ppm lead was prepared by addition of a suitable amount of lead nitrate to soluene and warming to 55^{o}C for approximately 10 minutes to dissolve the precipitate

formed on addition. Standards were prepared on the day of analysis by adding suitable amounts of stock solution to 5 ml of soluene and diluting to 10 ml with toluene. A linear curve with standards in soluene and toluene was obtained, with standard concentrations of 0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm of lead (Appendix 1, 2).

2.4.2.4 Recovery and precision

Recovery of added lead (0.1 and 0.2 ppm) to the placental samples was between 90 - 100%. The relative standard deviation with six placental samples was 7.3%.

2.4.2.5 Advantages of Soluene Technique

The main advantage of this method is its rapidity with few stages of handling involved thus reducing contamination.

The soluene technique also seems to have advantages over dry ashing where we found considerable losses of lead at higher temperatures. There were complete losses of lead when the samples were ashed at 550^{o}C and 600^{o}C for 20 and 22 h respectively.

Similarly, when work was attempted with wet acid digestions followed by chelation by A.P.D.C. (Ammonium pyrrolidine dithiocarbamate) and MIBK (Methyl isobutyl ketone) complete recoveries were not possible.

Thus the soluene technique developed seemed to compare better than dry and wet acid digestion techniques.

2.5 Determination of Lead in Acid Extracts

2.5.1 Still Birth Bone Samples

Still birth bone samples were cleaned by removing the soft tissues adhering to it. The samples (0.2 gm) were left overnight, at room temperature, in 1 ml of concentrated Nitric acid. The dissolved samples were then diluted just before analysis to 10 ml

with distilled water. The diluted bone samples could not be stored over longer periods because of calcium precipitation. It was therefore essential to dilute and thoroughly mix the dissolved sample just before analysis. The flameless atomic absorption analysis was then carried out on thoroughly mixed samples. Instrumental settings described for lead analysis in section 2.4 were used. Drying and charring temperatures were increased to 100°C and 500°C respectively. Drying time was reduced from 60 seconds to 30 seconds. These changes were essential because of different matrix effects of bone samples when compared with soluene samples.

2.5.2 Birmingham Soil Samples

The soil samples (≃ 1 gm) were boiled for at least 30 minutes in an equal mixture of water and concentrated nitric acid. After filtration the soil residue was reboiled for a further 30 minutes and the contents were refiltered. The two filtrates were then pooled for carbon furnace lead analysis using the instrumental conditions as described in sections 2.4 and 2.5.1.

2.6 Determination of Blood Lead

2.6.1 Determination of Blood Lead by Soluene Technique

Blood samples were prepared by solubilizing 0.5 - 1.0 ml of whole blood in 5 ml of soluene. The samples were diluted with toluene to 10 ml and aliquots (25 µl) were pipetted into a HGA 74 graphite furnace of a Perkin Elmer 360 AAS model. The samples were dried at 60°C for 60 seconds, charred at 550°C for 30 seconds and atomized at 2100°C for 10 seconds.

A linear calibration curve was obtained over the range from 0 - 0.4 µg/ml Pb. No lead peaks were observed for soluene, toluene

or soluene : toluene blanks. The co-efficient of variation with 0.1 ppm Pb standard was 3.4% and recoveries were between 102 to 106% when 0.1 and 0.2 ppm Pb was added to whole blood samples. This method was compared with a paper punched technique, using 0.03 ml of blood spotted onto Whatman No. 4 filter of 7.0 cm diameter. Lead determinations were made on a Varian Techtron AA6 AAS on the following instrumental conditions:

Lead lamp current	6.0 mA
Spectral band width	0.50 nm
Wavelength	217.0 nm
Power control to workhead (Arbitrary units)	
Ashing	5.6 seconds for 30 seconds
Atomization	5.2 for 2.5 seconds.

A comparison of blood lead values obtained on the same samples by soluene and paper punched techniques is given in Table 12. Statistical analysis using students t (corr coeff. = 0.891) and F tests showed that the difference between the two sets of results was not significantly different ($P < 0.05$). This method was used for blood lead analysis from hypertensive and normotensive patients (Appendix 5). However, when applied to cord and maternal blood samples, where the expected levels were less than 10 µg/100 ml, further improvements in the method were needed.

2.6.2 Determination of Blood Lead by Triton X Technique

Considering the problems concerning cord and maternal blood lead analysis by soluene technique, the use of Triton X was experimented. Meredith et al.[218] diluted blood samples in 1 : 5 ratio with 1% (W/V) Triton X - 100 and used 20 µl aliquots for lead assay by direct injection into the carbon furnace.

Table 12

Comparison of human blood lead concentrations determined by graphite furnace atomic absorption spectroscopy after solubilizing in soluene and amended paper punched disc techniques

	Patient's age	Sex	Concentration of lead in blood pg per 100 ml by	
			Soluene Technique	Paper punched disc technique
1	23	M	17	15
2	51	F	23	16
3	45	M	14	20
4	22	F	10	11
5	50	M	15	11
6	19	M	28	13
7	38	M	28	22
8	27	F	12	12
9	44	F	17	15
10	56	F	15	22
11	47	M	19	31
12	29	M	23	33
13	41	M	14	24
14	26	M	17	20
15	44	F	12	18
16	31	M	15	23
17	32	F	21	18
18	40	F	17	13
19	40	F	17	23
20	26	M	22	33
21	19	M	26	25
22	29	M	27	26
23	27	M	19	27
24	36	F	21	15
		Range	10 - 28	11 - 33
		Arith mean	18.7	20.2
		Standard deviation	5.1	6.5

Experiments with various dilutions of Triton X-100 and various blood and Triton X-100 ratios were carried out and finally Triton X-100 (0.1%) and blood to Triton X-100 ratio of 1 : 5 were used. Lead analysis was carried out on the dissolved samples by using the instrumental conditions described in section 2.4. By this technique the detection limit was lowered to 5 µg/100 ml, thus enabling accurate determination of maternal and cord blood lead levels.

2.7 Determination of Other Metals

2.7.1 Cadmium Determination by Soluene Technique

Although there have been a number of improved analytical methods[219-222] during the last 10 years, the determination of cadmium in biological materials is still far from satisfactory. Data reported on placental cadmium from unexposed population by different authors vary from 10 - 150 ppb[223-224] on wet weight basis. Similar variations have also been reported on blood cadmium from unexposed populations (4 ng/ml to 37 ng/ml).[225,226] Use of aqueous tetramethyl ammonium hydroxide for cadmium determination have been previously reported.[215] Our first studies on the use of soluene-350 for placental cadmium determination indicated that it was slightly contaminated with cadmium.[227] This observation was not confirmed in our later studies. Therefore it is important that each bottle of soluene should be examined for its cadmium content before its use.

2.7.1.1 Experimental

Sample Preparation and Instrumental Conditions

The placental samples used in this study were preserved at -20°C during 1971. These samples were thawed and weighed out in

500 mg portions into a 10 ml graduated pyrex test tube. Five ml of soluene was added to each sample and the samples were placed in a constant temperature water bath at 55°C for approximately 8 hours. The placental samples were dissolved during this time and were diluted to 10 ml in toluene. These samples were then analysed for cadmium on a Perkins Elmer model 360 AAS fitted with a deuterium background corrector and an HGA 74 graphite furnace (BodesnseewerkPerkin Elmer). The instrumental conditions used are given in Table 13.

Table 13

Instrumental Conditions for Cd Analysis by AAS

Wavelength:	224.8 nm
Spectral Slit width:	0.7 mm
Gas flow:	Argon with mini flow/gas stop setting
Sample size:	20 ml
Chart recorder:	5 mV full scale
Source:	E.D.L. (Electrodeless Discharge Lamp, Perkin Elmer)

Time and Temperature Settings

	Digit setting	Temperature (°C)	Time (seconds)
Drying	84	60	30
Charring	331	550	30
Atomization	661	1900	10

2.7.1.2 Glassware

All glassware was left in 2% Decon -90 overnight and washed once with dilute nitric acid, twice with tap water, twice with distilled water and dried in an oven and finally rinsed with toluene before use.

2.7.1.3 Standard Solutions

A stock solution of 100 ppm cadmium was prepared in soluene. Standard solutions were then prepared on the day of analysis in the range of 0 - 0.004 µg/ml by adding suitable amounts of stock solution to 5 ml of soluene and making up to 10 ml with toluene. Standard solutions were also prepared in placental samples of known concentration.

2.7.1.4 Results

The linearity of the standard curve was observed between 0.00 to 0.004 µg/ml of cadmium standard. No peaks were observed for soluene, toluene or soluene/toluene blank. The peak height for standards 0.001, 0.002 and 0.004 were 7, 20 and 45 respectively. Recoveries on the placental samples were in the range of 90 - 110%.

2.7.2 Determination of Cadmium in Acid Extracts

2.7.2.1 Still Birth Bone Samples

The still birth bone samples were acid digested as described in section 2.5.1 and were analysed on the experimental conditions described in section 2.7.1.

2.7.2.2 Birmingham Soil Samples

Birmingham soil samples were prepared as described in section 2.5.2 and were analysed on the experimental conditions described in section 2.7.1.

2.7.3 Determination of Mercury

2.7.3.1 Cold Vapour Technique

The cold vapour technique is possible because mercury is unique among metals. It does not oxidise readily in air and it has an appreciable vapour pressure at ordinary temperatures. These properties of mercury were first exploited for analytical

purposes by Woodson[228] who described an instrument for determining this metal in air. The technique was further improved by Ballard et al.[229-231] and applied in various modifications by Monkman et al.[232] and Pappas and Rosenberg[233] for various biological materials. During the course of atomic absorption experiments involving mercury solutions, Poluektov and Vitkun[234] observed that they obtained an unusual enhancement of mercury when aspirating a sample containing stannous chloride. Hatch and Ott[235] applied this principle for the determination of mercury in nickel and cobalt metal. Magos and Cernik[236] applied the same principle for mercury determination in urine, blood and kidney tissues. Various other authors[237-239] have also confirmed that the cold vapour stannous chloride method for atomic absorption mercury determination is substantially more sensitive than the standard solution nebulization sample technique.

A cold vapour mercury atomic absorption system consists basically of a light source emitting mercury resonance lines, an absorption cell and a detector system. In this work a Perkin-Elmer mercury analysis system fitted to a model 360 atomic absorption spectrophotometer was used.

Determination of mercury in biological materials by the cold vapour technique involves two distinct stages: destruction of organic matter and sample preparation, and measurement of the mercury in the absorption cell. Once a sample has been digested, the procedure for mercury analysis is basically the same regardless of the biological material to be tested. The digested sample is diluted to 100 ml with distilled water and treated with nitric acids and sulphuric acids in the presence of potassium permanganate

in order to oxidise all of the mercury present to the mercury (II) form (Hg^{2+}). The excess of permanganate is reduced with hydroxyl ammonium chloride and the mercury is reduced to metallic mercury with tin (II) chloride. An aerator is placed in the sample solution and a circulation pump moves the air trapped in the system through the solution, thus evaporating the mercury and carrying the vapour through the absorption cell.

Mercury vapour in atomic form absorbs the 253.7 nm radiation emitted from the light source. The change in energy is then detected and read out in the usual way on the atomic absorption spectrophotometer. The standards were prepared in the range 0.1 to 1 µg of mercury. The average digits for 0.1, 0.2, 0.5 and 1 µg Hg were 6, 17, 33 and 72 and a linear curve was obtained.

2.7.3.2 Placental Mercury Analysis

Deep frozen placental samples were defrosted and approximately 4 g of the material were weighed after removing excess of blood. Concentrated nitric acid was then added and the samples were placed in the water bath at 55°C for 2 - 4 hours. The samples were almost dissolved after this time. To complete the digestion the samples were then heated to 100°C for 5 minutes. These solutions were then diluted to 100 ml with water and mercury levels were determined as previously described (Appendix 4).

2.7.4 Determination of Calcium and Zinc

Still birth bone samples were wet ashed with nitric acid and analysed for calcium and zinc by flame atomic absorption spectroscopy at wavelengths of 422 and 213.9 nm respectively. A linear calibration curve was obtained by using calcium standards of 2.5, 5.0, 7.5 and 10.00 ppm concentrations. A linear calibration curve for zinc was obtained by using 0.5, 1.0, 1.5 and 2.00 ppm standards.

3. RESULTS AND DISCUSSION

3. Results and Discussion

With the methods thus developed (Chapter 2) analytical work was carried out on placental, foetal tissue and blood samples. One of the most important precautions needed in this work was environmental lead contamination during sample preparation.

When blank soluene samples were left open in the laboratory over a period of 120 hours (figure 2), a marked increase in lead levels was observed with time. Thus, throughout the entire study, precautions were taken to eliminate lead contamination during sample preparation stages. With these procedures it was possible to obtain lead free reagent blanks (figure 3).

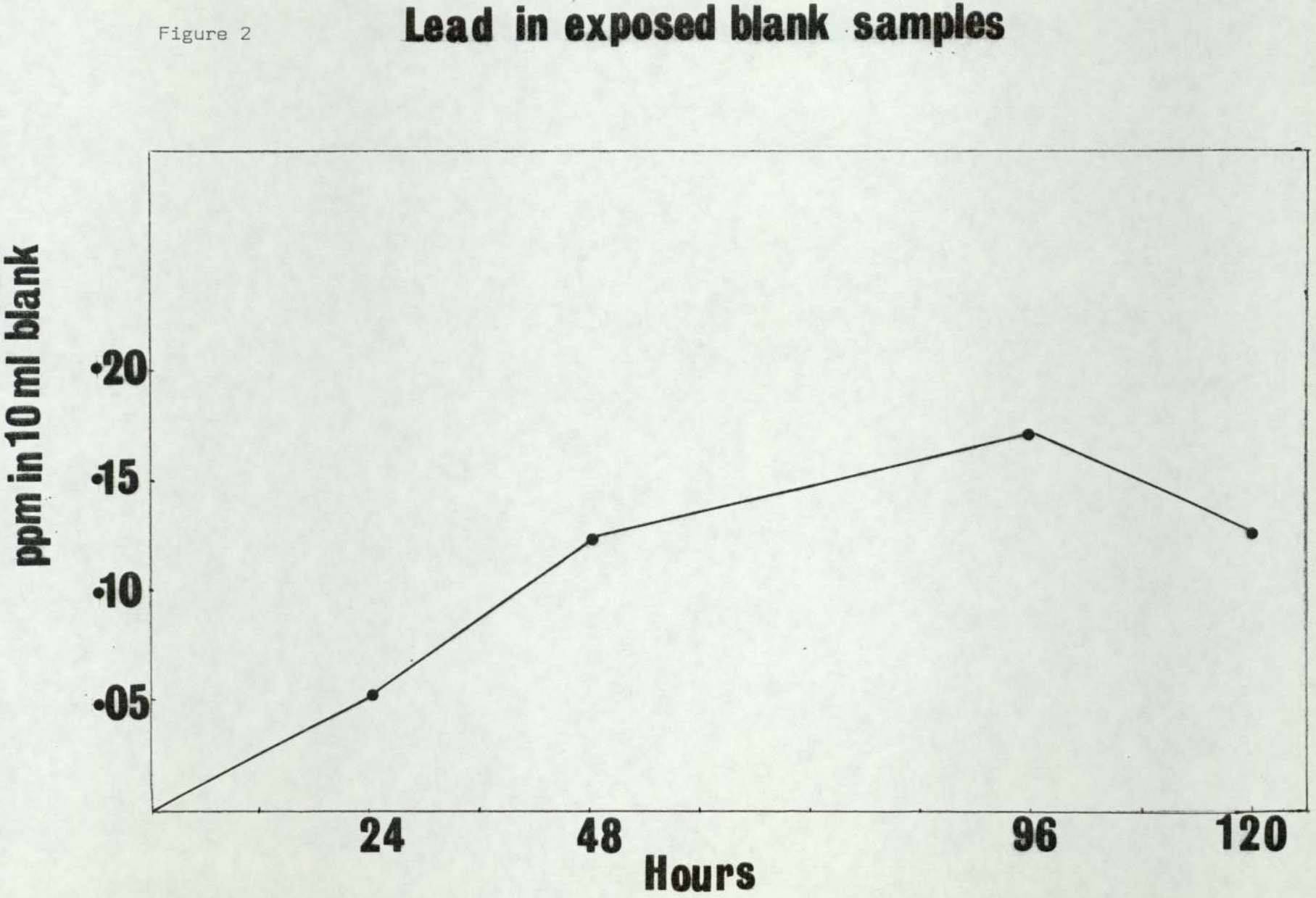
So as to eliminate any contribution of lead through glass during sample preparation, all glass equipment was thoroughly cleaned before its use and was checked for lead levels. A calibration curve of 0 to 0.4 ppm Pb (figure 4) was also used while analysing glass equipment for any possible lead contamination.

Further, to ascertain that overall lead pollution in Birmingham was not excessive when compared to urban areas of other industrial countries, lead in soils (200 samples) was analysed (figure 5).

Lead levels were higher in soils from inner residential areas and near motorways (M'W). Areas in city parks and rural localities were comparatively lower in soil lead. However, this data do confirm that lead pollution as reflected by soil lead in Birmingham is typical of industrial urban cities.

The present studies on placental, foetal tissues and blood samples are mainly from Birmingham and Stoke-on-Trent. The reported trace metal levels in human placenta are given in Table 14.

Figure 2


Lead in exposed blank samples
ppm in 10 ml blank
·20
·15
·10
·05
24
48
96
120
Hours

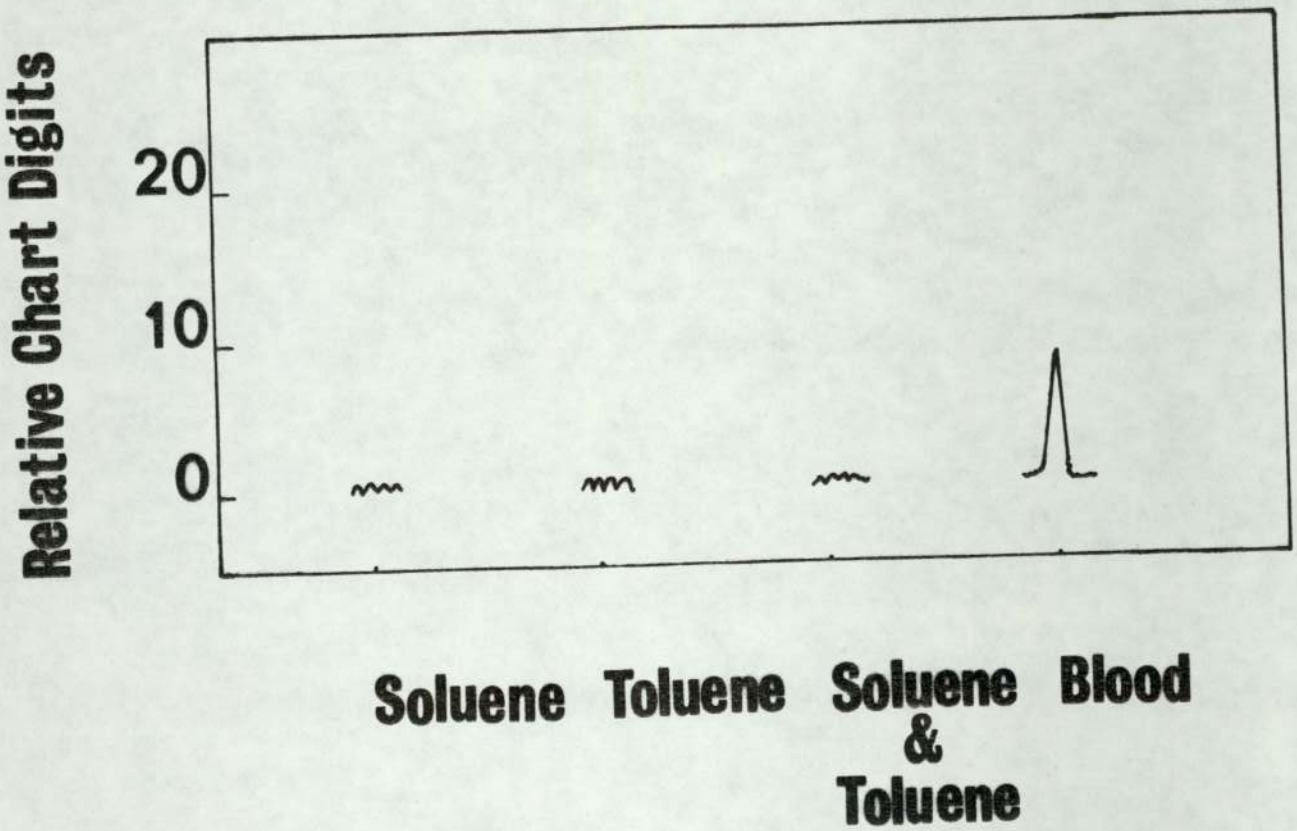


Figure 3:- Lead peaks in Soluene and Toluene as compared to blood or placental matrixes.

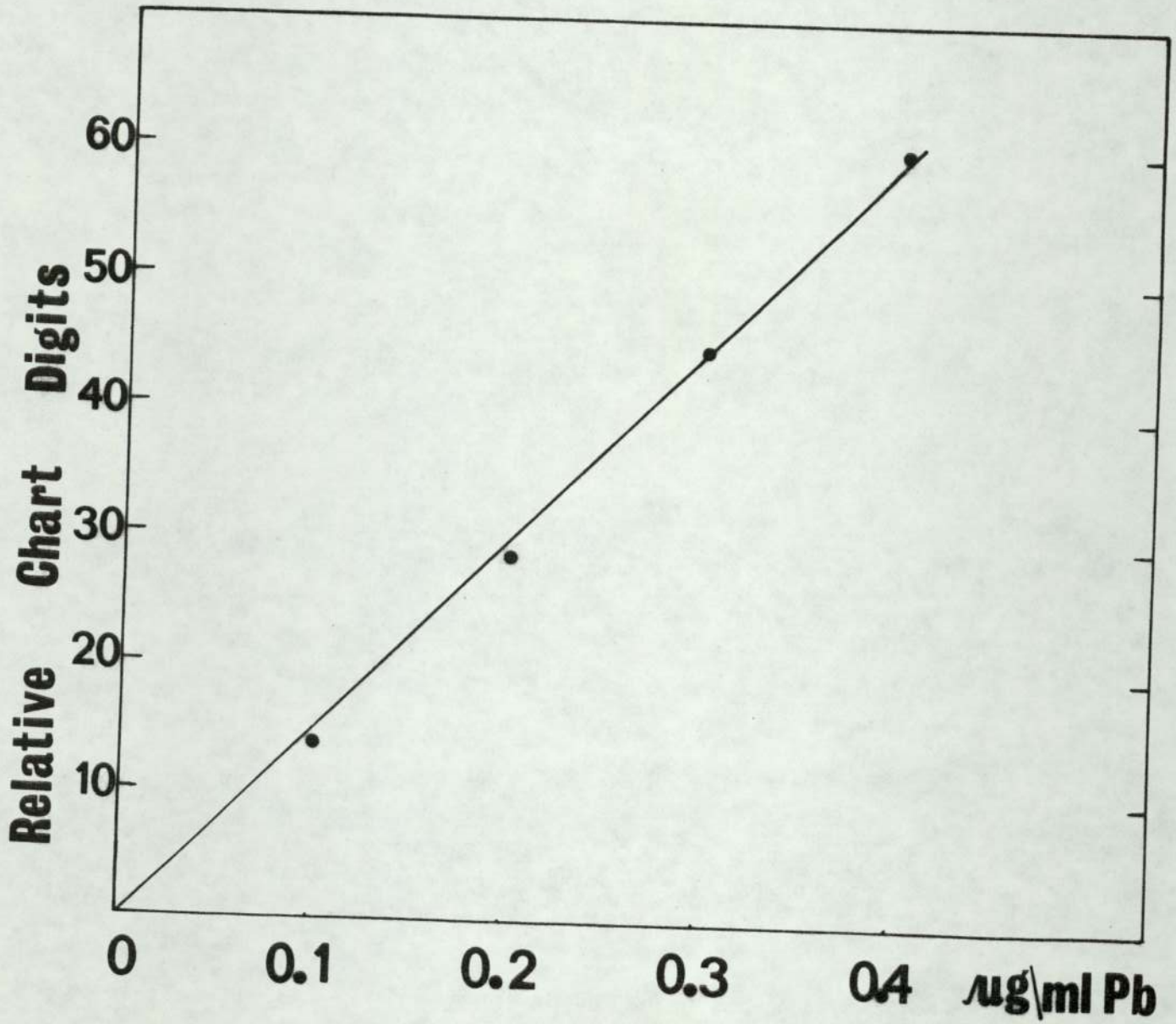


Figure 4:- Calibration curve for lead standards

Figure 5:-

Lead Levels In Birmingham Soils

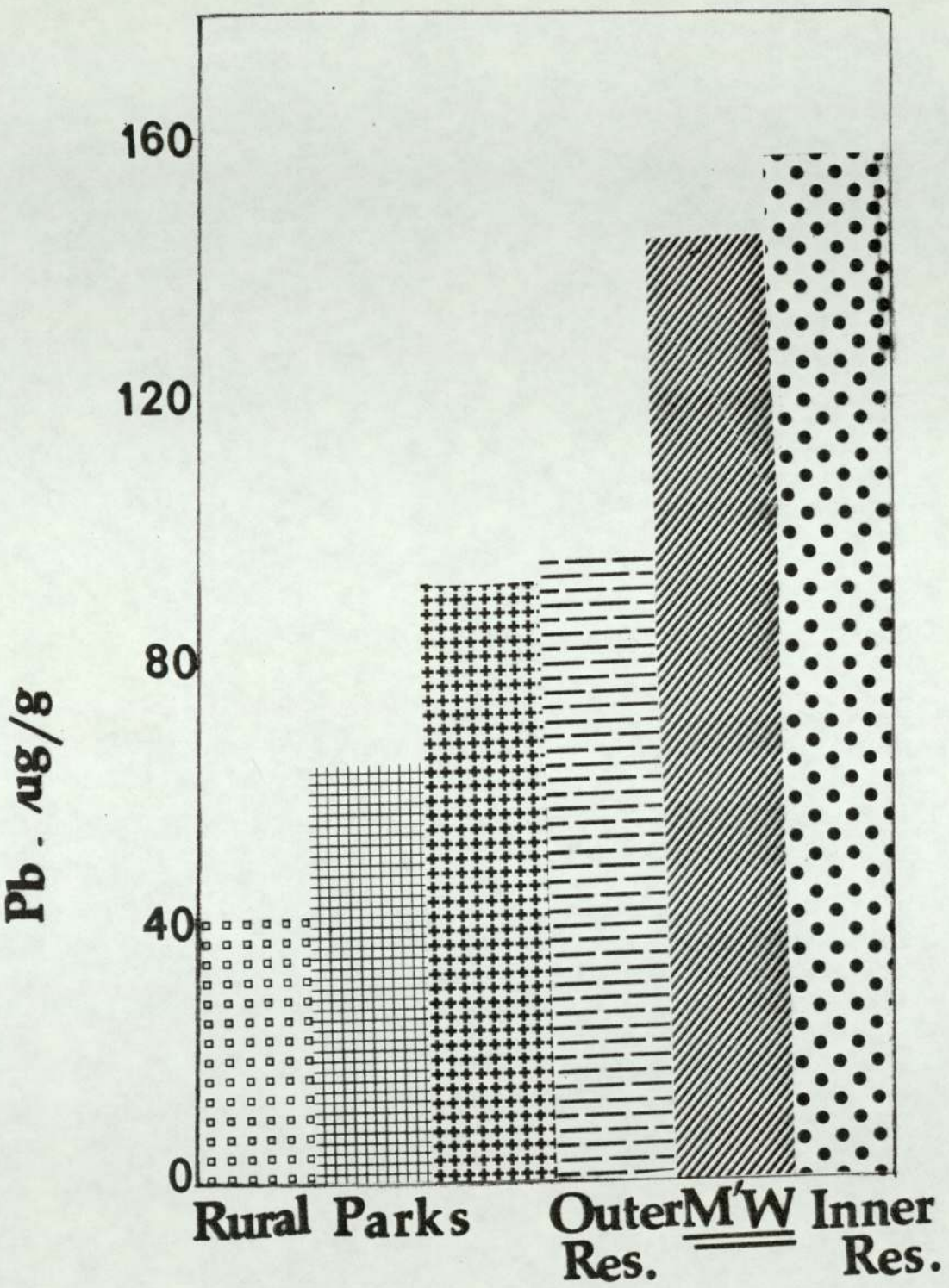


Table 14

The elemental composition of human placenta

Element	No. of samples	x 10^{-6}	Remarks
Al	60	1.10	
As	6	0.02	
	-	0.019	
	-	0.13	
Br	7	16	
	-	40.40	
	-	27.60	
Ca	-	855	Urban population
	-	1033	Rural population
	7	910	" "
	-	250	Up to mid term pregnancy
	-	300	Up to 30 weeks of pregnancy
	-	1000	At term
	118	10260	" "
	-	1230	" "
Cd	135	0.102	
	45	0.021	
	4	0.4	
	1	0.012	
Ce	-	0.0035	
	-	0.024	
Cl	15	2170	
Co	838	0.023	
	7	0.07	
	-	0.064	
	-	0.031	
Cr	60	0.036	
	3	0.4	
Cu	61	3.90	
	13	1.20	
	1	0.95	
	-	5.65	
F	25	0.15	
Fe	58	570	
	811	731	
	7	861	
	-	750	
	-	1434	
	-	1084	

Table 14 continued

Element	No. of samples	x 10^{-6}	Remarks
Hg	1061	0.12	
	6	0.06	
	-	0.10	
	9	0.072	
K	16	1430	
	-	1550	
La	7	0.021	
	-	0.015	
	-	0.006	
Mn	59	0.47	
Mo	7	0.06	
	-	0.30	
	-	0.14	
Na	-	7750	
	-	6700	
	16	1990	
	-	2500	
	-	2000	
Pb	234	1.83	
	55	0.35	
	6	1.4	
Rb	826	25	
	-	6.20	
	-	11.70	
Sb	7	0.06	
	-	0.073	
	-	0.016	
Sc	-	0.00028	
	-	0.00048	
Se	822	1.70	
	7	0.47	
	-	1.24	
	-	1.64	
	16	0.373	
Si	-	14	
	5	138	
Sn	4	1.10	
	-	6.20	
	-	18.40	

- not given

3.1 Placental lead in stored samples

The samples stored in a freezer during 1971 were provided by Dr. D. I. Rushton of the Birmingham Maternity Hospital. These samples have been dissected by a number of different laboratory staff over a considerable period of time. These samples also seem to have lost moisture during storage. It was further observed that placental samples over a period of three years lost approximately 15% of moisture. On thawing the 1971 placental samples also appeared more decomposed compared to fresh samples and this possibly changes matrix effect during lead determination. These stored samples were also kept in unsealed plastic containers.

Because of all these reasons, lead levels in stored samples were higher (0.9 ppm) than fresh samples (0.3 ppm) from Stoke-on-Trent. However, the results obtained on these stored samples were found comparable with lead determinations made by Birmingham environmental laboratories[240] (Appendix 3). At the same time lead levels in fresh placental samples were found in similar range with Cricklewood laboratories.[240] Thus comparisons with other laboratories[240] confirmed that lead levels were higher in cases of stored samples when compared to fresh samples. However, both these levels are within the range of reported placental lead values (Tables 14 & 21).

The lead levels in 1971 stored samples were higher (0.4 ppm) when compared with 1980 placental samples (0.15 ppm) from Birmingham. The placental lead levels were also equally distributed when samples from different parts of fresh placenta were analysed.

Within the stored samples analysed, placental lead was higher in samples from stillbirths (1.5) and neonatal deaths (1.7) when compared with samples from normal births. It was further noted that there was no significant correlation between birth weight and placental lead (figure 6). However, in case of premature babies placental lead levels increased with an increase in birth weight. No significant correlations were observed between placenta and gestation period (figure 8) and between younger and elder sibs (figure 9 & Appendix 3).

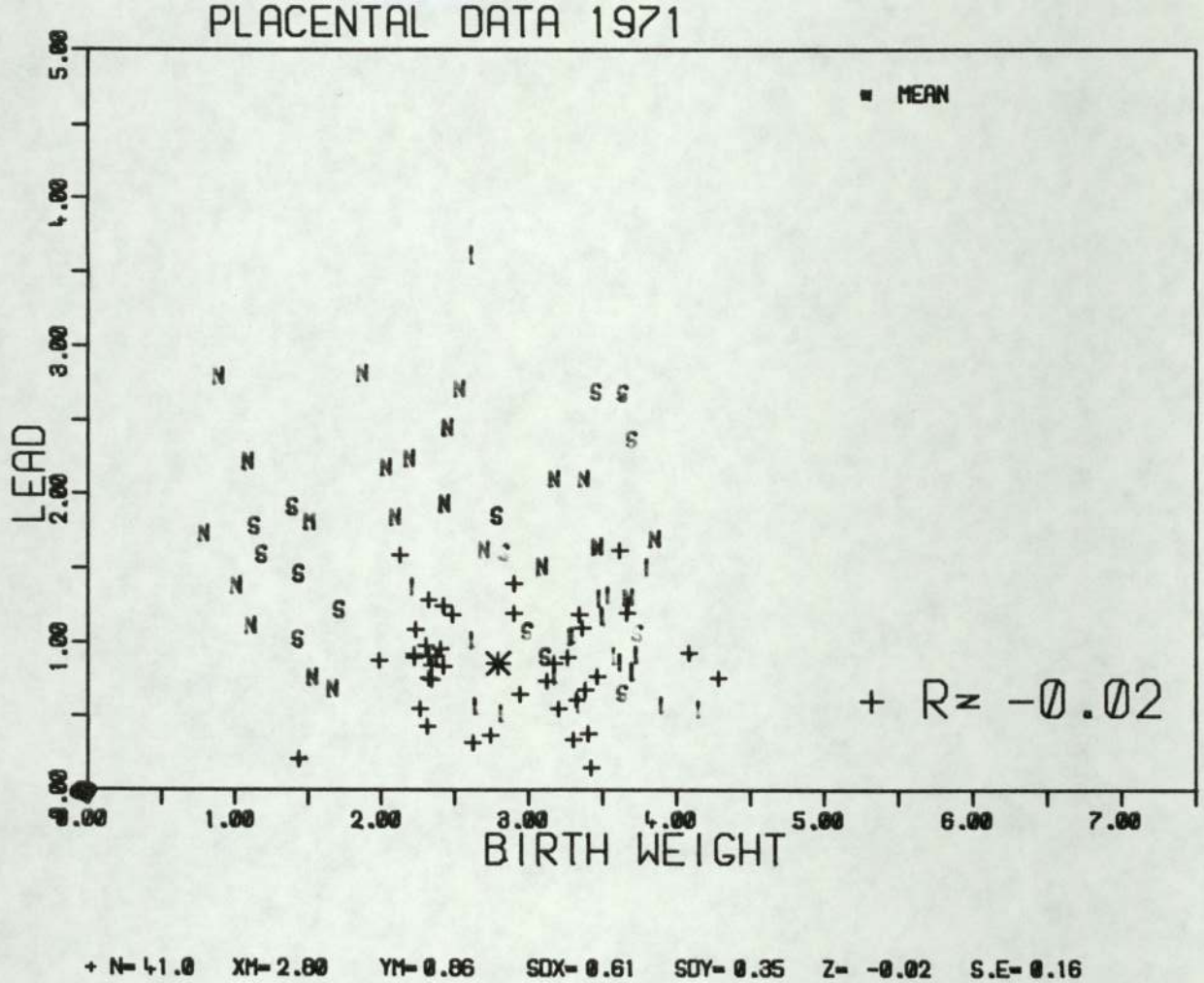


Figure 6:- Correlation between birth weight and placental lead.

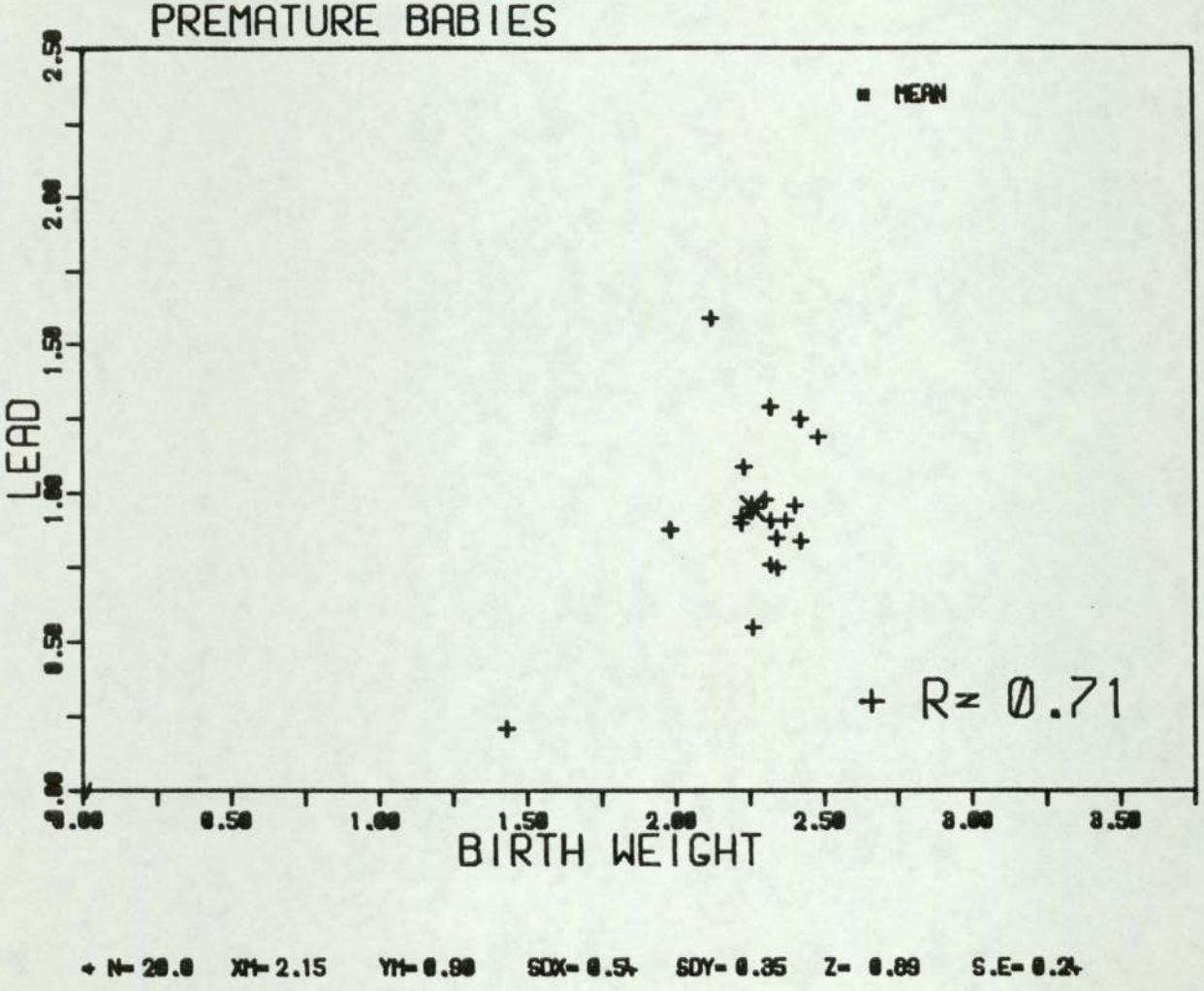


Figure 7:- Correlation between placental lead and birth weight from premature babies.

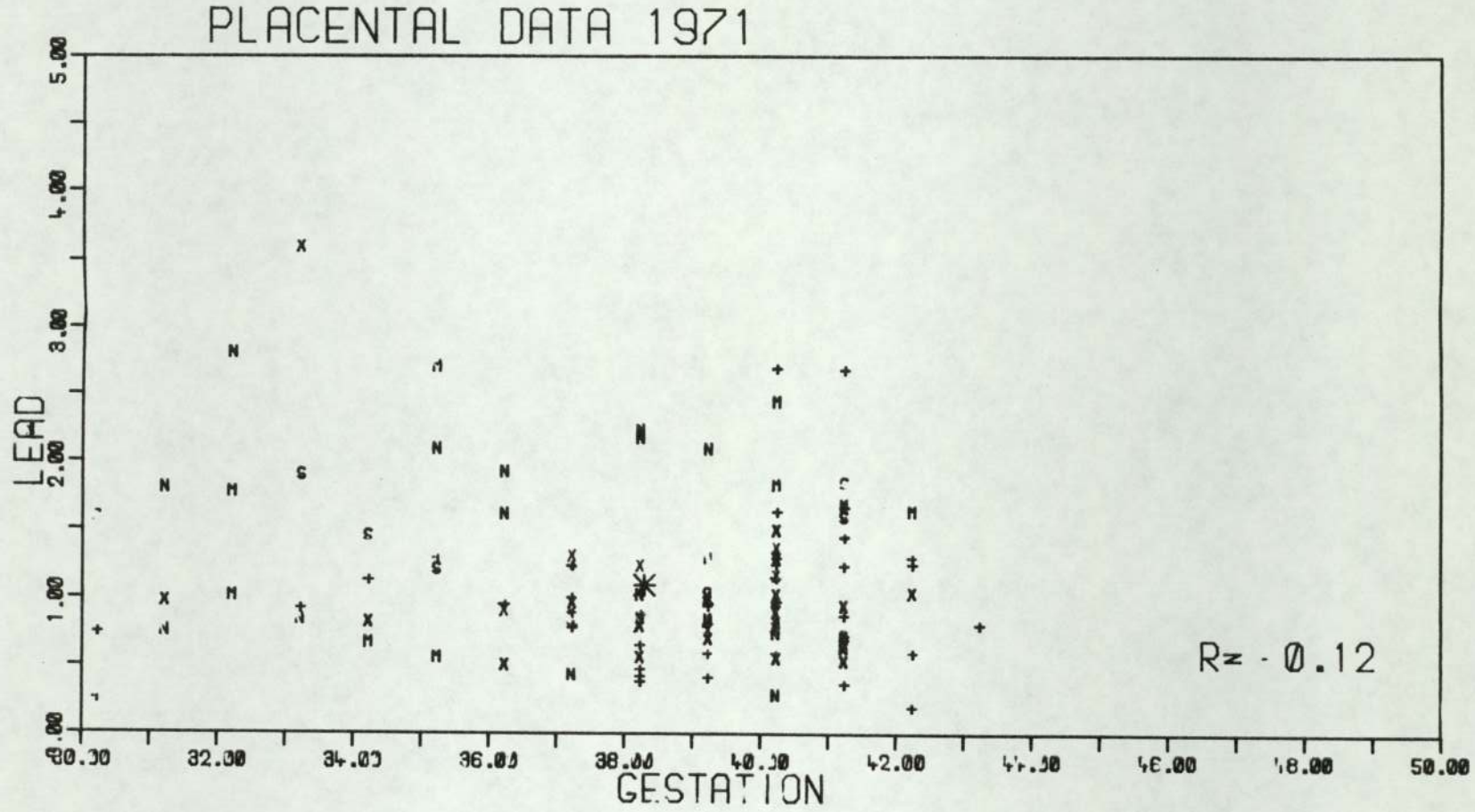


Figure 8:- Correlation between placental lead and gestation period.

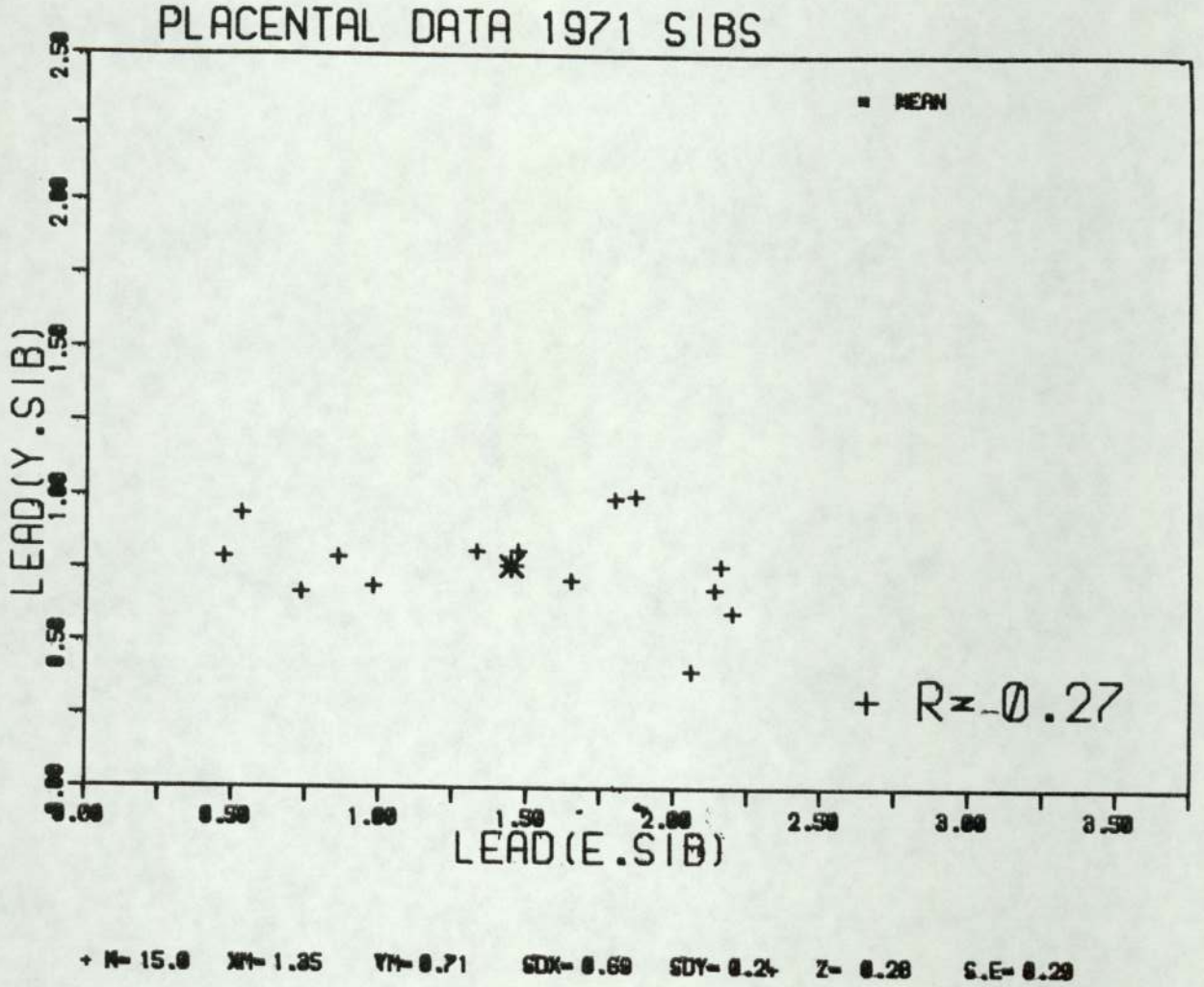


Figure 9:- Placental lead correlation between younger and elder sibs.

3.2 Placental cadmium levels in stored samples

Placental cadmium levels in the samples stored at the Birmingham Maternity Hospital during 1971 were determined by soluene technique described on page 74.

The placental cadmium levels in normal Indian and European births were similar (Tables 15, 16). The average cadmium level in placental samples from normal births was 10 ng/g wet weight basis, whereas in placental samples from European normal births was 11 ng/g.

The placental cadmium levels in malformed stillbirths and neonatal deaths (Table 17) were 16 ng/g, in stillbirths (Table 18) 19 ng/g and in neonatal deaths (Table 19) 21 ng/g.

No information was available on the smoking habits of the mothers and therefore no correlation could be worked out between placental cadmium and smoking habits.

Baglan et al.[241] have determined an average cadmium placental level of 17 ng/g (wet weight), whereas Thieme et al.[242] found average placental cadmium value of 3.50 for Munich and 17 ng/g for Essen. However, Friberg et al.[223] and Colluci et al.[226] have reported cadmium in placenta as 10 and 73 ng/g (wet weight) respectively. Lauwerys et al.[243-244] have found higher placental cadmium in smokers (15.7 ng/g) when compared with non-smokers (12.5 ng/g). Peerboom et al.[245] have also determined cadmium in placental samples from smokers and non-smokers. The placental cadmium levels in their study were 8 ng/g and 15 ng/g (wet weight basis) in non-smokers and smokers respectively.

Table 15

Cadmium in Indian placental samples from normal births

Mother's age (years)	Gestation period (weeks)	Mother's weight (kg)	Child's weight (kg)	Sex of child	Social class of father	Cd(ng/g fresh wt. basis
20	40	56.5	2.18	M	3	15
28	36	58.1	3.69	M	4	16
36	38	50.5	3.64	F	5	7
21	40	78.0	3.46	M	3	13
37	37	77.5	3.50	M	4	9
39	42	52.2	3.26	F	4	16
26	-	-	-	-	4	13
26	-	-	-	-	3	14
20	40	48.5	3.44	F	-	9
20	36	45.5	2.78	M	3	8
26	40	85.0	3.76	F	3	17
18	42	47.0	3.45	M	3	3
34	40	69.0	3.24	F	4	3
32	38	48.5	3.86	M	4	2
24	41	76.0	4.11	F	5	16
28	40	61.0	3.66	M	-	5
19	39	53.0	2.58	F	4	15
28	28	57.5	3.66	F	4	1
Arith. mean 26.8	38.8	60.2	3.39	-	3.7	10 ± 6

- = no information

Table 16

Cadmium in European placental samples from normal births

Mother's age (years)	Gestation period (weeks)	Mother's weight (kg)	Child's weight (kg)	Sex of child	Social class of father	Cd(ng/g fresh wt. basis)
16	41	46.0	2.90	F	3	10
33	40	62.5	3.66	F	3	27
24	40	65.0	3.36	M	3	26
21	40	57.1	3.26	M	-	14
32	42	81.5	2.90	F	3	19
32	42	81.5	2.90	F	3	23
38	41	73.0	2.62	M	3	12
32	40	-	4.08	F	-	17
40	38	62.5	3.32	-	-	2
32	42	58.5	3.20	F	3	3
33	37	47.0	3.12	M	3	9
33	42	83.0	3.42	M	3	1
39	38	59.5	3.40	M	1	4
35	39	55.0	2.74	F	4	18
30	38	76.2	3.30	F	3	5
23	-	-	-	-	-	10
20	39	54.5	3.46	M	3	7
22	41	57.5	3.60	F	2	13
28	41	58.0	2.94	F	3	7
33	41	-	3.38	F	-	5
24	41	57.0	3.61	M	3	7
25	41	66.5	3.34	M	3	5
22	43	49.5	4.28	M	1	6
27	38	65.5	2.31	F	3	11
Arith. mean 28.9	40.2	62.7	3.26	-	2.8	11 ± 7

- = no information

Table 17

Cadmium in European placental samples from malformed stillbirths and neonatal deaths

Mother's age (years)	Gestation period (weeks)	Mother's weight (kg)	Child's weight (kg)	Sex of child	Social class of father	Nature of malformation	Cd(ng/g fresh wt. basis)
17	-	-	-	-	3	Anencephaly	10
-	-	-	-	-	-	Multiple malformations	30
22	42	71.0	3.43	F	3	Meningocele	8
30	32	49.0	1.10	F	3	Anencephaly	8
26	36	62.0	2.67	M	3	Diaphragmatic hernia	11
24	41	58.0	3.82	F	3	Haemangioma liver	28
30	32	55.5	1.40	M	3	Anencephaly	6
23	47	63.7	2.42	M	2	Chromosomal abnormality (ring D)	23
31	40	58.0	2.06	F	3	Chromosomal abnormality (trisomy 18)	12
24	35	-	2.50	M	3	Congenital tumour of CNS	21
Arith. mean 25.2	38.1	59.6	2.43	-	2.9	-	16 ± 9

- = no information

Table 18

Cadmium in European placental samples from stillbirths without malformations

Mother's age (years)	Gestation period (weeks)	Mother's weight (kg)	Child's weight (kg)	Sex of child	Social class of father	Cd(ng/g fresh wt. basis)
34	35	52.2	1.68	F	3	18
26	41	61.5	3.60	M	3	37
25	40	76.0	3.08	F	3	29
27	30	82.2	1.15	F	3	16
27	33	-	1.36	M	4	14
-	-	-	-	-	-	4
30	41	50.0	2.75	F	3	14
20	41	62.0	2.80	F	-	37
30	34	68.5	1.40	M	3	5
Arith. mean 27.4	36.9	64.6	2.23	-	3.1	19 ± 12

- = no information

Table 19

Cadmium in European placental samples from neonatal deaths

Mother's age (years)	Gestation period (weeks)	Mother's weight (kg)	Child's weight (kg)	Sex of child	Social class of father	Cd(ng/g fresh wt. basis)
-	28	-	-	-	-	21
28	-	-	-	-	3	11
31	36	-	2.67	M	-	21
29	32	65.5	1.84	M	3	38
24	35	55.0	3.14	M	3	29
21	38	52.0	2.00	M	3	15
18	29	48.5	1.06	F	3	16
20	38	63.2	2.16	F	3	28
17	39	66.5	3.64	M	-	24
33	39	74.5	3.34	M	4	31
19	31	63.0	1.50	M	3	18
Arith.mean 24.0	34.5	61.0	2.37	-	3.1	23 ± 8

- = no information

3.3 Placental and stillbirth tissue lead levels from occupationally exposed women

This study was carried out on the samples from the Stoke-on-Trent area, provided by Dr. J. G. Dathan). The main aim of this investigation was to study the effect of occupational lead exposure on placental lead levels. The control samples of infant bones were provided by Dr. H. A. Waldron of the London School of Hygiene and Tropical Medicine. These samples consisted of cot deaths (age under one week) and accidental death (age under 18 weeks).

The placental samples from Stoke-on-Trent were received fresh and samples for analysis were dissected, behind the umbilical cord, from the maternal side of placenta (Figures 10 and 11). These samples were stored for 6-12 months at -20^{o}C before analysis.

There were 8 cases of the women working in the pottery industries (Table 20) where there was no recent exposure to lead. The placental lead levels in these cases ranged from 0.16 to 0.41 µg/g fresh weight basis with an arithmetic mean of 0.29 $\pm$ 0.09 µg/g. The average age in this group was 28 years. Because there was no recent occupational expesure to lead in this group, the placental lead value of 0.29 µg/g has been considered as the control value for the Stoke-on-Trent placental samples.

The Stoke-on-Trent placental samples analysed by the Cricklewood laboratories also averaged 0.31 µg/g fresh weight basis (177 samples).

The reported average placental lead values are generally greater than 0.3 µg/g fresh weight basis. In all reported cases (Table 21) the placental lead levels are at least twice as high than the average maternal blood lead values. However, in one report[244] average placental lead levels (0.11 µg/g) are slightly higher than their average maternal blood lead levels (10.2 µg/100 ml). These authors also state that their levels in general are low because of their acid digestion procedure. Many authors[247-250] have further concluded from their work that during pregnancy placenta does accumulate lead.

Figure 10:- Human placenta (maternal side)

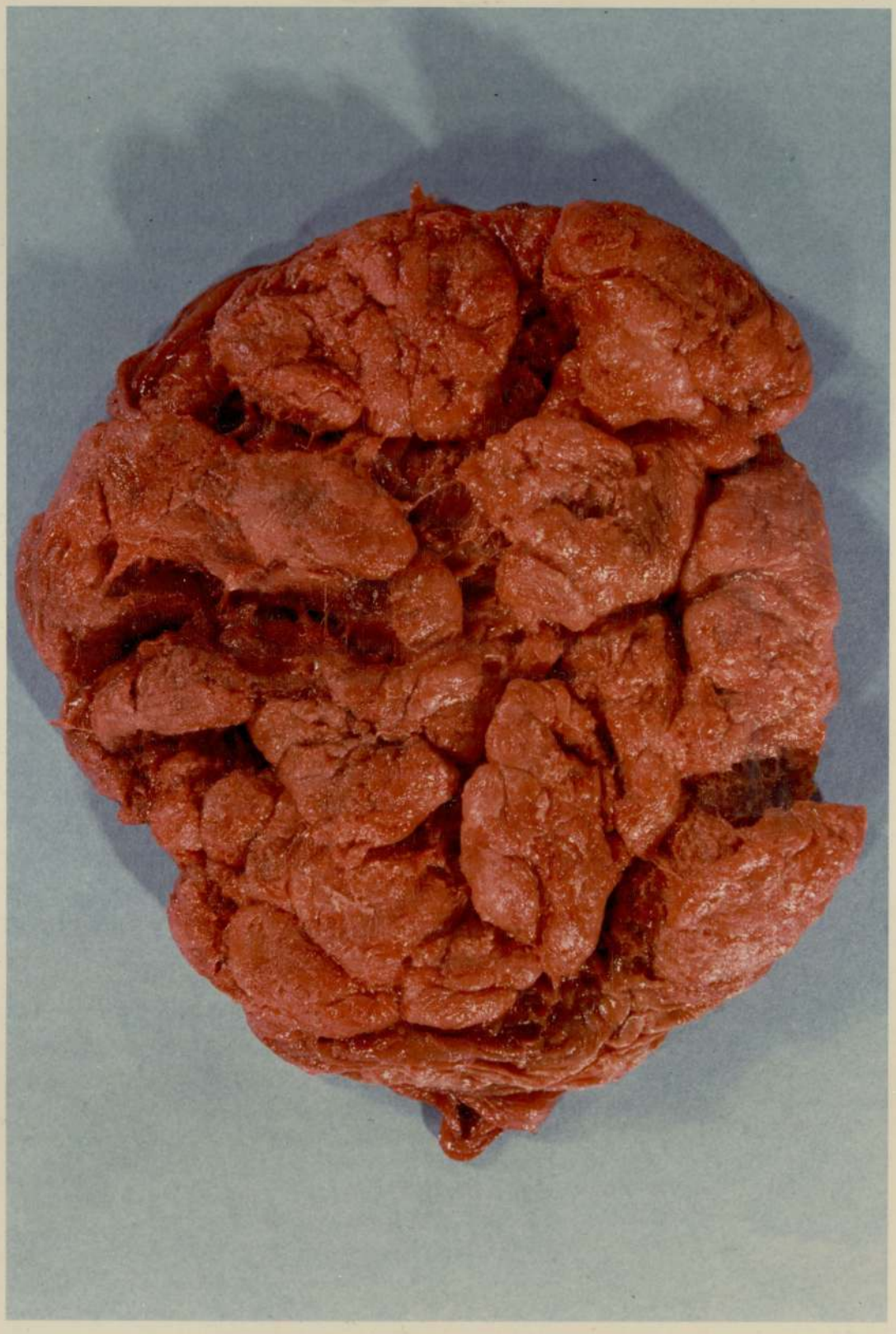


Figure 11:- Human placental (foetal side)

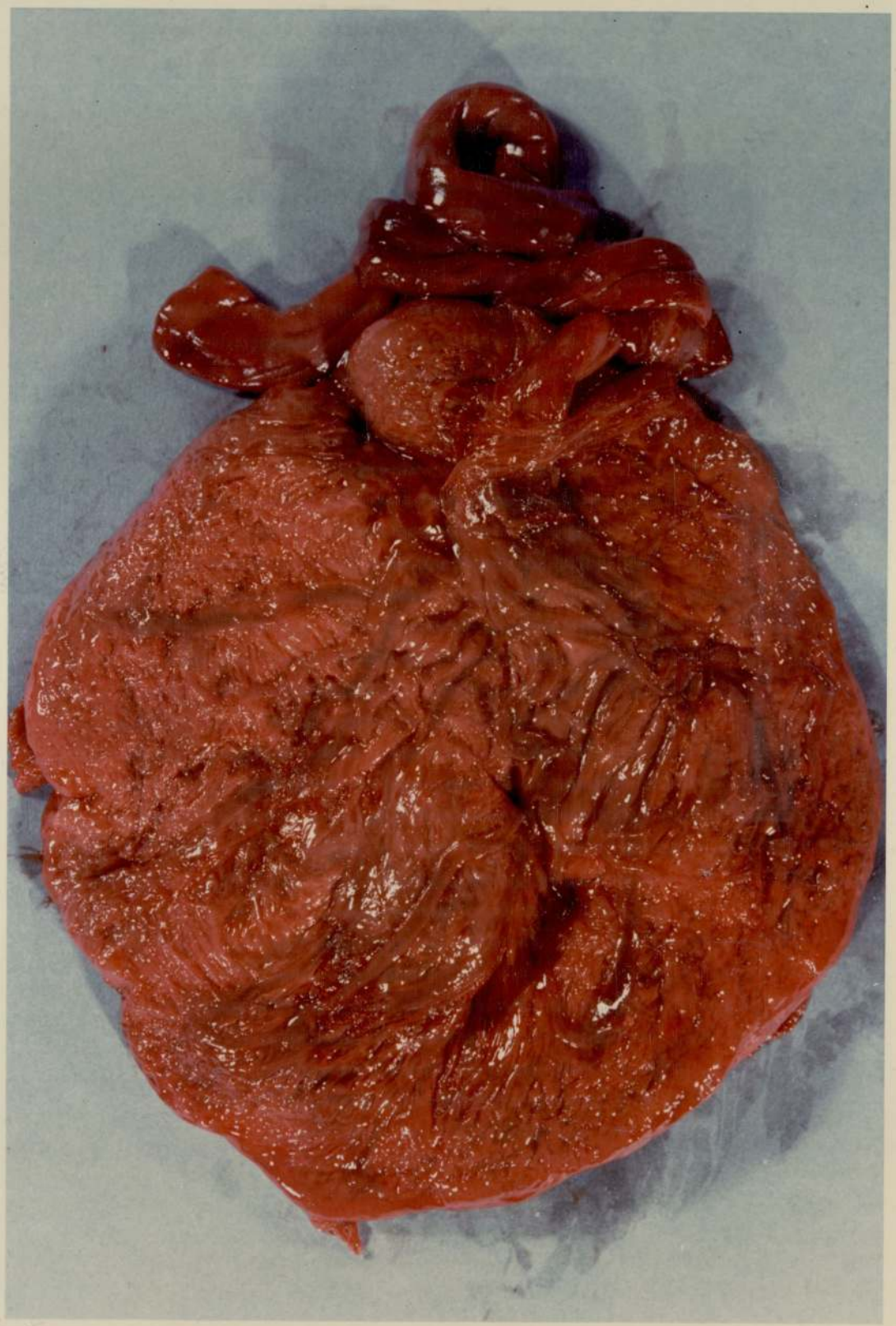


Table 20

Placental lead in samples from normal deliveries where mothers did not work during last two years. (Deliveries in 1976)

Age (years)	Occupation	No. of years worked	Year in which exposure ceased	Placental lead µg/g
25	Paintress	3	1972	0.16
33	Cuphandler and fetler	3 6	1967	0.21
30	Processor	2	1968	0.27
28	Lithographer	6 months	1963	0.23
32	Cup sprayer	10	1973	0.35 & 0.32 (twins)
26	Lithographer	12	1973	0.37
18	Paintress	1	1973	0.41
31	Lithographer	14	1974	0.29

Arith. mean = 0.29 $\pm$ 0.09 µg/g fresh weight basis

Table 21

Reported placental lead levels (µg/g fresh weight basis)

Author	Year	Country	Pb	Reference
Dawson et al.	1968	U.S.A.	67.3	251
Colluci et al.	1973	"	0.96	226
Baglan et al.	1974	"	0.30	241
Karp and Robertson	1977	"	0.29	252
Bagchi et al.	1939	India	0.32	253
Horiuchi et al.	1959	Japan	0.57	247
Wagner et al.	1974	Austria	17.3	250
Maruna et al.	1975	"	51.9	249
Einbrodt et al.	1973	Germany	0.56	248
Thieme et al.	1974	"	0.40	242
Englehardt et al.	1976	"	0.31 - 0.39	254
Schaller et al.	1977	"	0.24 - 0.32	255

3.3.1 Placental lead and length of occupational exposure

There is an increase in placental lead values with a corresponding increase in length of occupational exposure (Table 22). The average placental lead value (0.30 µg/g) where occupational exposure is < 1 year, is similar to the value found for the control group (Table 20). However, where the length of occupational exposure is greater than 3 years the placental lead levels are higher (0.43 µg/g) than the control group (0.29 µg/g).

Within occupational exposure group of > 3 years, the placental lead levels are higher where exposure is > 6 years (0.46 µg/g) when compared with samples occupational exposure of 3-6 years (0.36 µg/g) (Table 23).

There is also an increase in placental lead levels with an increase in mothers' age (Table 24). Thus, for mothers' average ages of 19, 26 and 33 years the placental lead values are 0.30, 0.35 and 0.51 µg/g respectively. This increase in placental lead values with increasing age is due to longer exposure periods of mothers in higher age group. However, with increasing age there is a possibility that more lead is also mobilised during pregnancy along with bone calcium.

3.3.2 Placental lead and nature of occupation

The placental lead levels were significantly higher ($p < 0.01$) among paintresses (0.70 µg/g) when compared with lithographers and transferrers (0.34 µg/g). Removal from consideration of one paintress with an exceptionally high lead value (1.70 µg/g) still gave an average value of 0.54 µg/g (Table 25). The placental lead levels from a miscellaneous group (0.25 µg/g) were still lower than lithographers and transferrers. In the miscellaneous group

Table 22

Placental lead and length of exposure from normal births (Pb, µg/g fresh wt. basis)

	Occupational Exposure		
	<1 year	1 - 3 years	>3 years
	0.29	0.31	0.08
	0.27	0.17	0.27
	0.55	0.21	0.28
	0.20	0.67	0.32
	0.24	0.50	0.40
	0.24	0.23	0.40
		0.38	0.21
		0.42	0.31
		0.32	0.30
		0.22	0.34
		0.28	0.11
		0.28	0.66
			1.70
			0.30
			0.20
			0.54
			0.46
			0.56
			0.62
			0.48
			0.57
			0.53
			0.49
			0.44
			0.22
			0.25
No. of samples	6	12	26
Pb (µg/g)	0.30	0.33	0.43
Standard Deviation	0.13	0.14	0.30
Standard Error	0.05	0.04	0.06

Table 23

Placental lead and length of exposure in normal births (Pb, µg/g fresh wt. basis)

	Occupational Exposure	
	3 - 6 years	>6 years
	0.28	0.08
	0.11	0.46
	0.66	0.40
	0.54	0.31
	0.57	0.30
	0.22	0.34
	0.27	1.70
	0.21	0.30
	0.20	0.32
	0.35	0.62
	0.40	0.48
	0.53	0.49
		0.44
		0.25
No. of samples	12	14
Pb (µg/g)	0.36	0.46
Standard Deviation	0.18	0.38
Standard Error	0.05	0.10

Table 24

Placental lead and mothers age in samples from normal births

(Pb, µg/g fresh wt. basis)

	Mothers Age		
	< 20 years	21 - 29 years	30 years
	0.31	0.08	0.21
	0.55	0.29	0.27
	0.41	0.27	0.35
	0.17	0.28	0.29
	0.20	0.32	1.70
	0.21	0.16	0.54
	0.29	0.27	0.56
	0.11	0.38	0.48
	0.67	0.40	0.44
	0.28	0.40	0.25
	0.22	0.23	
	0.24	0.21	
	0.24	0.31	
		0.30	
		0.37	
		0.42	
		0.34	
		0.66	
		0.30	
		0.20	
		0.32	
		0.46	
		0.62	
		0.50	
		0.57	
		0.53	
		0.49	
		0.22	
		0.28	
		0.23	
Samples	13	30	10
Arith. mean (Pb)	0.30 µg/g	0.35 µg/g	0.51 µg/g
Standard deviation	0.16	0.14	0.44
Standard error	0.04	0.03	0.14

Table 25

Placental lead in different occupations (Pb, µg/g fresh wt. basis)

	Lithographers	Transferrers	Paintress	Miscellaneous	
	0.27	0.08	0.40	Aerographer	0.29
	0.32	0.27	0.66	Colour	
	0.31	0.40	1.70	Glazier	0.38
	0.27	0.55	0.56	Selector	0.21
	0.42	0.48	0.62	Caster	0.30
	0.34	0.44	0.50	Lining	0.17
	0.30	0.22	0.49	Sorter	0.20
	0.67	0.25		Backstamper	0.21
	0.20			Print Room	0.29
	0.57			Enameller	0.11
	0.53			Dipping	
	0.28			House	0.32
	0.28			Warehouse	
	0.22			Cleaner	0.23
	0.24			Cuphandler	0.28
	0.24				
No. of Samples	16	8	7		12
Pb (µg/g)	0.34	0.34	0.70		0.25
Standard deviation	0.14	0.16	0.45		0.07
Standard error	0.03	0.06	0.17		0.02

Table 26

Placental lead in different occupations and length of occupational exposure

	Lithographer	Transferer	Paintress	Miscellaneous
Placental lead	0.34	0.34	0.70	0.25
Average no. of years of exposure	4.2	6.7	9.6	2.7
No. of samples	16	8	7	12

in seven cases (selector, caster, lining, sorter, backstamping, warehouse cleaner and cup handler) there is no exposure to lead at all.[256] The placental lead levels in these seven cases average 0.23 µg/g. In other five cases exposure to lead is minimal except in the case of aerographer (one sample only), where average blood lead levels (30 µg/100 ml) are even higher than for paintresses (24 µg/100 ml).[256] The higher placental lead levels in paintresses is due to higher lead exposure compared to most other occupations studied where lead exposure is minimal. The length of occupational exposures was also greater (9.6 years) among paintresses when compared to other groups (Table 26).

3.3.3 Placental lead levels in stillbirth samples

The placental lead levels in stillbirth samples were higher (0.45 µg/g) when compared with the control group (0.29 µg/g) (Tables 27, 20). The average age of mothers in this group was 26 years. There were no details of the nature of the mother's occupation in this group. In another group of normal birth samples where there were complications of the pregnancy (foetal distress) the average placental lead levels were similar (0.43 µg/g) to the levels obtained for stillbirths (Table 28). However, the average placental lead levels from malformed live births (Table 29) were similar (0.32 µg/g) when compared with the control group (0.29 µg/g). The average age of mothers (22 years) in this group seems to indicate a lower length of occupational exposure. In all the cases, where the nature of occupations were known (Table 29), exposure to lead was minimal or none at all.

In the kidney and liver samples from stillbirths, lead levels averaged 0.48 and 0.42 µg/g fresh weight basis respectively (Table 30). Reported stillbirth kidney levels range from 0.19[253]

Table 27

Placental lead in stillbirth samples

Age	Placental Lead	Remarks
27	1.43	Gestational age 32 weeks, males, 1.505 and 1.410 kg, Cardiac failure
26	0.15	Full term male, 345 g, Placental insufficiency
18	0.70	32 weeks, 1.430 kg, male, Intrauterine death
22	0.28	37 weeks, female, 2.720 kg, Haemorage into tumor
-	0.80	-
22	0.20	Cord around baby's neck
21	0.20	42 weeks male, 4.6 kg, Interauterine death
22	0.20	31 week male, 1.007 kg, Eclampsia
38	0.95	Previously one stillbirth, 1973. 2 miscarriages 3 normal pregnancies (all babies died soon after birth)

Table 27 continued

Age	Placental Lead	Remarks
-	0.50	-
24	0.30	Female, 1.580 kg, multiple congenital defects
-	0.26	-
28	0.26	Female, 36 weeks, 2.438 kg, Intrapartum anoxia
-	0.56	Female, 30/34 weeks, 1.730 kg
30	0.44	Female, 3.799 kg. Previous abortion (1973) and three normal pregnancies
28	0.37	Male, 35 weeks, 2.345 kg
-	0.51	Male, 30 weeks, 745 g
-	0.56	Female, 38 weeks, 2.485 kg
-	0.15	Female, Intrauterine death, 1.170 kg

No. of samples = 19

Placental lead = 0.45 µg/g

Standard deviation = 0.32

Standard error = 0.07

Average age = 26 years

Table 28

Placental lead in normal births where foetal distress was observed

Occupation	No. of years worked	Age	Pb (µg/g)
Paintress	2½	23	0.25
Tile Selector	3	20	0.50
Lithographer	3	18	0.24
Cold Printer	5 months	18	0.15
Lithographer	6	22	0.62
Lithographer	6	22	0.45
Paintress	9½	31	0.75
Paintress	15 months	24	0.50
Paintress	3	-	0.53
Transferer	5 weeks	22	0.27

No. of samples = 10

Placental Pb = 0.43 µg/g

Standard Deviation = 0.19

Standard Error = 0.06

Average Age = 22 years

Table 29

Placental lead in case of malformed live births

Malformation observed	Age	Occupation	Length of exposure	Placental lead
Hydrocephalic and spina bifida	22	Dipping House	1½ years	0.23
Anencephalic and spina bifida	30	-	-	0.25
Spina bifida	21	-	-	0.57
Anencephalic	23	Transferer	3 years	0.19
Anencephalic	24	-	-	0.41
Anencephalic	19	Rist Assembly	-	0.26
Anencephalic	19	-	-	0.24
Anencephalic	24	Typist	-	0.24
No fingers on left hand	20	Tile Selector	-	0.50

- = No information

No. of samples = 9

Pb = 0.32 µg/g

Average age = 22 years

to 0.64[257] µg/g wet weight basis and reported liver levels range from 0.59[247] to 0.68[257] µg/g wet weight basis.

Table 30

Lead levels in stillbirth kidney and liver samples (Pb, µg/g)

	Kidney	Liver
	0.51	0.23
	0.49	0.31
	0.69	0.35
	0.41	0.02
	0.38	0.36
	0.56	0.68
	0.36	0.43
	0.40	0.54
	0.67	1.12
	0.51	0.54
	0.30	0.08
Arithmetical mean	0.48 ± 0.13	0.42 ± 0.30

3.3.4 Lead levels in stillbirth bone samples

Lead levels in Stoke-on-Trent rib samples ranged from 0.73 to 3.75 µg/g wet weight basis, with an arithmatical mean of 2.01 µg/g. Ninety percent of the samples had lead levels more than 1 µg/g (Table 31). Lead levels in the skull bones ranged between 0.36 to 2.53 µg/g, with an average of 1.15 µg/g wet weight basis (Table 31).

The average wet wt./dry wt. and wet wt./ash wt. ratios for stillbirth rib samples were 1.85 and 3.23 respectively. In the skull bone samples, wet/dry and wet/ash weight ratios were 1.39 and 2.38 respectively.

In the cot death rib samples the moisture ratios were similar to the stillbirth rib samples. The wet/dry and wet /ash wt. ratios for cot death rib samples were 2.0 and 3.6 respectively. Thus the cot death rib samples were comparable to stillbirth rib samples.

Table 31

Lead levels in Stoke-on-Trent rib and skull bone samples (Pb, µg/g wet weight basis)

	Rib samples	Skull bone samples
	1.23	1.26
	2.21	1.27
	-	0.54
	1.89	-
	1.97	0.95
	3.75	2.53
	2.55	0.36
	1.41	-
	1.05	-
	0.73	1.55
	3.25	1.06
Arithmetical mean	2.01 ± 0.97	1.19 ± 0.67

Lead levels in cot death rib samples averaged 0.72 ± 0.46 µg/g wet weight basis (Table 32). Cadmium levels in cot death ribs and stillbirth ribs and skull bones (Tables 32, 33) were comparatively lower when compared with cadmium in ribs (5.2 µg/g wet weight basis) and vertebra (2.2 µg/g) bone samples reported by Bryce-Smith et al.[258]

Table 32

Lead and cadmium levels in cot death rib samples (μ g/g, wet weight basis)

	Pb	Cd
	1.50	0.070
	0.27	0.012
	1.71	0.01
	0.48	0.021
	0.41	0.01
	0.54	0.01
	0.66	0.01
	0.86	0.01
	0.43	0.01
	0.71	0.012
	0.82	0.018
	0.26	0.01
No. of samples	(12)	(12)
Arithmetical mean	0.72 ± 0.46	0.02 ± 0.02

Table 33

Cadmium levels in stillbirth rib and skull bone samples

	Ribs	Skull bones
	0.072	0.066
	0.070	0.044
	0.108	0.100
	0.108	0.090
	0.169	0.185
	0.121	0.075
	0.167	0.106
	0.219	
No. of samples	(8)	(7)
Arithmetical mean	0.128 ± 0.05	0.095 ± 0.04

Calcium and zinc levels in stillbirth rib bone samples averaged 34 ± 32 mg/g and 115 ± 37 µg/g wet weight basis respectively (Table 34). Calcium and zinc levels in cot death rib samples averaged 54 ± 11 mg/g and 111 ± 25 µg/g respectively (Table 34).

Table 34

Calcium and zinc levels in stillbirth and cot death rib samples

Ca(mg/g)		Zn(µg/g)	
stillbirths	cot deaths	stillbirths	cot deaths
6	55	77	151
26	51	109	123
27	72	121	108
42	42	145	76
7	75	174	116
107	65	136	143
19	45	112	107
34	40	46	69
	47	113	111
	55		85
	48		129
	49		108
No; of samples (8)	(12)	(9)	(12)
Arithmetical mean 34	54	115	111

Thus calcium levels are higher in cot death rib samples compared to stillbirth ribs, whereas zinc levels tend to be similar in both the cases.

3.3.5 Blood lead levels in Stoke-on-Trent occupationally exposed women

There was little variation between the lead levels in maternal blood samples taken just before birth (12 ± 5 µg/100 ml, 65 samples) whatever the maternal occupation or length of employment but when ante-natal blood samples (17 ± 7 µg/100 ml) are grouped, an occupational variation is apparent (Table 35). Similarly there was little variation in cord blood samples (10 ± 5 µg/100 ml, 65 samples). None of the maternal or cord blood samples had lead levels higher than 35 µg/100 ml.

A highly +ve significant correlation (r = +0.40) between lead levels in maternal and infant blood lead but insignificant correlation between maternal blood lead and placental lead (r = +0.16) were observed. A weak negative correlation (r = -0.20) was also observed between infant blood lead and placental lead (Figs. 12-14).

The maternal and cord blood levels from Stoke-on-Trent are similar to those found for occupationally unexposed population from Birmingham, where maternal and cord blood lead levels averaged 11 ± 6 µg/100 ml (43 samples) and 10 ± 5 µg/100 ml (43 samples) respectively.

Table 35

Antenatal blood in different occupation groups

	Transferer	Lithographer	Paintress
Pb µg/100 ml	12	19 ± 9	24 ± 9
No. of samples	(2)	(27)	(11)

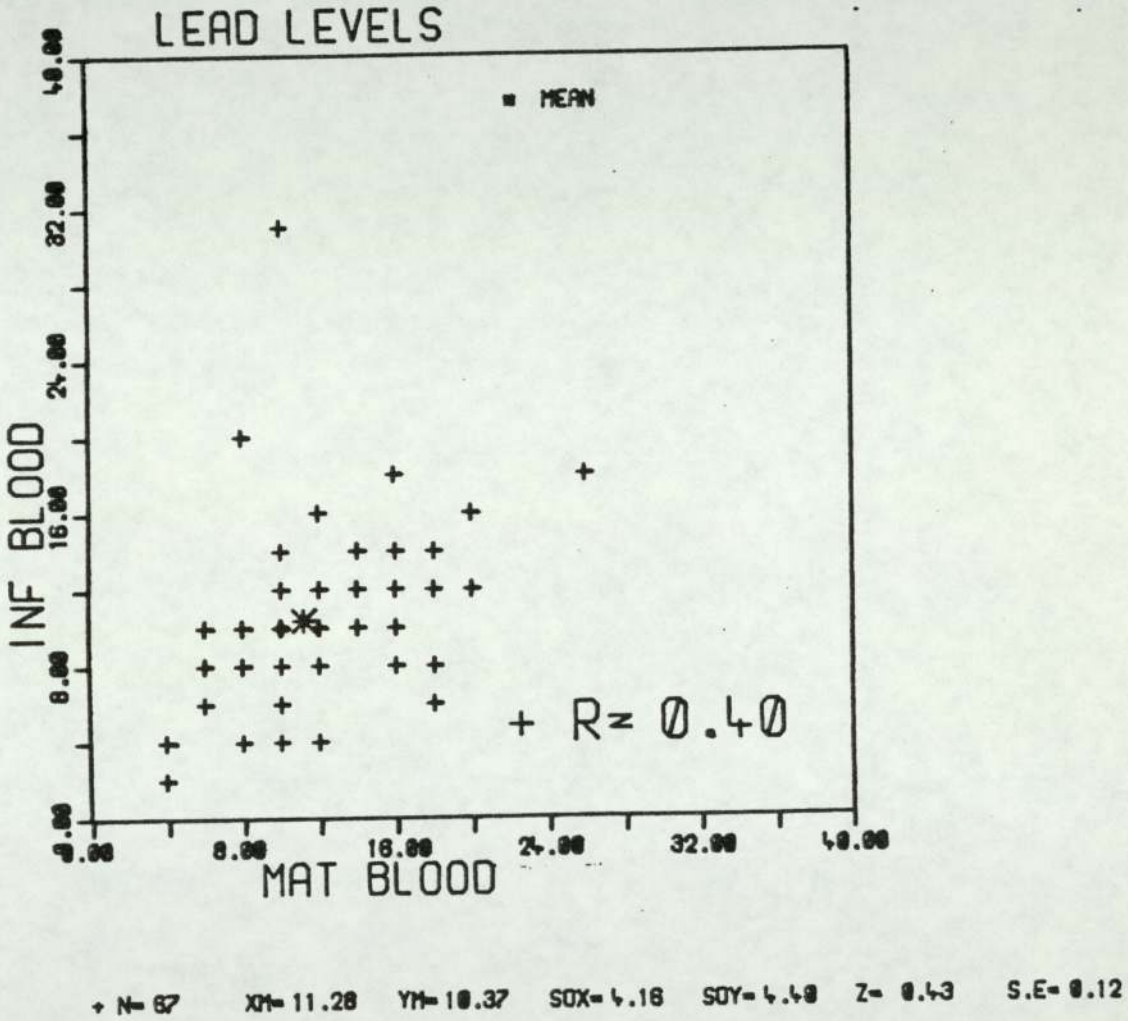


Figure 12:- Correlation between maternal blood lead and infant blood lead.

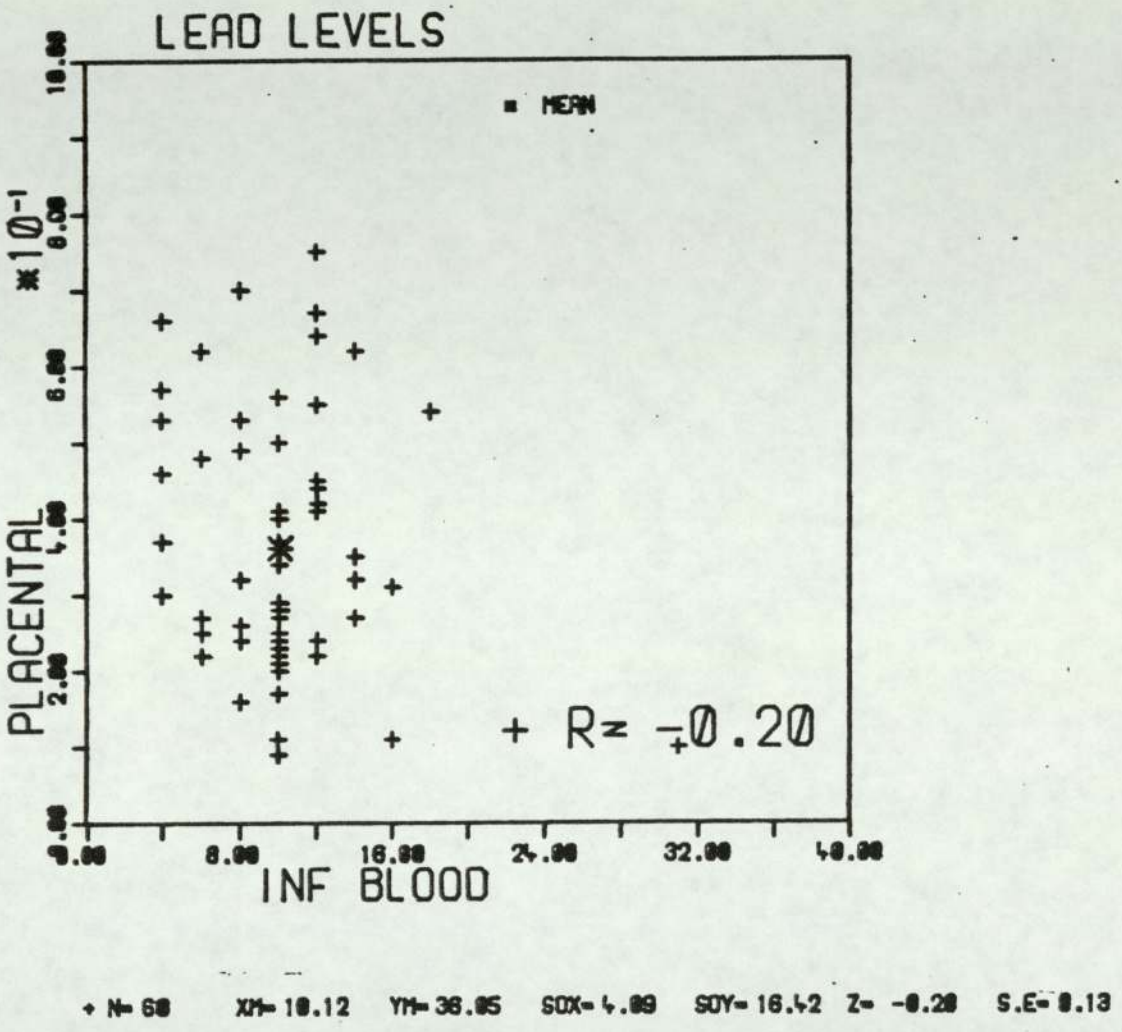


Figure 13:- Correlation between infant blood lead and placental lead.

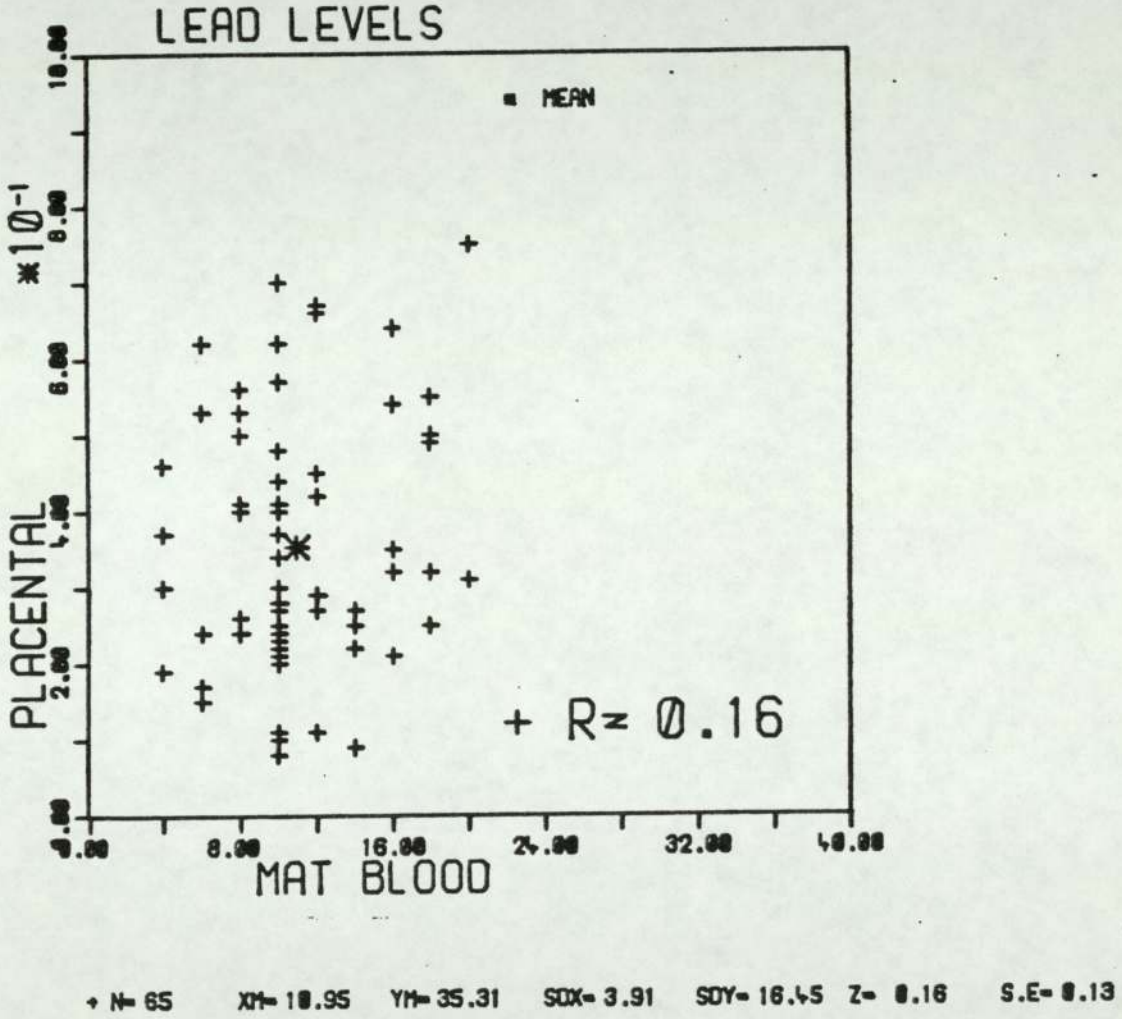


Figure 14:- Correlation between maternal blood lead and placental lead.

3.4 Overall results and general discussion

The fact that one can determine lead levels in human placenta and other foetal tissues does not necessarily mean that these levels are harmful. One of the main reasons that one can detect these levels is the rapid improvement in analytical techniques over the past 10 years. Thus caution must be exercised and reported ultra trace metal levels (<1 ppm) must not be considered as absolute levels or as if these were molecular weights of a particular tissue. Reported data on placental trace metals (Table 14) and placental lead (Table 21) further illustrates this point, as in many cases the difference in reported trace metal level is more than 100 times. Thus there is an urgent need for developing international lead standards for human placental matrix.

However, most trace metal studies become more useful if their levels are compared and conclusions drawn within its own group of samples.[246]

The literature contains several cases of foetal damage which appears to be directly due to the presence of abnormally high quantities of lead in the mother's environment.

Thus females who worked in lead industries before marriage miscarried three times more frequently than those who were housewives.[113] Reid[100] reported an average of 133.5 stillbirths and miscarriages in 100 women lead workers compared to 47.6 in women millworkers without lead exposure. Deaths under one year of age were 271 per 1000 live births of lead workers, 214 per 1000 of millworkers and 150 per 1000 of housewives.

In a study[114] on 104 Japanese women lead workers with blood lead ranging from 11.0 to 317 µg/100 ml, a detailed study of their pregnancy before and after beginning lead work shows an increase in spontaneous abortion to 84.2/1000 pregnancies from a prelead rate of 45.6/1000; the miscarriage rate for 75 comparable employees not exposed to lead was 59.1/1000 pregnancies. In contrast to this study, in the Rotherham epidemic of lead poisoning no increased foetal wastage was found.[259] In the Rotherham lead poisoning outbreak exposure was caused by the burning of battery cases. The exposure was over a shorter period of time than would be expected from industrial exposure. It is possible that chronic exposure has greater influence on foetal development than does short term relatively high dose exposure.

Measurable amounts of lead have been reported in stillbirth tissues despite the absence of any known maternal lead exposure. Thus, in a study by Kehoe et al.[260] four of five stillborn human foetuses showed the presence of small amounts of lead in their tissues. The lead was found in the bones of three, in the liver, brain and skin of two and in the intestinal tract of one.

Another study[257] on four stillbirths reported liver, kidney and femur lead levels as 0.68, 0.63 and 1.73 µg/g wet weight basis respectively.

Hansman and Perry[261] studied ten foetuses between 11 to 24 weeks of gestation using whole body ash analysis. They found eight of these foetuses to have measurable amounts of lead. The highest lead level was 27.7 µg/100 g. The mother of this

infant was suspected of being mildly lead-intoxicated. Horiuchi et al.[247] studied sixteen stillbirths, where deaths were due to maternal disease except lead poisoning, and reported bone, liver and kidney lead levels as 0.85 (16 samples), 0.65 (12 samples) and 0.32 (6 samples) µg/g wet weight basis respectively.

In cases where mothers were lead-poisoned, the foetal tissue lead levels were higher when compared with the above mentioned foetal tissue lead levels from mothers who were not excessively exposed to lead. Thus Karlog and Moller[262] have reported a case history of a mother poisoned with lead in which the stillbirth bone and liver lead levels were 21 µg/g wet weight basis in each tissue. Kidney and muscle samples were reported to contain 1 µg/g Pb wet weight basis in each case. The authors concluded that the foetal death was due to maternal lead poisoning. Similarly, Wilson[115] reported kidney and liver lead levels in a foetal death which was probably due to excessive maternal lead exposure through drinking water. The kidney and liver lead levels were 2 and 5.3 µg/g wet weight basis. In three other pregnancies where excessive lead intake from drinking water was a possibility, congenital defects (nystagmus, albinism and hemangioma) were observed in the offsprings.

Palmisano[116] reported a case of a 10 week old infant with evidence of neurological defects, intrauterine growth retardation and postnatal failure to thrive which was the conceptus of a 33 year old woman with a history of long term ingestion of lead through moonshine whisky.

Hickey[263] has also presented statistical evidence that deaths from congenital malformations are related to atmospheric lead concentrations.

Lanzola et al.[264] found mean lead levels in the vertebrae of 46 neonates, stillborns or dying in the first twenty days of life, which ranged from 43.7 µg/g in 1967 to 52.5 µg/g ash weight basis (≃ 20 µg/g wet weight basis). The stillbirth bone lead levels are close to the lowest level found in adults by these authors (56 µg/g ash weight basis). This emphasises the excessive exposure to which the stillborn infants were exposed and which resulted in bone lead concentrations higher by a factor of 12 or more than those found in normal babies.[265]

In another study on stillbirth bone lead levels, Bryce-Smith et al.[258] have reported rib and vertebra lead levels. Lead levels in ribs ranged between 0.4 to 24.2 µg/g wet weight basis with an average of 5.7 µg/g (26 samples). Lead levels in vertebra stillbirth samples ranged between 0.2 to 13.2 µg/g wet weight basis with an average of 2.9 µg/g (46 samples). These levels were 5-10 times higher compared to their control group (0.2 to 0.6 µg/g) which comprised of three cot deaths, one congenital heart disease, one viral pneumonia and one road accident.

The bone lead levels in stillbirths, reported by Lanzola et al.[264] and Bryce-Smith et al.[258] are higher when compared with our study (2.01 µg/g wet weight basis). However, stillbirth rib lead level in our study were higher when compared with our control group of ribs from cot and accidental deaths (0.72 µg/g).

3.4.1 Placental lead and length of occupational exposure to lead

Our studies from occupationally exposed population from Stoke-on-Trent showed that with longer periods of exposure there was an increase in placental lead values. Thus the placental lead levels were higher where the occupational exposure to lead was >3 years (0.42 µg/g) when compared with placental lead levels where occupational exposure to lead was <1 year (0.30 µg/g). Mothers in higher age groups also had higher placental lead. Thus placental lead values were 0.30, 0.35 and 0.51 µg/g where the mothers' average ages were 19, 26 and 33 years respectively. Mothers in higher age groups also have longer periods of occupational exposure than mothers in younger age groups.

However, with increasing age there is a possibility that more lead is also mobilised along with bone calcium during pregnancy.

Similarly, a heavy exposure was also reflected in higher placental lead levels. Thus, placental lead levels were significantly higher among paintresses (0.70 µg/g), where lead exposure is known to be high,[256] when compared with lithographers or transferrers (0.34 µg/g) where there is minimal exposure. In the miscellaneous group, where women are not exposed to lead, the placental lead values were 0.25 µg/g.

The studies on occupationally exposed women in the pottery industries show that placental lead levels are a reflection of lead exposure and maternal age. Data from Thieme et al.[242] also suggests that higher maternal lead exposure may result in higher placental lead levels. Thus for rural, urban and lead mining areas the placental lead levles were 0.10, 0.35 and 0.62 µg/g wet weight basis. Schaller,[255] however, did not find any relationship between lead content of human placenta and the degree of exposure to lead in the environment. On the otherhand, Roels et al.[266] found a slight but statistically significant effect of environmental lead pollution (urban and industrial > semi-rural > rural) on lead uptake by pregnant mothers.

These studies further confirm our observations that placenta does reflect, possibly because of its unique life time of several months, an integrated environmental and occupational exposure to lead besides the effects of maternal age.

3.4.2 Foeto-maternal blood lead relationship

If the placenta does not operate as a complete barrier for lead one would expect there to be some correlation between maternal and infant blood lead levels. Together with other workers we find this to be so. Average maternal (12 µg/100 ml) and cord blood lead levels (10 µg/100 ml) for occupationally exposed women were in the similar range as reported by many other workers for normal population. However, ante-natal blood lead levels were higher (17 µg/100 ml) than maternal blood taken at the time of birth.

There was a significant +ve correlation (r = +0.40) between maternal blood and cord blood further confirming that lead crosses

the placental barrier. There were no significant correlations between placenta and maternal blood and between placenta and cord blood. These observations thus suggest that the degree of lead exposure of the foetus depends directly on the level of exposure of the mother.

Harris and Holley[267] did not find any significant differences between the blood lead values found in the pregnant mothers and in their newborn offspring. In 24 maternal and cord bloods, lead levels averaged 12.3 and 13.2 µg/100 ml respectively. No determination was found to be above 20 µg/100 ml. These levels were lower when compared with infant blood lead levels reported by Robinson et al.[141] In their study the infants ranged in age from 5 hours to six days and average blood lead levels were 17 µg/100 ml.

Scanlon,[268] in 1971, reported the lead concentrations in cord blood of urban (13 samples) and suburban infants (15 samples) to be 22.1 and 18.3 µg/100 ml respectively. The difference was not considered to be statistically significant. Rajegowda et al.[269] determined cord blood determinations in 100 samples and reported a mean of 14.6 µg/100 ml. Kubasik et al.[270] determined 20 cord blood samples and average blood lead levels were 13.8 µg/100 ml. Th. Haas[271] studied 294 newborn infants and mothers blood lead levels. Infant blood lead levels (14.98 µg/100 ml) were significantly lower than maternal blood lead levels (16.89 µg/100 ml). They also found a +ve correlation (r = 0.538) between maternal blood lead and cord blood lead.

Ninety eight cord and maternal blood samples showed a high correlation (r = +0.6377) of lead levels in infants (10.1 µg/100 ml) and corresponding mothers (10.3 µg/100 ml).[272]

Zetterlund et al.[273] determined lead in 541 umbilical cord samples. The mean concentration of lead was 7.6 µg/100 ml. The blood lead values were also determined for 297 mothers with a mean of 8.7 µg/100 ml. There was a significant correlation between blood lead levels of the mother and the infant studied in 253 pairs (r = +0.62).

Clark[274] reported infant and maternal blood lead levels from a lead mining area in Zambia. The mean blood lead levels were 37 µg/100 ml and 41.2 µg/100 ml for infant and maternal blood (122 samples). These levels were significantly higher compared to their control population (31 samples) where infant and maternal blood lead levels were 11.8 and 14.7 µg/100 ml respectively. The control and lead mining area groups showed a significant correlation (r = 0.56 and 0.77 respectively) between lead concentrations in the blood of mothers and infants cords.

Ryu et al.,[275] Singh et al.[276] and Timpo et al.[277] have reported case histories of mothers and infants, where excessive maternal exposure was reflected in infants blood.

These studies further confirm our observation that lead crosses the placental barrier[278, 279] and may thus damage the foetus in the case of excessive maternal exposure.

3.4.3 Maternal lead exposure and foetal health

From the foregoing discussion it is clear that lead crosses the placental barrier and may thus damage the foetus. Recent studies by David et al.[80] and Needleman et al.[89] have shown that the learning abilities in children are related to lead exposure. This raises the possibility of the harmful effect

of excessive maternal lead exposure on the development of the foetal brain. In a study from Glasgow, Beattie et al.[280] reported that the probability of mental retardation was significantly increased where water lead in the homes, during pregnancy and later, exceeded 800 µg/l. The authors determined water lead levels in the homes of 77 mentally retarded children and 77 non-retarded matched controls and in the homes occupied by their mothers during pregnancy. The water lead content was significantly higher in the retarded group and the probability of mental retardation was significantly increased when water lead exceeded 800 µg/l. Blood lead levels were also significantly higher in the retarded group. The authors concluded that lead contamination of water and the higher intake of lead during pregnancy may be one of the factors in aetiology of mental retardation. However, this study was not designed to investigate the effect of pre-natal lead exposure on the development of mental abilities of the offspring. Thus there is an urgent need for further research into the effects of prenatal exposure of lead and its relationship to the subsequent learning abilities of the child. Such a study should involve isolating mothers with higher (i.e. with blood lead > 35 µg/100 ml) and lower lead burdens (i.e. with blood lead < 35 µg/100 ml). Infants' blood lead and mothers' placental and blood lead levels should then be determined to evaluate their environmental lead exposure and this could then be followed up by psychological tests of the child to correlate environmental lead exposure to the subsequent learning ability.

3.4.4 Association of lead with calcium in human placenta

In calcified human placenta it was observed that there was profuse calcification on the maternal side of placenta (figure 15) and in between placental cotyledons (figure 16). The calcified human placenta was further studied with Cambridge 150 Scanning Electron Microscope attached with 'Kevax' Energy Disperser. Figures (17-20) show calcium crystals in situ in human placenta. Figures 21 & 22 are of calcium crystals isolated from human placenta, whereas figures 23-26 are of non-calcified human placenta. The presence of calcium along with phosphorus was much higher in calcified placenta when compared with non-calcified placenta (figure 27).

Furthermore, isolated calcium crystals contained 20 ppm lead and 4% of calcium. Lead levels in non-calcified placenta range 0.2-0.3 ppm and calcium levels 400 ppm.

The chemical composition along with E.C.M. data confirm that lead is associated with calcium crystals in human placenta. There is also a possibility that calcium is returned to maternal circulation from foetus (as the data of low calcium in stillbirth bones suggests) and that lead is deposited along with it in placenta in the form of calcium granules. Thus the comparatively higher placental lead levels in stillbirth samples are possibly a reflection of calcium accumulation in placenta from foetus.

However, from an ethical point of view,[281-283] even in the absence of any direct causal evidence of the subclinical effects of lead, it is only prudent to ensure that wherever possible lead should be removed from the environment of the pregnant mother. For the women working in the lead industires the Health and Safety Commission,[284] has also proposed that: "In order to safeguard the developing foetus from lead (1) pregnant women should be suspended from lead work and (2) a woman of child bearing capacity should not be employed in lead works where her blood lead concentration exceeds 1.9 µmol/l" (Appendix 7).

Figure 15:- Calcium deposits on the maternal side of human placenta (magnification 6 times)

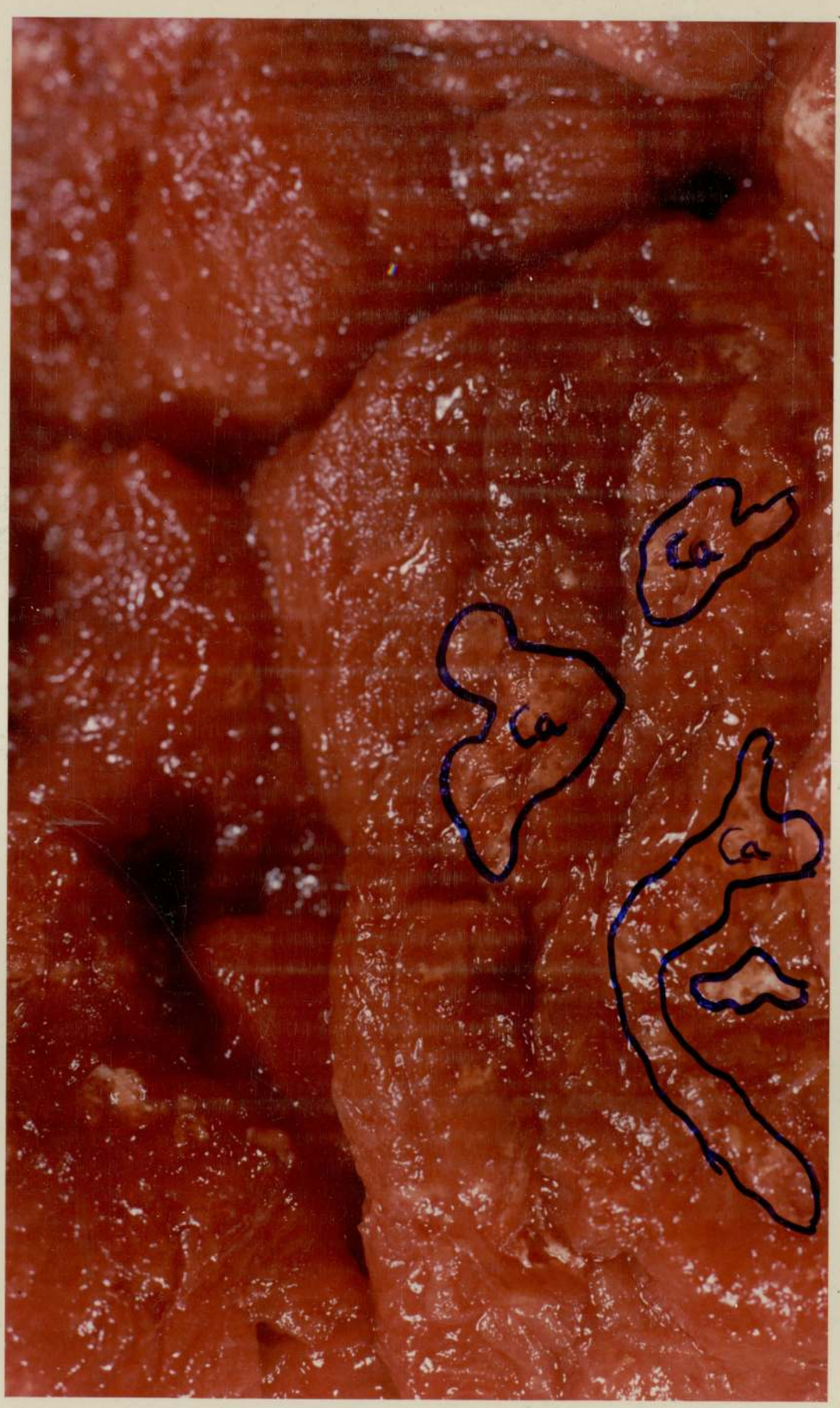


Figure 16:- Calcium deposits between placental cotyledons (magnification 6 times)

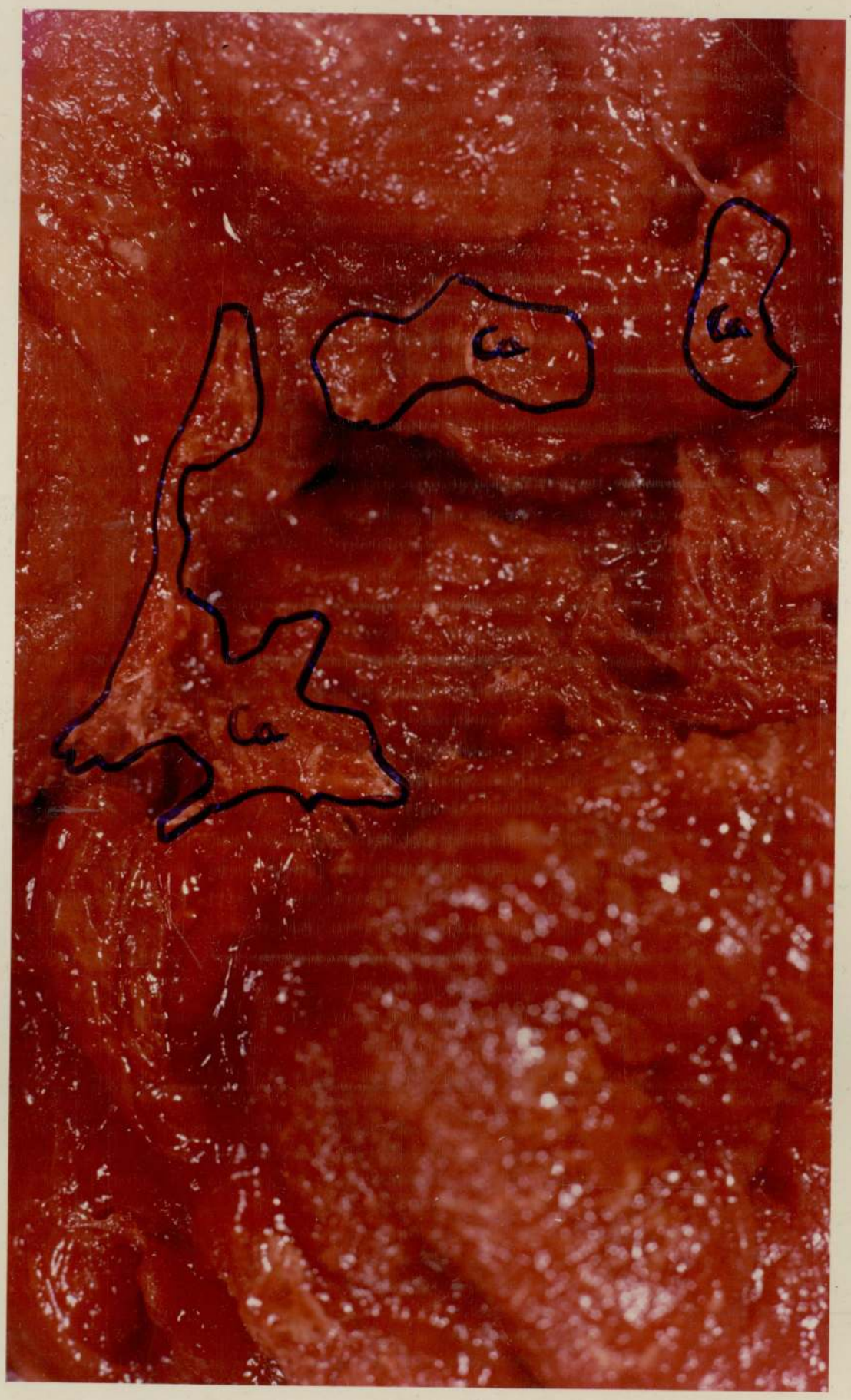


Figure 17:- Electron micrograph of human placenta showing in situ calcium deposition.

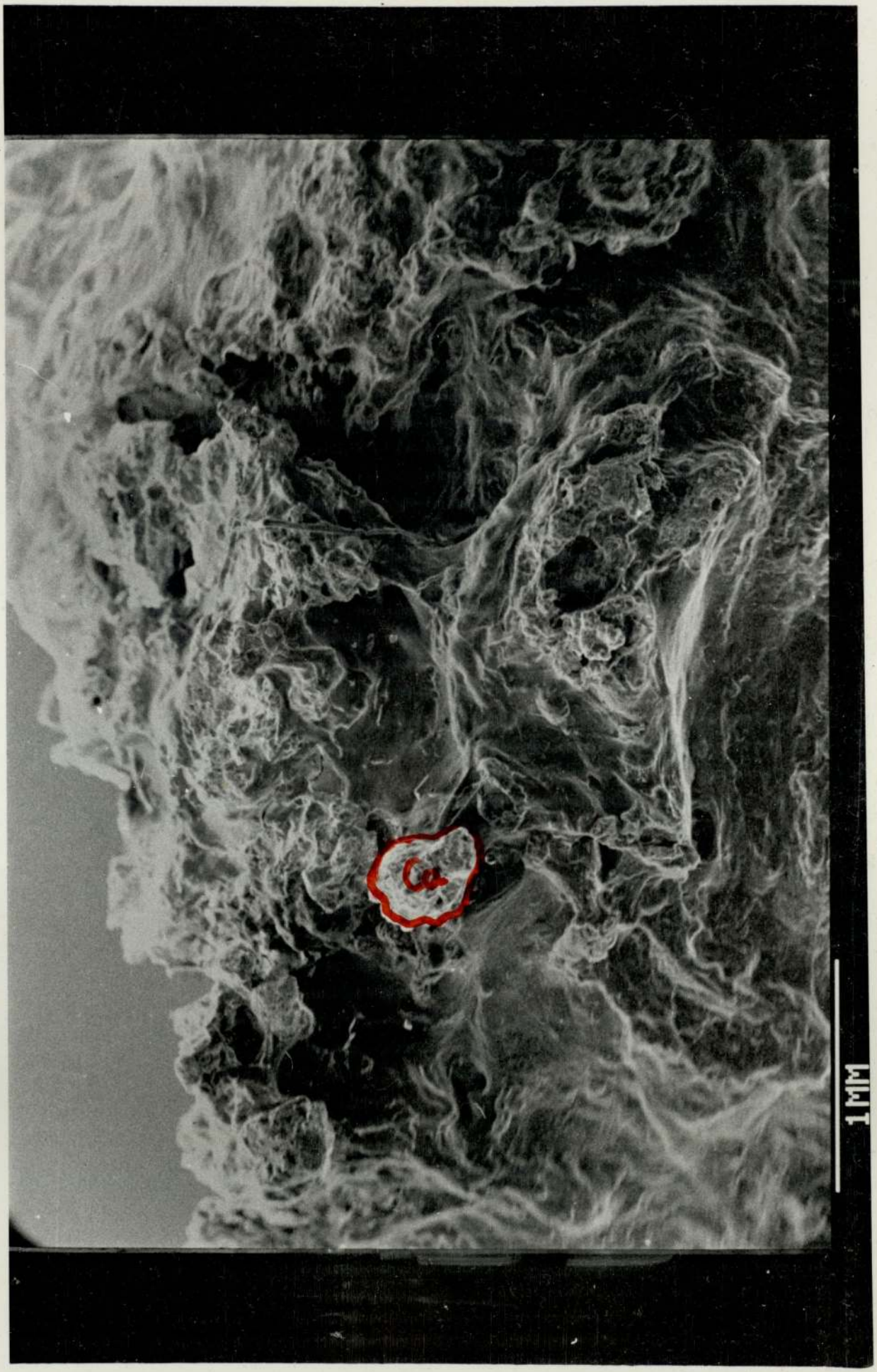


Figure 18:- Electron micrograph of human placenta showing in situ calcium deposition.

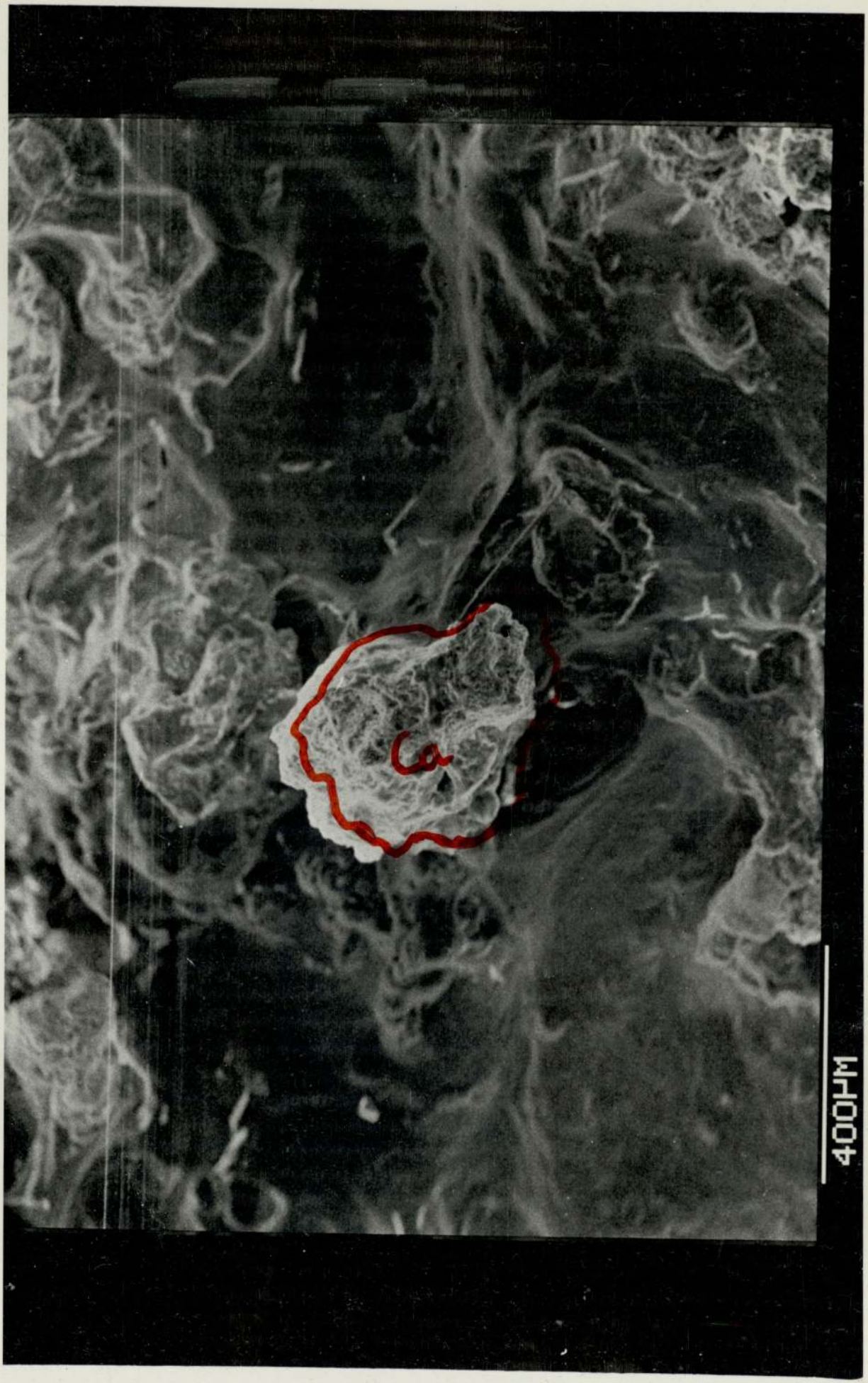


Figure 19:- Electron micrograph of human placenta showing in situ calcium deposition.

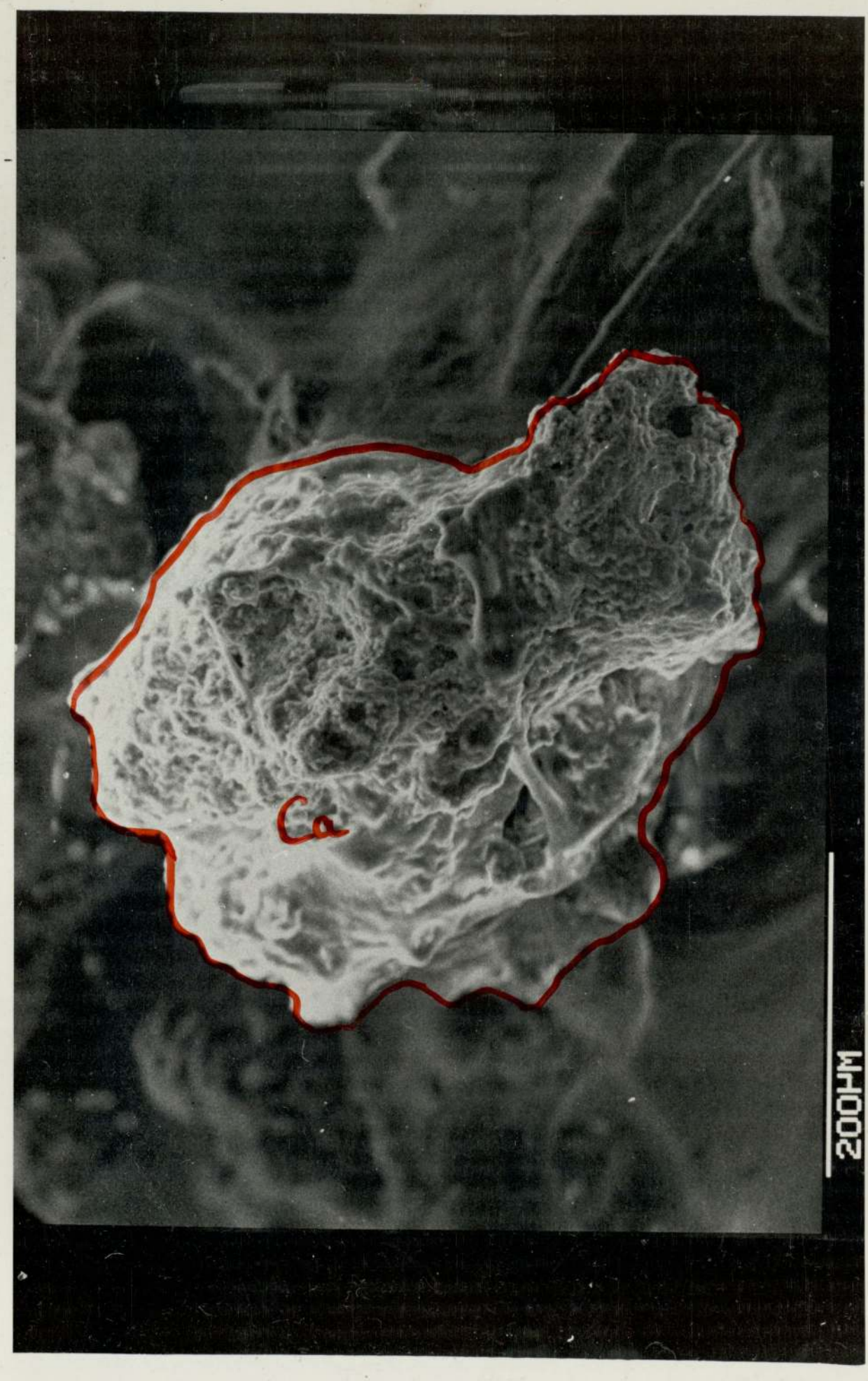


Figure 20:- Electron micrograph of human placenta showing in situ calcium deposition.

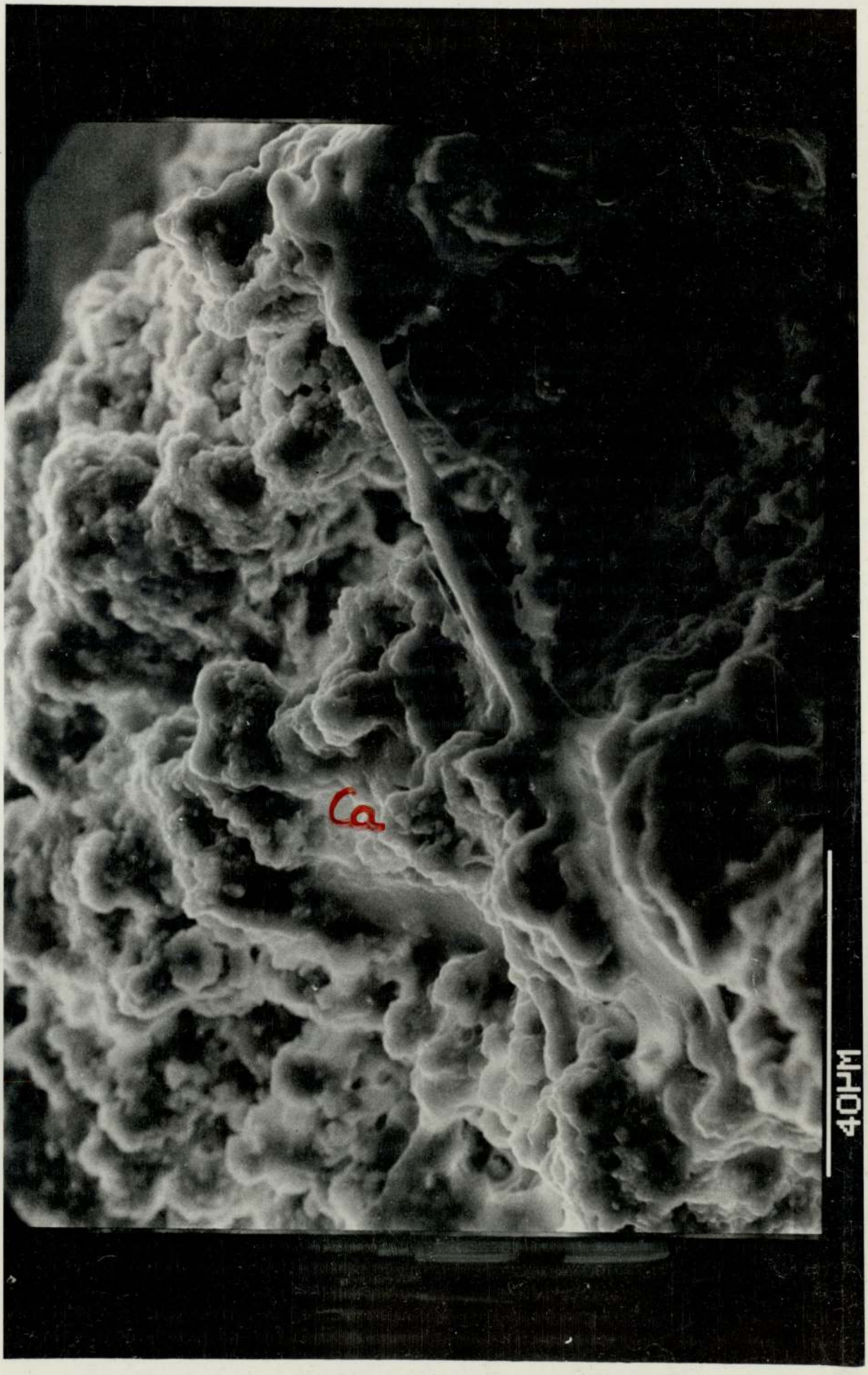


Figure 21:- Electron micrograph of an isolated calcium crystal from human placenta.

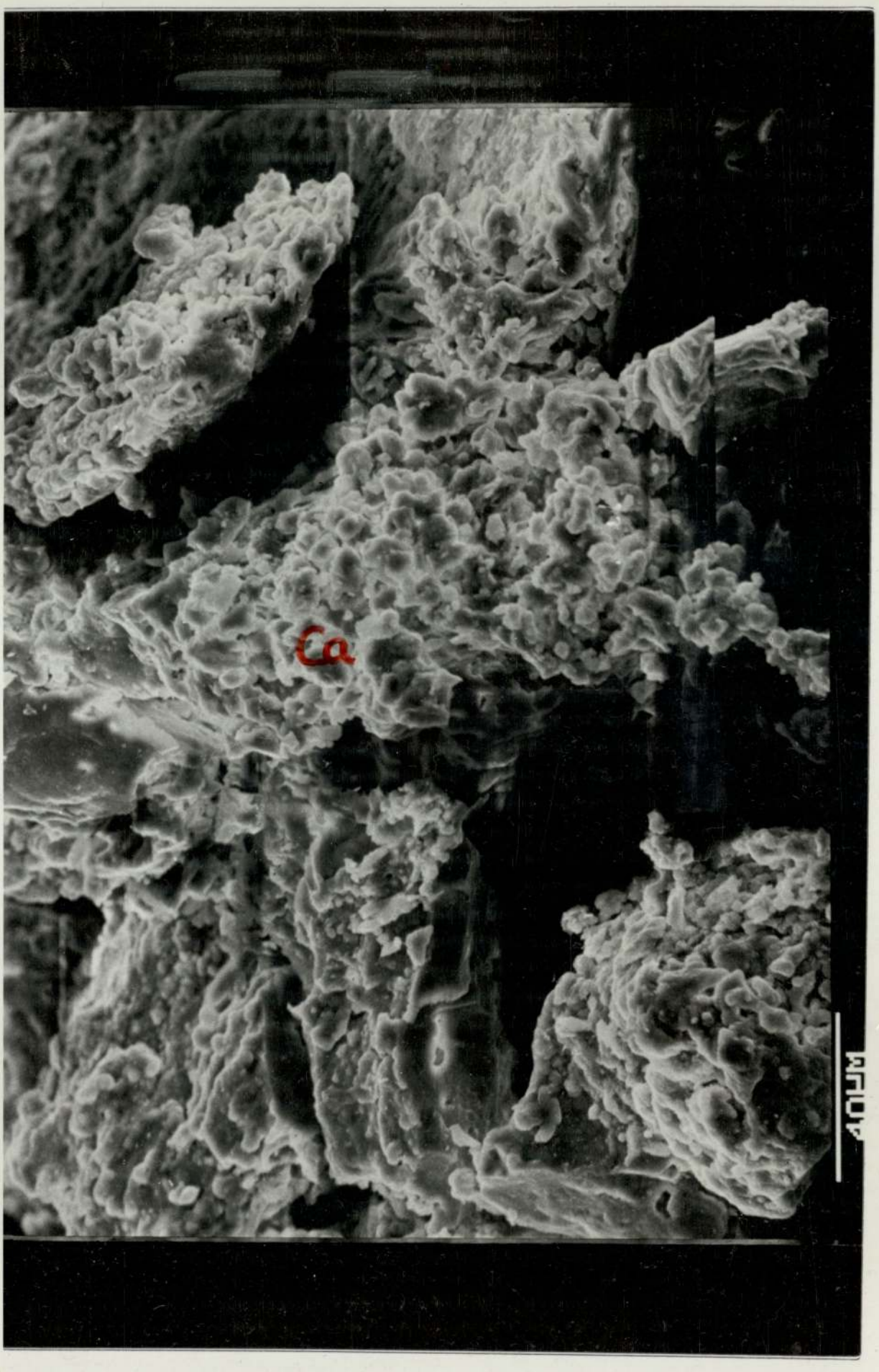


Figure 22:- Electron micrograph of an isolated calcium crystal from human placenta.

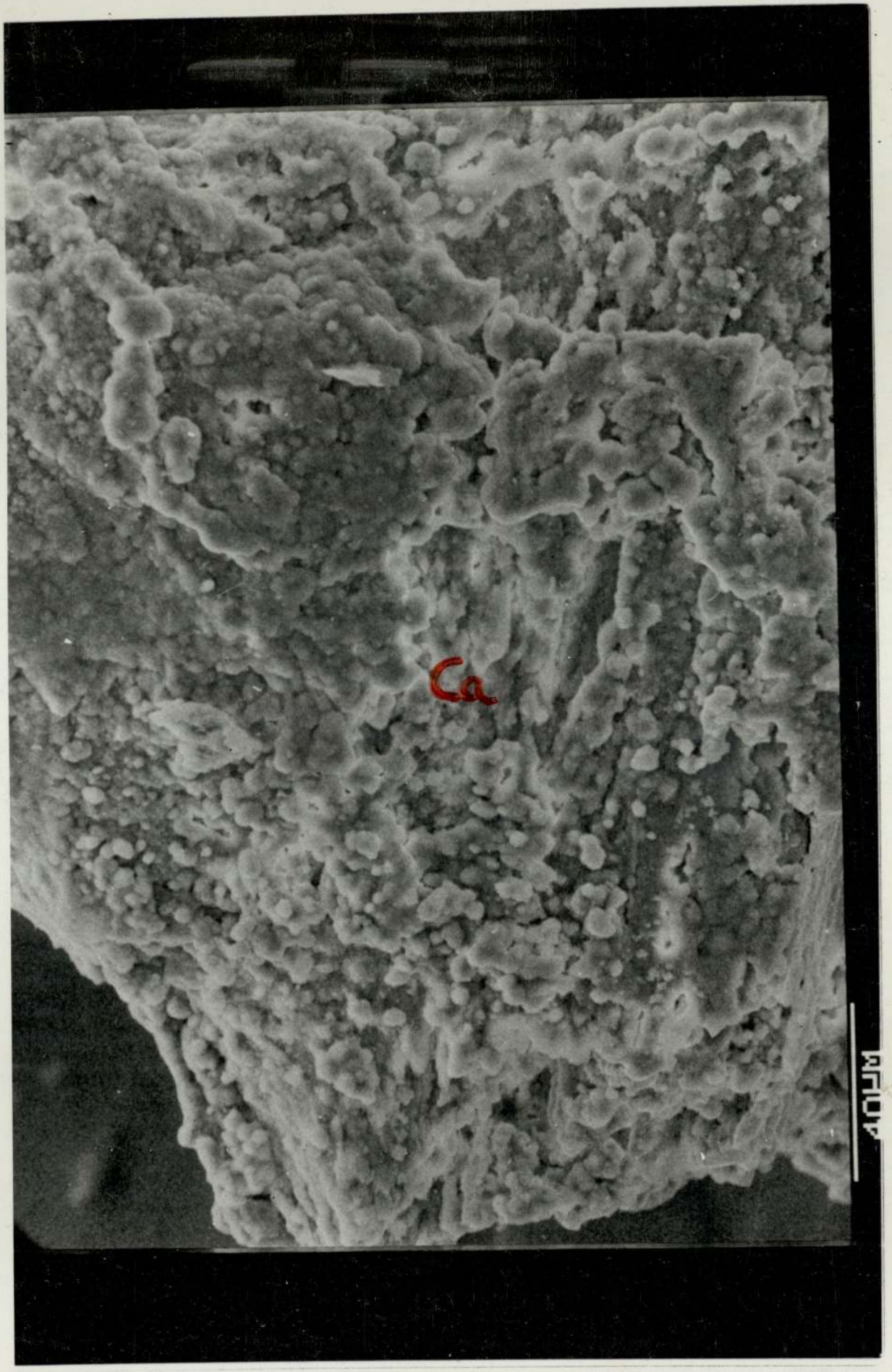


Figure 23:- Electron micrograph of a non-calcified human placenta.

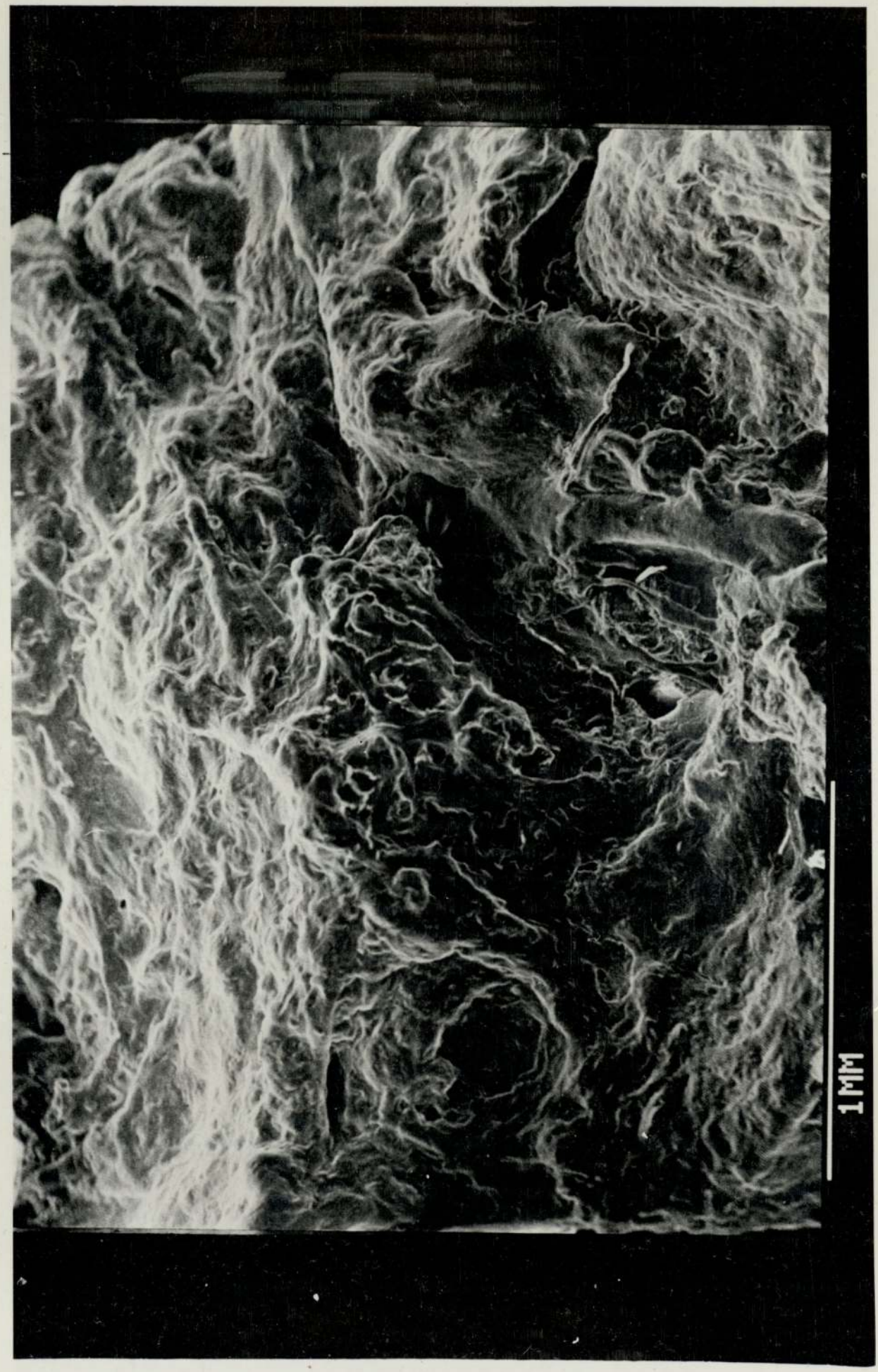


Figure 24:- Electron micrograph of a non-calcified human placenta.

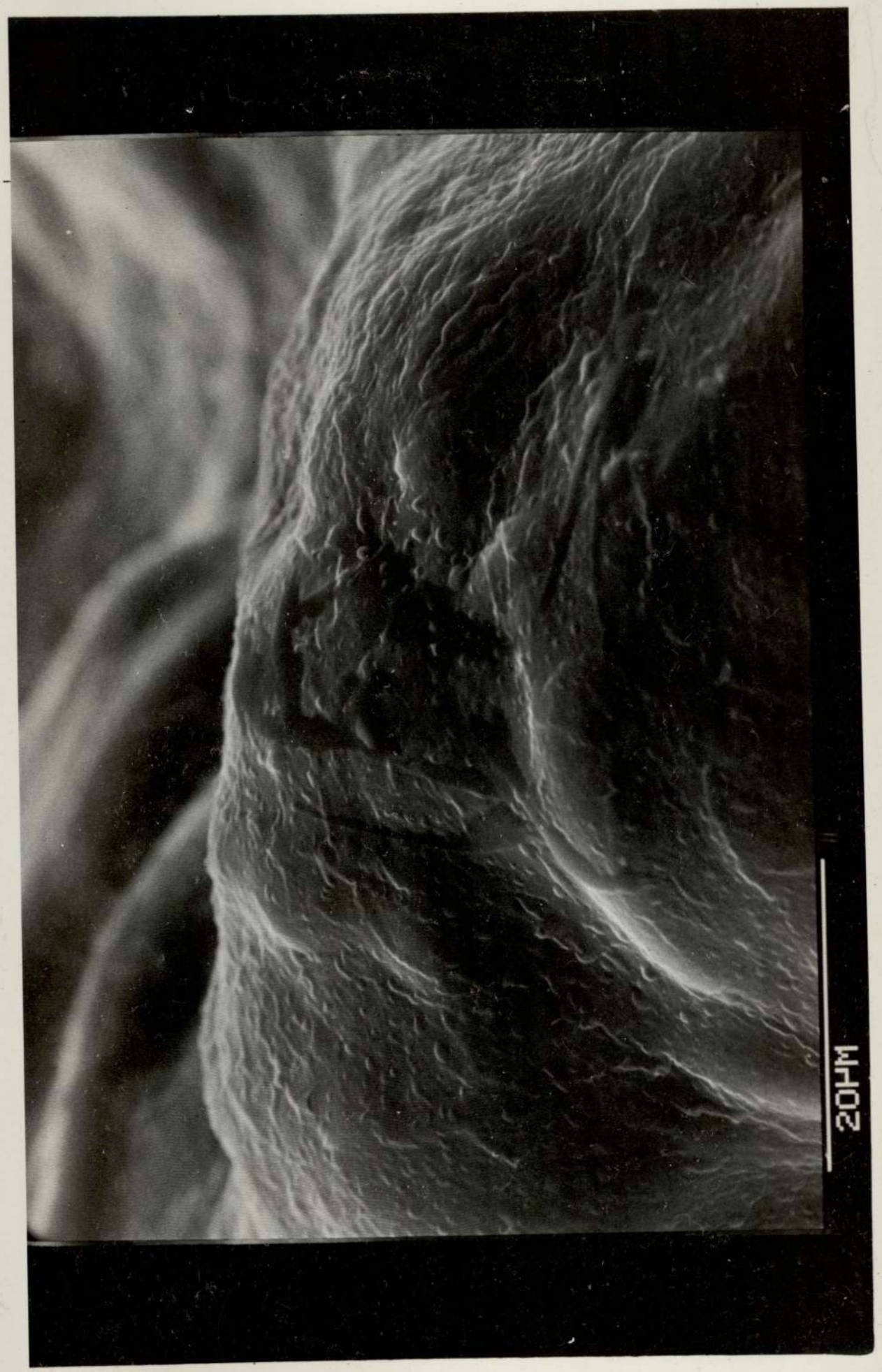
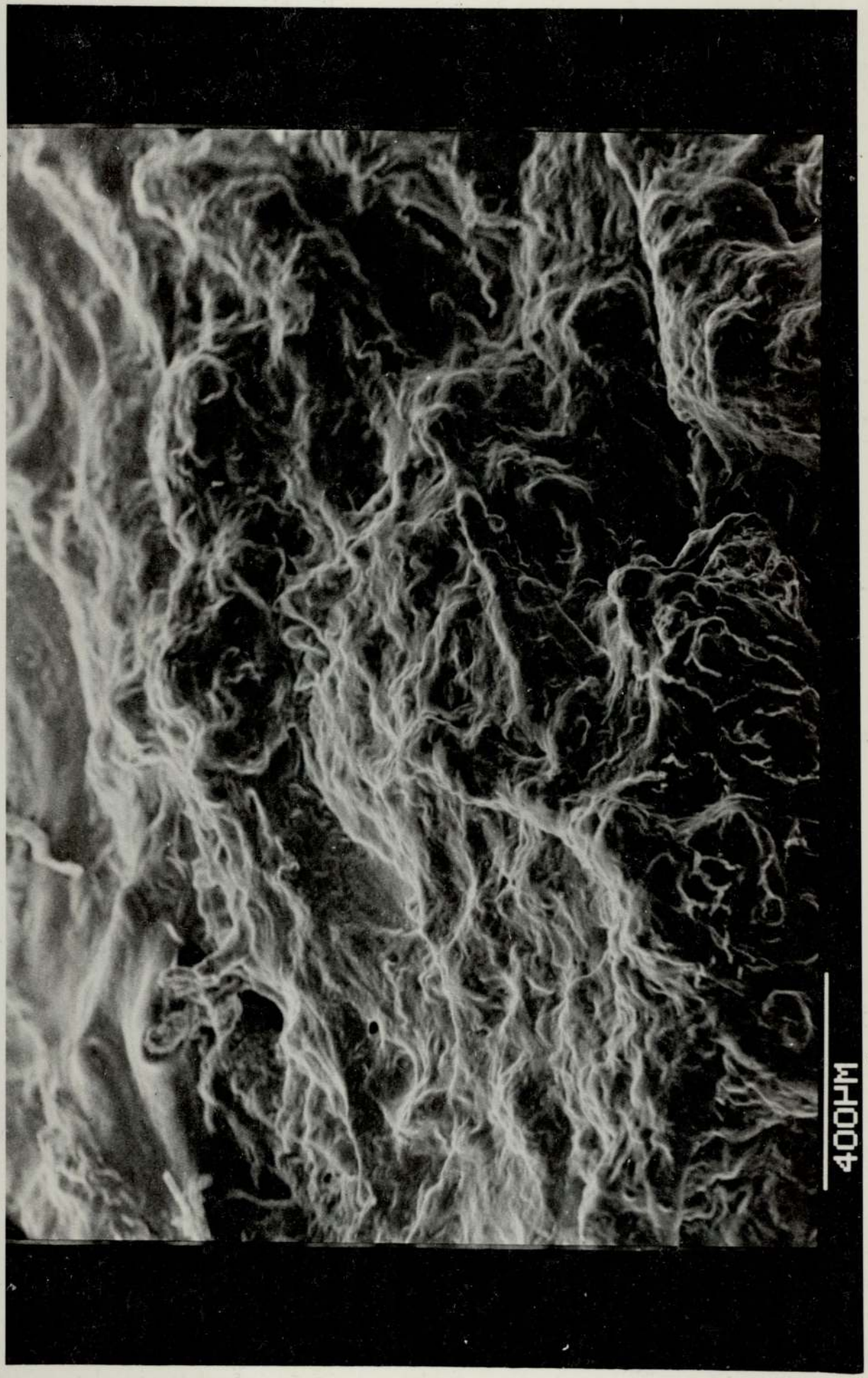


Figure 25:- Electron micrograph of a non-calcified human placenta.

Figure 26:- Electron micrograph of a non-calcified human placenta.



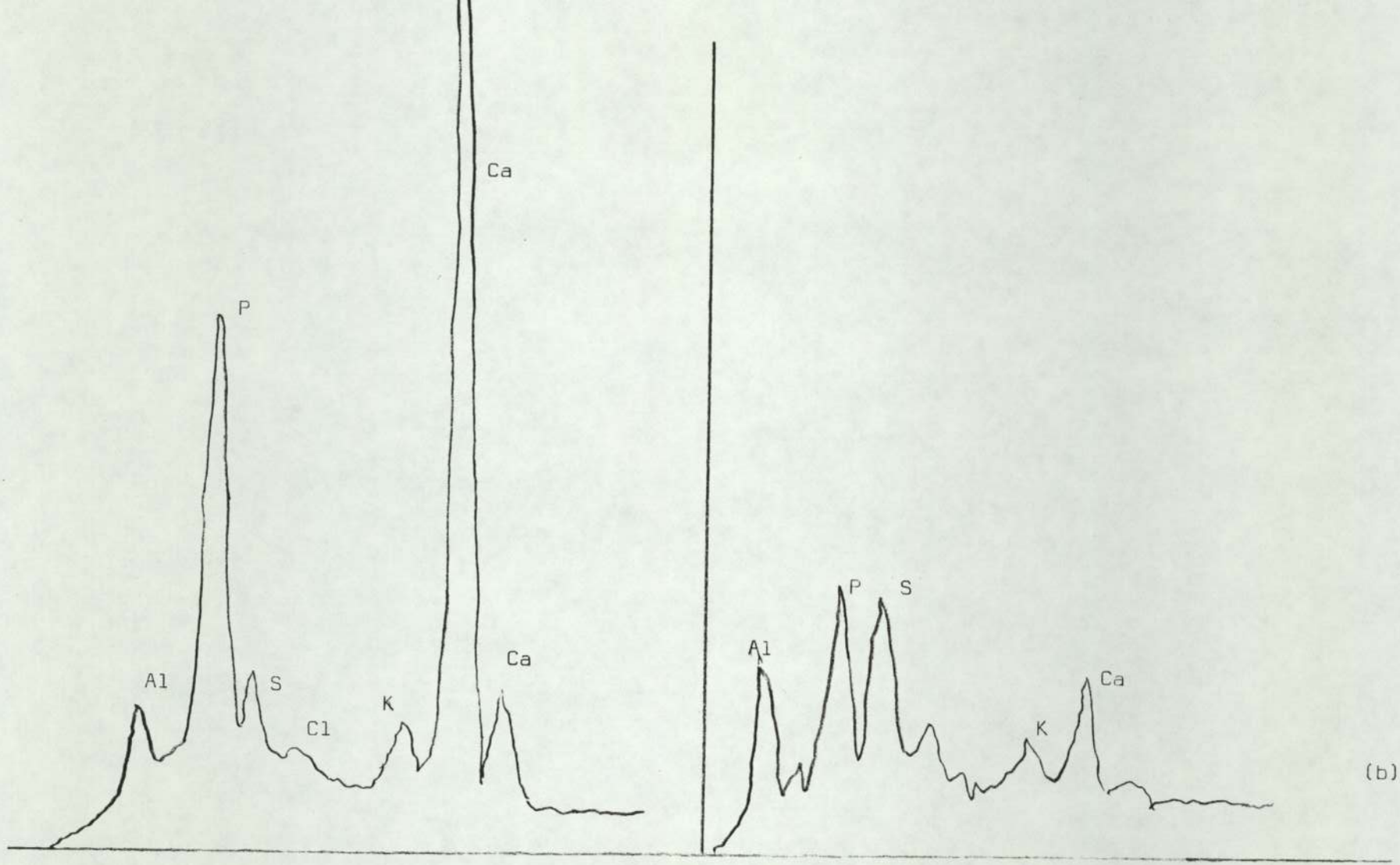


Figure 27:- Chemical composition of a calcified human placenta (a) and non calcified human placenta (b) as determined by scanning Electron Microscope attached with 'Kevax' Energy Disperser.

4. CONCLUSIONS

The placental lead levels seem to be associated with calcium deposition in human placenta. Comparatively higher lead levels in stored and fresh stillbirth samples when compared with normal birth samples are possibly due to higher calcium deposition as shown by low calcium in stillbirth bones and accumulation of calcium crystals in human placenta.

In occupationally exposed women comparatively higher placental lead is in cases where not only exposure to lead is for longer periods but mothers' average age is also higher. Thus, a possibility exists that, besides longer exposure period, during pregnancy more calcium and lead are mobilised in cases of mothers in higher age group.

There is no correlation between placental lead and maternal and cord blood lead. Significant positive correlations exist between maternal blood lead and cord blood lead. Thus it is more appropriate to measure maternal and cord blood lead levels to monitor environmental lead exposure to mother and foetus. The utility of placenta thus seems to be limited. However, as placenta does not offer a complete barrier to lead, in cases of excessive maternal exposure damage to foetus is possible.

5. APPENDICES

Appendix 1

Sample preparation using tissue solubilization by soluene-350 for lead determinations by graphite furnace atomic absorption spectrophotometry.

TECHNICAL NOTES

SAMPLE PREPARATION USING TISSUE SOLUBILIZATION BY SOLUENE — 350™ FOR LEAD DETERMINATIONS BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROPHOTOMETRY

P. J. Barlow and A. K. Khera
Departments of Building (Environmental Health Section) and Pharmacy
University of Aston in Birmingham
Gosta Green
Birmingham B4 7ET, United Kingdom

ITRODUCTION

Atomic absorption spectroscopy is perhaps the most suitble method for the determination of metals in animal tisues, and its use is widespread. The destruction of organic atter in animal and human tissues is a prerequisite for etal ion determination to prevent interference to the analis and also to release any metal ions that may be comexed with such tissues. The two common methods used for is purpose are dry ashing and wet oxidation with various ids. Both methods have the disadvantage that relatively rge quantities of sample material are required, and also ere is possibility of metal ion loss during the preparation age. Some workers have been able to prevent this loss but ly with specific samples and elements. Even with chelaon and extraction with APDC (ammonium pyrrolidine thiocarbamate) and MIBK (methyl isobutyl ketone) rectively, complete recovery is not always possible.

Considering these problems it was decided to investigate e use of Soluene - 350 (Packard Instrument Company d.), a quaternary ammonium hydroxide tissue solubiler specifically formulated for use with toluene and xylenesed scintillation counting solutions (1). The use of oluene - 100 has been reported for the determination of , Cu, Fe, and Mn in animal tissue by flame atomic abrption spectroscopy (2), and Soluene - 350 is reported be an improvement over Soluene - 100 (1). The use of unspecified quaternary ammonium hydroxide soluizer is also reported for flame determination of Cd, Ni, d Zn in rat lungs (3) and for flameless determination of , Pb, Cd, Mn, and Zn in human tissues (4).

XPERIMENTAL

The samples were analyzed on a Perkin-Elmer Model 360 omic absorption spectrophotometer fitted with a Deuteum Background Corrector and an HGA-74 Graphite Furce (Bodenseewerk Perkin-Elmer). The results were rded on a Perkin-Elmer Model 056 strip chart recorder. strument conditions are shown in Table I.

eagents

1) Soluene - 350
2) Toluene (AR)
3) Nitric acid (Aristar)
4) Lead nitrate (BDH standard for atomic absorption purposes)

andard Solutions

A stock solution of 100 ppm lead was prepared by addion of a suitable amount of lead nitrate to Soluene and arming to 55°C for approximately ten minutes to dissolve e precipitate formed on addition. Standards were prered on the day of analysis by adding suitable amounts of stock solution to 5 ml of Soluene and making up to 10 ml with toluene.

Glassware

All glassware was washed once with dilute nitric acid, twice with tap water, twice with deionized water, and finally rinsed with toluene and dried in an oven before use.

TABLE I
Instrumental Conditions

Wavelength	283.3 nm
Spectral slit width	0.7 nm
Gas flow	Argon with Mini flow/gas stop setting
Sample size	20 µl
Chart recorder	2mV full scale
Source	E.D.L. (Electrodeless Discharge Lamp, Perkin-Elmer)

Time and Temperature Settings

	Digit Setting	*Temp °C*	*Time (sec)*
Drying	84	60	60
Charring	297	450	30
Atomization	730	2100	10

Preparation of Samples

Samples were prepared by removing excess blood by means of cotton sponges, homogenizing in a rotary tissue homogenizer for approximately 5 minutes without the addition of water and preserved by freezing at −20°C. The preserved samples were then weighed out in 500-mg portions, the placental tissue being thawed before weighing. For liver, which becomes a viscous material after homogenization, the frozen tissue was used. The weighed samples were transferred to numbered 10-ml test tubes (research grade). Five ml of Soluene was added to each sample, and the test tubes were placed in a constant temperature water bath at 55°C. The placental tissue was completely dissolved in approximately four hr giving a clear solution, while the liver samples took longer (up to 24 hr). The solutions obtained varied in color from pale yellow to dark brown, the color being independent of blood content of the tissue. The dissolved samples were transferred to 10-ml volumetric flasks and made up to the mark with toluene. These samples were then used directly for lead determination by flameless atomic absorption spectroscopy.

During the sample preparation the following observations were also made:

a) At lower temperatures the dissolving power of Soluene was considerably reduced. Soluene did not dissolve placental tissue at room temperature even when kept overnight.

b) Under the specified conditions decidua vera and umbilical cord samples dissolved in approximately 2 hr.

TABLE III
Recovery of Lead Added to Liver and Placental Samples

Porcine Liver			*Human Placenta*		
Sample No.	*Added Lead (ppm)*	*Recovery %*	*Sample No.*	*Added Lead (ppm)*	*Recovery %*
3	0.1	98	108	0.1	100
8	0.2	104	100	0.2	90

) to 0.4 ml of water can be dissolved in 1 ml of Solu-e. This means stock standard solutions can be pre-red in Soluene.

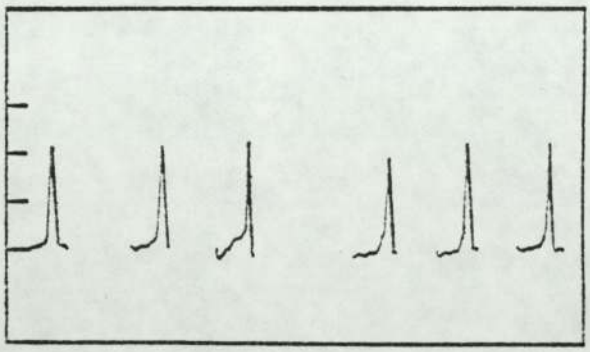
TS AND CONCLUSIONS

results for the lead content of the liver and placental es and the recovery of added lead are given in Tables III. Moisture content of fresh liver and placental es was 71% and 83% respectively.

TABLE II
Lead in Liver and Placental Samples
(μg/g Wet Weight)

Porcine Liver		*Human Placenta*	
ple No.		*Sample No.*	
1	2.55	98	1.60
2	2.63	99	1.40
4	2.28	100	1.30
5	2.14	101	1.30
11	1.97	102	1.40
12	2.15	103	1.40
ean	2.29		1.40
S.D.	10%		7.3%

reproducibility of the method is shown in Figure 1. 2 shows the linearity of the standard curve. No were observed for Soluene, toluene, and blank (1:1 e/toluene).



Precision for 0.1-ppm standard sample (2 ng/20 ul).

he above data and graphs show, Soluene - 350 seems an excellent material for the solubilization of liver acental tissue and possibly for many other kinds of The method provides a simple and reproducible ure for preparing a homogenous sample for direct absorption spectroscopy and has the following ad-es:

a) The method is rapid with few stages of handling involved thus reducing contamination.

b) Loss during sample preparation is much reduced or absent.

c) The organic-based solubilizer enhances the sensitivity for the metals to be investigated.

d) The same samples can be used for liquid scintillation (1) and for gas chromatography (5).

e) There is no loss of lead during storage at room temperature.

In this laboratory Soluene - 350 has also been shown to be useful for the solubilization of bird feathers and hair samples.

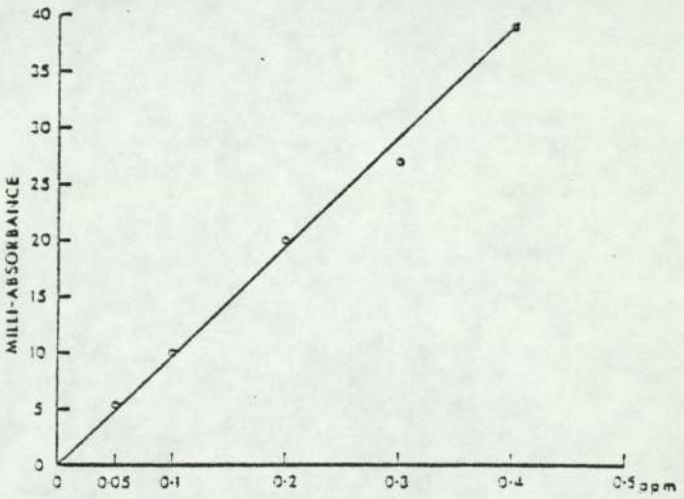


Fig. 2. Calibration graph for 0.00 to 0.40 ppm Pb.

ACKNOWLEDGMENT

A. K. Khera acknowledges support from the Department of Health and Social Security and the European Environmental Research Establishment.

Received September 19, 1975

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Appendix 2

Some analytical problems concerning trace metal analysis in human placentae.

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Some Analytical Problems Concerning Trace-metal Analysis in Human Placentae

A. K. Khera and D. G. Wibberley

Department of Pharmacy, University of Aston in Birmingham, Birmingham, B4 7ET

There are few reports on trace-metal analysis of human placentae. In the present paper, analytical problems concerning placental lead are described. Some preliminary work on placental cadmium is also reported.

Lead in Human Placentae

Lead is known to cross the placental barrier.[1–3] It is also known to cause various kinds of congenital anomalies in animals[4–8]; there are reports to suggest that it may cause sterility, abortion and congenital malformations in human beings[9–11] but these reports are not substantiated by lead analysis. However, the reported values of lead in normal human placentae are approximately 0.5–1.0 $\mu g\ g^{-1}$ on a fresh mass basis.[12–14]

In most of the work that has been reported determinations were made by using dry-ashing techniques, the samples being dried overnight, followed by ashing at 550 °C for 16 h, dissolving the ash with various acids and finally determining the metal contents by flame atomic-absorption spectroscopy. In a separate study we found that dry ashing at such high temperatures caused an excessive loss of lead, in agreement with the radiochemical studies for lead in blood and other biological materials.[15,16] In our studies, there were considerable losses of placental lead when the ashing temperature and duration were 500 °C and 22 h. There were complete losses of lead when the samples were ashed at 550 and 600 °C for 20 and 22 h, respectively.

It was also observed that excessive blood adhering to the placenta was also a cause of variations in results obtained. In all subsequent work excessive blood was therefore removed with cotton sponges. By use of this technique, oven-dried placentae produced a constant composition (16–18%, *m/m*). Further work was then attempted with wet-acid digestions. With these procedures complete recovery was not always possible.

On considering these problems, it was decided to investigate the use of Soluene-350, a quaternary ammonium hydroxide tissue solubiliser specifically formulated with toluene. This product is manufactured by Packard Instrument Company Ltd.[17] Soluene-100 has been used[18] for the determination of zinc, copper, iron and manganese in animal tissues by flame atomic-absorption spectroscopy. Determinations of cadmium, nickel and zinc in rat lungs by flame atomic-absorption spectroscopy and using an unspecified quaternary ammonium hydroxide have also been reported.[19]

Placenta samples in Soluene were prepared in the following manner. Excessive blood was removed by means of cotton sponges, the samples were homogenised in a tissue homogeniser for approximately 5 min, without addition of water, and preserved by storing at −20 °C. The preserved samples were thawed and weighed out in 500-mg portions into glass test-tubes.

portions (5 ml) of Soluene were added to each tube and the samples warmed in a water-bath for approximately 4 h. The solubilised samples were then diluted to 10 ml with toluene. The blank and standard samples were also prepared in Soluene and toluene.

The samples thus prepared were analysed directly on a Perkin-Elmer Model 360 atomic-absorption spectrophotometer fitted with a deuterium background corrector and an HGA 74 graphite furnace. The experimental conditions used have been reported.[20] The analytical results obtained for lead levels in normal human placentae are given in Table I.

TABLE I

LEAD IN NORMAL HUMAN PLACENTAE

Placenta	Lead/ $\mu g\ g^{-1}$ (fresh mass)
5	0.57
6	0.48
7	0.37
8	0.37
9	0.59
10	0.36
11	0.15
12	1.40
13	0.89
14	0.17
25	0.63
26	0.62
27	0.82
28	1.32
29	0.44

Arithmetic mean, 0.63
Range, 0.15–1.40
Standard deviation, 0.38

Placenta samples in Soluene were sent to other laboratories for confirmation of the results. The results obtained were in close agreement with the results shown here.

The Soluene method for placental analysis appears to have the following advantages.

(*i*) The method is rapid. Few stages of sampling are involved and there is less chance of sample loss or contamination.

(*ii*) There is no loss of lead at room temperature over at least a 3-month storage period.

The technique was not successful when oven-dried or ashed placental samples were used.

Cadmium in Human Placentae

For similar reasons to those described for the determination of lead, dry ashing and wet-acid digestions were not suitable for the determination of cadmium. Preliminary work indicated the presence of cadmium as a contaminant in Soluene, but its absence in aqueous tetramethyl ammonium hydroxide. The results for placental cadmium obtained by using aqueous tetramethyl ammonium hydroxide, 0.02 $\mu g\ g^{-1}$ of cadmium (fresh mass), are close to the reported cadmium levels in normal human placentae. Further work on the details of the determination of cadmium is continuing at the present time.

The present work was carried out under contract No. 8304 of the E. C. Environmental Research Programme. The authors acknowledge financial support.

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Detection Limits and Trace Analysis

The following are summaries of three of the papers presented at a Joint Meeting of the Atomic Spectroscopy Group with the Modern Methods of Analysis Group of the Sheffield Metallurgical and Engineering Society and the Spectroscopy Group of the Institute of Physics held on April 22nd and 23rd, 1976.

Monitoring Residual Elements in Steels by Atomic-absorption Spectroscopy

M. S. Taylor

Firth Brown Ltd., Atlas Works, P.O. Box 114, *Sheffield, S4 7US*

The stature and significance of "residual elements" present in steels and related alloys continue to grow with increasing knowledge of their individual and collective effects upon the ultimate performance of metallurgical products under commercial conditions. Where materials have to operate satisfactorily in high-duty applications, be it either in a hostile environment, or under extremes of temperature and stress, there are valid metallurgical reasons for controlling both the nature and concentration of certain elements within very close tolerances. It is in this area that atomic-absorption spectroscopy has proved to be particularly valuable.

In our laboratories, this technique is used extensively for both routine and research purposes to monitor a range of elements at residual concentrations, which are not otherwise readily evaluated by the more conventional quality control processes. These elements include calcium, magnesium, zinc, lead, bismuth, silver and antimony. In addition to these elements, but for rather different reasons, nickel has now also become of interest.

Optimum instrumental parameters used to determine all of these elements are given in Table I.

TABLE I
OPTIMUM INSTRUMENTAL PARAMETERS

	Element							
Parameter	Ag	Bi	Ca	Mg	Ni	Pb	Sb	Zn
Wavelength/nm	328.1	223.1	422.7	285.2	352.5	217.0	217.6	213.8
Slit width/μm	50	100	100	50	50	200	100	100
Lamp current/mA	4	8	7	5	5	5	8	6
Observation height/mm	5	7	5	5	4	7	10	7
Fuel	C_2H_2	C_2H_2	C_2H_2	C_2H_2	C_2H_2	C_2H_2	C_2H_2	Natural gas
Support gas	Air	Air	N_2O	Air	Air	Air	Air	Air

Calcium, Magnesium, Zinc and Nickel

These elements are determined by using a sample solution loading of 1% *m*/*V*. Calcium and magnesium are determined together in 5% *V*/*V* hydrochloric acid containing 0.2% *m*/*V* of

Appendix 3

Lead levels in human placentae from normal and malformed births.

Journal of Medical Genetics, 1977, 14, 339-345

Lead levels in human placentae from normal and malformed births

D. G. WIBBERLEY, A. K. KHERA, J. H. EDWARDS, AND D. I. RUSHTON

From the Department of Pharmacy, University of Aston in Birmingham, and Birmingham Maternity Hospital and University of Birmingham, Birmingham

SUMMARY Placental lead levels were studied in a series of Birmingham births classified by stillbirth, neonatal death, or survival beyond one week. There was an appreciable range of lead levels even in normal births (0·15-3·56 μg/g) but nevertheless average results showed a pronounced excess of lead in those who failed to survive both birth and the neonatal period. There was no association of placental lead with impaired birthweight among survivors but, in common with other authors, we noted a seasonal variation. The placentae from Indian women had similar lead levels to those from European women and lower values were found in the normal sibs of stillbirths and neonatal deaths. The possibility is discussed that under conditions of impaired fetal health in late pregnancy the placenta may concentrate lead.

The possibility exists that current environmental levels of heavy metals such as lead, cadmium, and mercury may present a hazard to the fetus. It is undoubtedly true that exposure of the mother to abnormally high levels of any of these metals can result in fetal death or abnormality. The best documented cases are those associated with mercury, and in particular the Minnemata Bay (Matsumota *et al.*, 1965) and Iraq incidents (Bakir *et al.*, 1973), but in addition several workers (Cantarow and Trumper, 1944) have shown the increased liability to abortion of women employed in industries with a high exposure to lead. The presence of lead in human fetal tissues has been reported and high levels of lead in bone have been reported in two studies of stillbirths (Lanzola *et al.*, 1973).

All three metals have been shown to be capable of crossing the placental barrier in animal studies and to cause fetal malformations and deaths (Ferm and Carpenter, 1967), and both synergistic and antagonistic reactions of lead (Ferm, 1969) and cadmium are known (Holmberg and Ferm, 1969). In view of such evidence it seemed to us that if current environmental levels of these heavy metals is in any way responsible, or partly responsible, for fetal abnormality then one would expect a correlation between the heavy metal level in placental tissue and some malformations. To examine this assumption we have embarked upon a detailed analysis of heavy metals in the placentae of stillbirths, normal births, and abnormal births. There are relatively few published reports (Table 1), and no previous workers have attempted this type of correlation. The present report describes our initial findings with respect to lead.

Table 1 *Reported metal levels in normal human placentae*

Author	*Country*	*Year*	*Metal level (μg/g fresh wt).*		
			Pb	*Cd*	*Hg*
Baglan *et al.*	U.S.A.	1974	0·30	0·017	—
Collucci *et al.*	U.S.A.	1973	0·96	0·068	—
Einbrodt *et al.*	Germany	1973	0·56	—	—
Horiuchi *et al.*	Japan	1959	0·57	—	—
Suzuki *et al.*	Japan	1971	—	—	0·071
Thieme *et al.*	Germany	1974	0·40	—	—
Thûrauf *et al.*	Germany	1975	—	0·07	—

Materials and methods

Placental samples from births at the Birmingham Maternity Hospital have been stored at −20°C for the past six years. In the present work 1971 samples were studied from the 1st week of each month and it was shown that the moisture levels (mean level 79·6%) compared favourably with those of fresh samples (80%).

Placental samples were obtained from:

(1) All neonatal deaths and stillborn births.

Received for publication 22 March 1977

(2) The next two normal births whose mothers did not have Indian names.

(3) The next birth to a mother with an Indian name.

(4) Placentae from sibs of all those births were then obtained using hospital numbers of mothers on an ordered computer listing which printed out only women who delivered at the hospital more than once since 1970.

(5) Finally, a series of 24 premature normal births (less than 2·5 kg) was defined by a consecutive series with non-Indian names in 1971.

The samples were thawed, excessive blood removed, the tissue dissolved in Soluene, and the lead estimated by flameless atomic absorption spectroscopy. We have described our solubilisation procedure (Barlow and Khera, 1975) and the problems concerning trace metal analyses in analytical papers (Khera and Wibberley, 1976). Results have been checked by double blind experiments in our laboratories and by assays in other laboratories by alternative procedures. Extremely high attention to environmental contamination was required to ensure reproducible results.

Results

The lead levels in 126 analyses of placental samples are recorded in Tables 2-9. There is an appreciable variation between the lead levels in samples from normal births to non-Indian women whom we will refer to as European (Table 3, mean level 0·93) and those in samples from malformations (Table 4, mean level 1·49), neonatal deaths (Table 5, mean value 1·73), or stillbirths (Table 6, mean value 1·45). There was no clear relation with cause of death; in fact the three highest values included the rare condition of trisomy 18 (about 1 in 3000 births), a ring D chromosome, and a congenital cerebral tumour: both the latter conditions are of exceptional rarity (less than 1 in 50 000 births).

Another group of samples were those from Asian mothers from the Indian subcontinent (i.e. India, Pakistan, and Bangladesh) and these showed average levels of 1·13 μg/g. Our 1971 results also appear to show a seasonal variation in lead levels in normal births with the lowest levels occurring in May for the European births and in September for the Asian births. Dawson *et al.* (1968) and Baglan *et al.* (1974) have both reported seasonal variations with minimum levels in summer months. If placental lead is solely an indicator of maternal exposure then one would have expected sibs to have closely similar levels. This has not so far proved to be the case (Table 7).

In order to evaluate the possible contribution of low birthweight to both high lead levels and stillbirth or neonatal death, a series of placentae from premature European babies was then studied (Table 8). No correlation was found between low birthweight for these normal births and high mean lead values. The mean values for the various categories are

Table 2 *Lead in Indian placental samples from normal birth*

Delivery data	*Mother's age (y)*	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Pb (μg/g fresh wt basis)*
January	20	40	56·5	2·18	M	3	1·33
January	28	36	58·1	3·69	M	4	0·87
January	27	39	63·5	3·14	M	3	0·73
January	36	38	50·5	3·64	F	5	1·20
January	21	40	78·0	3·46	M	3	1·13
January	37	37	77·5	3·50	M	4	1·27
January	39	42	52·2	3·26	F	4	1·00
May	26	—	—	—	—	4	2·16
May	25	40	98·0	3·54	F	3	0·86
May	26	—	—	—	—	3	1·37
May	28	33	61·7	2·58	M	4	3·56
May	20	40	48·5	3·44	F	—	1·25
May	20	36	45·5	2·78	M	3	0·47
May	26	40	85·0	3·76	F	3	1·46
September	18	42	47·0	3·45	M	3	0·52
September	34	40	69·0	3·24	F	4	0·99
September	32	38	48·5	3·86	M	4	0·53
September	24	41	76·0	4·11	F	5	0·53
September	28	40	61·0	3·66	M	—	0·75
September	19	39	53·0	2·58	F	4	0·97
September	28	28	57·5	3·66	F	4	0·76
Arith. mean	26·8	38·9	62·5	3·34	—	3·7	1·13 ± 0·68

Range 0·47-3·56 Pb μg/g fresh wt basis
Arith. mean for Jan. 1·08 μg/g fresh wt basis
May 1·59 μg/g fresh wt basis
Sept. 0·72 μg/g fresh wt basis
— = no information.

Table 3 *Lead in European placental samples from normal births*

Delivery data	*Mother's age (y)*	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Pb (μg/g fresh wt basis)*
January	16	41	46·0	2·90	F	3	1·40
January	33	40	62·5	3·66	F	3	1·20
January	24	40	65·0	3·36	M	3	1·10
January	21	40	57·1	3·26	M	—	0·90
January	32	42	81·5	2·90	F	3	1·20
January	32	42	81·5	2·90	F	3	2·65
May	38	41	73·0	2·62	M	3	0·32
May	32	40	—	4·08	F	—	0·93
May	40	38	62·5	3·32	M	—	0·61
May	32	42	58·5	3·20	F	3	0·55
May	33	37	47·0	3·12	M	3	0·74
May	33	42	83·0	3·42	M	3	0·15
May	39	38	59·5	3·40	M	1	0·38
May	35	39	55·0	2·74	F	4	0·37
May	30	38	76·2	3·30	F	3	0·34
May	23	—	—	—	—	—	0·79
May	20	39	54·5	3·46	M	3	0·77
May	22	41	57·5	3·60	F	2	2·64
September	28	41	58·0	2·94	F	3	0·65
September	33	41	—	3·38	F	—	0·68
September	24	41	57·0	3·61	M	3	1·62
September	25	41	66·5	3·34	M	3	1·19
September	22	43	49·5	4·28	M	1	0·76
September	27	38	65·5	2·31	F	3	0·43
Arith. mean	28·9	40·2	62·7	3·26	—	2·8	0·93 ± 0·64

Range 0·15-2·65 μg/g fresh wt. basis
Arith. mean for Jan. 1·41 μg/g fresh wt basis
May 0·72 μg/g fresh wt basis
Sept. 0·89 μg/g fresh wt basis
— = no information.

Table 4 *Lead in European placental samples from malformed stillbirths and neonatal deaths*

Delivery date	*Mother's age (y)*	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Nature of malformation*	*Pb (μg/g fresh wt basis)*
11.2.71	17	—	—	—	—	3	Anencephaly	1·37
19.2.71	—	—	—	—	—	—	Multiple malformations	1·51
11.3.71	22	42	71·0	3·43	F	3	Meningocele	1·60
22.3.71	30	32	49·0	1·10	F	3	Anencephaly	1·74
17.5.71	23	27	52·0	1·08	M	3	Microcephaly	1·07
19.5.71	25	35	72.0	3·30	F	3	Persistent ductus arteriosus	0·53
1.6.71	26	36	62·0	2·67	M	3	Diaphragmatic hernia	0·64
15.6.71	24	41	58·0	3·82	F	3	Haemangioma liver	1·65
29.7.71	30	32	55·5	1·40	M	3	Anencephaly	0·98
24.9.71	25	39	59·5	3·58	F	4	Extra fingers	0·82
18.10.71	23	47	63·7	2.42	M	2	Chromosomal abnormality (ring D)	2·41
5.11.71	31	40	58·0	2·06	F	3	Chromosomal abnormality (trisomy 18)	2·41
25.11.71	24	35	—	2·50	M	3	Congenital tumour of CNS	2·67
Arith. mean	25·0	36·9	60·1	2·49	—	3·0	—	1·49 ± 0·69

Range 0·53-2·67 μg/g fresh wt basis
— = no information.

Table 5 *Lead in European placental samples from neonatal deaths*

Mother's age (y)	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Pb (μg/g fresh wt basis)*
—	28	—	—	—	—	0·83
28	—	—	—	—	3	1·42
19	36	53·0	2·40	M	3	1·89
31	36	—	2·67	M	—	1·58
29	32	65·5	1·84	M	3	2·77
24	35	55·0	3·14	M	3	2·06
21	38	52·0	2·00	M	3	2·14
18	29	48·5	1·06	F	3	2·18
20	38	63·2	2·16	F	3	2·20
17	39	66·5	3·64	M	—	1·26
33	39	74·5	3·34	M	4	2·06
19	31	63·0	1·50	M	3	0·72
37	27	72·7	0·98	M	2	1·32
23	31	57·5	1·48	F	3	1·77
Arith. mean 24·5	33·8	61·0	2·18	—	3·0	1·73 ± 0·57

Range 0·72-2·77 μg/g fresh wt basis
— = no information.

Table 6 *Lead in European placental samples from stillbirths without malformations*

Mother's age (y)	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Pb (μg/g fresh wt basis)*
34	35	52·2	1·68	F	3	1·18*
26	41	61·5	3·60	M	3	0·61
25	40	76·0	3·08	F	3	0·86*
27	30	82·2	1·15	F	3	1·55*
27	33	—	1·36	M	4	1·87*
—	—	—	—	—	—	2·19
30	41	50·0	2·75	F	3	1·81
20	41	62·0	2·80	F	—	1·56
30	34	68·5	1·40	M	3	1·42*
Arith. mean 27·4	36·9	64·6	2·23	—	3·1	1·45 ± 0·50

Range 0·61-2·19 μg/g fresh wt basis
— = no information.
*Macerated.

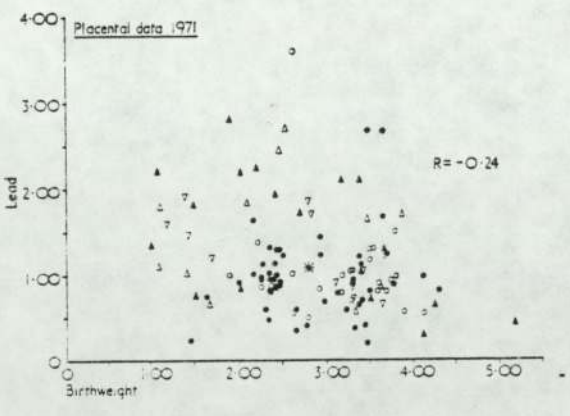

Fig. 1 *Distribution of lead levels by birthweight for stillbirths (▽), neonatal deaths (▲), malformed stillbirths and neonatal deaths, (△) and surviving births (● European and ○ Indian). The correlation coefficient refers to the non-immigrant surviving births.*

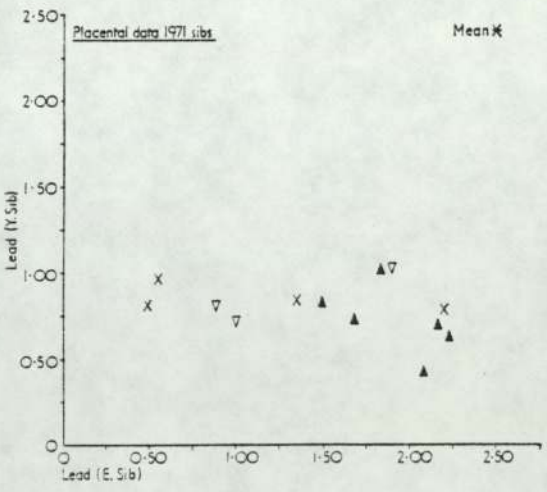

Fig. 2 *Distribution of lead levels in normal sibs to Indian births (×) and to European stillbirths (▽) and neonatal deaths (▲).*

Table 7 *Placental lead in sibs of 1971 samples*

Racial group	*Nature of previous birth*	*Nature of present birth*	*Mother's age (y)*	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Pb (μg/g fresh wt basis)*
Indian	Normal	Normal	28	37	70·0	3·15	M	4	0·93
Indian	Normal	Normal	27	40	72·5	3·26	F	3	0·79
Indian	Normal	Normal	22	41	57·2	3·74	F	4	0·91
Indian	Normal	Normal	28	39	67·5	3·24	F	4	0·67
Indian	Normal	Normal	27	40	46·5	2·90	M	4	0·78
Indian	Normal	Normal	28	40	67·5	3·52	F	3	0·76
Indian	Normal	Malformed	22	34	52·0	2·22	M	4	0·79
Indian	Malformed	Malformed	24	39	46·2	2·34	F	3	0·81
Indian	NND	Died (RDS)	32	33	47·0	2·00	M	4	0·81
Indian	Malformed	Died due to infection	29	31	67·0	1·86	M	3	0·94
European	Normal	Normal	21	39	53·0	2·62	M	3	0·55
European	Normal	Normal	21	41	71·0	3·72	M	3	0·83
European	NND	Normal	20	40	52·5	4·10	M	3	0·25
European	NND	Normal	21	38	44·5	2·40	F	4	0·82
European	NND	Normal	28	40	58·5	3·48	M	3	0·71
European	NND	Normal	21	41	62·2	4·22	F	3	0·60
European	NND	Normal	26	37	58·5	5·12	F	3	0·40
European	Stillbirth	Normal	26	39	53·0	3·39	M	4	1·00
European	Stillbirth	Normal	31	41	54·0	3·26	F	3	0·69
European	Stillbirth	Normal	26	39	67·5	3·26	M	3	0·79
European	NND	Malformed	33	38	60·5	3·36	M	3	0·99
Arith. mean			25·8	38·4	58·5	3·19		3·4	0·75±0·19

Indian	European
Range 0·67-0·94	0·25-1·00 μg/g fresh wt basis
Arith. mean 0·82	0·59 μg/g fresh wt basis

Table 8 *Placental lead in European premature birth samples*

Mother's age (y)	*Gestation period (w)*	*Mother's weight (kg)*	*Child's weight (kg)*	*Sex of child*	*Social class of father*	*Pb (μg/g fresh wt basis)*
23	39	44·5	2·34	M	2	0·75
28	30	—	1·62	F	5	0·70
28	40	77·0	2·12	M	4	1·59
24	34	59·5	2·23	F	1	1·09
31	37	68·0	2·40	M	3	0·96
23	39	53·0	2·22	F	—	0·90
33	40	—	2·30	M	1	0·98
27	30	57·0	1·43	M	3	0·21
30	40	58·2	2·42	F	3	1·25
31	37	59·5	2·48	F	—	1·19
27	40	61·0	2·26	M	4	0·55
25	39	49·0	2·22	F	3	0·92
24	42	49·0	2·42	M	4	1·25
38	33	47·5	1·98	M	3	0·88
33	36	59·0	2·32	M	4	0·91
32	37	62·5	2·32	F	3	0·76
27	35	51·0	2·40	M	—	1·25
27	38	54·5	2·42	F	3	0·84
27	40	69·5	2·32	F	3	1·29
28	37	57·0	2·34	M	2	0·85
32	39	54·5	2·37	M	2	0·91
27	—	—	2·43	—	—	0·87
23	—	—	2·38	—	—	1·11
27	—	—	2·12	—	—	0·97
Arith. mean 28·1	37·2	57·4	2·24		2·9	0·96±0·28

Range 0·21-1·59 μg g fresh wt.
— = no information.

Table 9 *Summary of placental lead results*

Nature of samples	*Pb (μg/g fresh wt basis)*
Normal Indian samples	1·13±0·68
European samples from	
(a) Normal births	0·93±0·64
(b) Normal premature births	0·96±0·28
(c) Malformed stillbirths and neonatal deaths	1·48±0·69
(d) Neonatal deaths	1·73±0·57
(e) Stillbirths	1·45±0·50
Indian and European sibs samples	0·75±0·19

collected together in Table 9 and expressed graphically in Figs. 1 and 2.

Discussion

Our first conclusion at this state is that the results appear to be significant and to justify further work. In particular we wish to examine other groups such as Asian samples where stillbirth or neonatal death has resulted. Further work concerning the background of the mother is also required. Just as blood-lead levels increase with urban environment (Waldron and Stofën, 1974) it is obviously possible that placental lead levels could show some variation within the Birmingham environment, and Thieme *et al.* (1974) have shown that placental lead levels were higher for mothers in dense urban areas. A previous blood-level survey in Birmingham by Betts *et al.* (1973) has implicated 'Surma' as a possible reason for higher Asian blood lead levels and in another family study the blood lead level of an Asian mother who did use 'Surma' was found to be higher than that of a father who did not (Warley *et al.*, 1968). However, the Indian placental lead leveis were not particularly high: in a fairly random survey of Indian households about a third of women used 'Surma', which is mostly lead sulphide (75% PbS). The higher placental lead levels in the stillbirths and neonatal deaths themselves of course do not prove that current environmental lead levels are having any adverse effect on the fetus. It could be argued at this stage that placental lead is an environmental indicator rather than an environmental hazard and as such its correlation with the other well-known environmental indicators such as social class and home environment should be investigated further.

In preliminary work which we have carried out on placental specimens from Stoke-on-Trent where some of the mothers were working in the pottery industry no high values were obtained and mean values were only half those found in similar Birmingham samples.

If substantiated by further work, our findings concerning placental lead levels in stillbirths and neonatal births are remarkable. In only 7% of the normal births were placental lead levels greater than 1·5 μg/g whereas 61% of the stillbirths or neonatal deaths had levels greater than this. This does not of course mean that lead must be a causal factor in such deaths but it certainly suggests the necessity for further lead analyses on stillbirth tissues. The alternative explanation, that lead accumulates in times of fetal stress, is one which the present evidence appears to support. For an assessment of this proposal we have planned further work which will include in addition the analyses of maternal tisues or fluids.

We are grateful to Mr Robert Lancaster and Mr Jagdish Parekh for writing computer programmes and Miss Helena Haimila for technical assistance. We are also grateful to the Department of Health and Social Security and to the Environmental Research Programme of the European Communities (Research Contract No: 8304) for financial help.

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Requests for reprints to Professor D. G. Wibberley, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET.

Appendix 4

Some analytical problems in the determination of mercury in biological materials by a cold vapour technique.

TABLE III

SEPARATION OF THREE RED DYES ON SILICA GEL G USING ETHANOL - BUTAN-1-OL - WATER (9 + 2 + 1 V/V) AS DEVELOPING SOLVENT AT 25 °C AND THEIR QUANTITATIVE DETERMINATIONS

Method	Mass of each dye in admixture/μg	Amount of dye recovered/μg			No. of separations and measurements
		Amaranth	Erythrosine	Rhodamine	
Elution*	9.7	8.9	9.2	9.0	10
Reflectance ..	9.5	9.3	9.6	9.2	10
Scanning	9.1	9.0	8.9	9.4	10

* With 80% V/V ethanol - water and absorbance measurement at the wavelengths listed in Table II.

the times required were 45 min (elution), 40 min (reflectance using the layer-removal apparatus) and 20 min (scanning). Reliable determinations by the elution and reflectance methods therefore take similar times. Silica gel and alumina are satisfactory but Kieselguhr less so for reflectance determinations.

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Some Analytical Problems in the Determination of Mercury in Biological Materials by a Cold Vapour Technique

P. J. Barlow and D. R. Crump

Department of Construction and Environmental Health, University of Aston in Birmingham, Birmingham, B4 7ET

A. K. Khera and D. G. Wibberley

Department of Pharmacy, University of Aston in Birmingham, Birmingham, B4 7ET

Most mercury occurs in nature as a red crystalline sulphide called cinnabar. Until fairly recently, mercury has been used widely in medicine, but the toxic properties of mercury and its compounds have been recognised since ancient times. The mechanism of mercury toxicity is related to the strong affinity between mercury compounds and sulphydryl (SH) groups in biological materials, particularly in enzymes.[1,2]

Industrial use of mercury, estimated[3] at about 10 000 tonnes per annum, may cause pollution problems because it creates high levels of mercury in very limited locations. Therefore, it is most important to reduce the emission of mercury to the environment and it is the task of the analytical chemist to measure low levels of mercury accurately, so that even small changes in mercury concentrations can be detected.

There are a large number of analytical techniques that can be used for the determination of mercury, including mass spectrometry, gas - liquid chromatography, atomic-absorption spectrometry with electrothermal atomisation, neutron activation analysis, colorimetric analysis and cold vapour atomic-absorption spectrometry.

Cold Vapour Technique

The method utilises the fact that mercury is the only element (other than inert gases) that has an appreciable vapour pressure at room temperature and of which the vapour is almost wholly monoatomic. Mercury has a low affinity for oxygen; a relatively high con-

centration of mercury atomic vapour can be maintained in air at room temperature. The relatively high vapour pressure also means that no thermal energy is required for vaporisation and atomisation of elemental mercury. A cold vapour mercury atomic-absorption system consists basically of a light source emitting mercury resonance lines, an absorption cell and a detector system. In the work reported in this paper a Perkin-Elmer mercury analysis system fitted to a Model 360 atomic-absorption spectrophotometer was used.

Determination of mercury in biological materials by the cold vapour technique involves two distinct stages: destruction of organic matter and sample preparation, and measurement of the mercury in the absorption cell. Once a sample has been digested, the procedure for mercury analysis is basically the same regardless of the biological material to be tested. The digested sample is diluted to 100 ml with distilled water and treated with nitric and sulphuric acids in the presence of potassium permanganate in order to oxidise all of the mercury present to the mercury(II) form (Hg^{2+}). The excess of permanganate is reduced with hydroxylammonium chloride and the mercury is reduced to metallic mercury with tin(II) chloride. An aerator is placed in the sample solution and a circulation pump moves the air trapped in the system through the solution, thus evaporating the mercury and carrying the vapour through the absorption cell. Mercury vapour in atomic form absorbs the 253.7-nm radiation emitted from the light source. The change in energy is then detected and read out in the usual way on the atomic-absorption spectrophotometer.

Standard solutions were prepared on the day of analysis from a 1 000 $\mu g\ ml^{-1}$ mercury stock solution. The standards were prepared in the range 0.1–1.0 μg of mercury and a rectilinear graph was obtained.

Sample Preparations

Human Hair Samples

Hair samples were prepared using Digby decomposition vessels (Digby Chemical Services, London). A 100-mg amount of hair was digested with 3 ml of concentrated nitric acid for 60 min at 140 °C. The solutions were then diluted to 100 ml with water.

Soil Samples

Soil samples were prepared by digesting 1 g of dry soil with 20 ml of concentrated nitric acid for 1½ h. The samples were gently heated on an electric hot-plate, filtered and the filtrate diluted to 100 ml with water.

Plant Samples

These samples were prepared in the same way as soil samples.

Milk Samples

The milk samples were prepared by digesting 2 ml of pasteurised milk with 5 ml of concentrated nitric acid at 110 °C for 60 min in the Digby decomposition vessels.

Placental Samples

Deep-frozen placental samples were defrosted and approximately 4.0 g of the material were weighed after removing excess of blood. Concentrated nitric acid (20 ml) was then added and the samples were placed in a water-bath at 55 °C for 2–4 h. The samples were almost dissolved after this time. To complete the digestion the samples were heated to approximately 100 °C for 5 min. These solutions were then diluted to 100 ml with water.

Roman Bone and Soil Samples

A considerable amount of Roman bones and soils have been collected from a Roman cemetery in the South of England; in order to estimate and compare the levels of a number of heavy metals with those in present-day samples. To date only a small number have been examined.

The bone samples were prepared by crushing approximately 1.5 g of bone and placing it in 20 ml of concentrated nitric acid. The mixture was heated in a water-bath at 55 °C for

2–4 h, which resulted in complete digestion, and the solutions were then diluted to 100 ml with water.

Roman soil samples were prepared in the same way as described for soil samples.

Teeth Samples

Mercury is commonly used in the amalgam used in teeth fillings. There is a possibility that some of this mercury could be released into the digestive system, particularly when foods of low pH are consumed. To examine this possibility, teeth samples with and without mercury fillings were kept for 24 h in solutions of pH 4 and 7 and the resulting mercury released was then determined.

Results and Discussion

The results of the determination of mercury in soils, hair, placentae and bones are given in Tables I and II.

TABLE I

MERCURY LEVELS IN SOILS

Samples	No. of samples	Range/ng g^{-1}	Mean/ng g^{-1}
Agricultural soils (Cheshire) ..	9	33–131	51
Rural soils (Birmingham) ..	4	19–30	26
Urban soils (Birmingham) ..	9	30–180	99
Roman soils	6	29–88	50

TABLE II

MERCURY LEVELS IN HUMAN HAIR, PLACENTAE AND BONES

Sample	No. of samples	Range/ng g^{-1}	Mean/ng g^{-1}
Hair	10	330–5200	110
Placentae	4	6.80–14.70	9.80
Bones (Roman)	6	33.6–151.0	81.9

The mercury levels given in Tables I and II are in close agreement with those reported by other workers. Mercury was also determined in nine grass samples, and was found to range between 37 and 88 ng g^{-1} dry mass with a mean of 64 ng g^{-1}. Mercury levels in milk were below the detection level of 0.01 μg ml^{-1}. When a known amount of mercury in solution was added to placental, soil and hair samples before wet digestion, the recoveries were 86, 90 and 95% respectively; therefore, only a small amount of the mercury present in solution was lost during the digestion and preparation of samples.

Mercury in Teeth

Mercury levels extracted from teeth samples with and without fillings at various pH values are given in Table III. The results indicate that mercury levels are higher from teeth with fillings than without fillings.

TABLE III

MERCURY LEVELS EXTRACTED FROM TEETH

Filling present	pH	Mercury level/ng
No	7	20
No	4	40
Yes	7	44
Yes	4	162
Yes	7	29
Yes	4	191

Problems with the Analytical Method

The most significant analytical problems were contamination from glassware, reagents and the laboratory environment.

The precautions taken to prevent contamination were as follows:

1. The glassware:
   (i) all glass equipment was soaked in 2% Decon 90 concentrate overnight;
   (ii) rinsed twice with hot tap water;
   (iii) washed with concentrated nitric acid;
   (iv) again rinsed twice with hot tap water;
   (v) rinsed twice with distilled water and allowed to drain;
   (vi) all glassware were then covered during storage.
2. A reagent blank must be run in order to verify the purity of the reagents. When complex sample preparation is necessary, recovery studies should be made and standards should be carried through the same digestion procedure. Actual operation of the system should be preceded by completion of all sample preparation requirements.
3. All precautions should be taken to avoid mercury-contamination from the laboratory.
4. A large volume (up to 100 ml) of distilled water is used and therefore it is necessary always to test the water before carrying out any analysis, as even slight contamination can have a large effect. Also, an acid wash of the B.O.D. bottle between each determination was found to be essential.
5. During wet digestion all samples should be completely digested.
6. It is most important to exclude water vapour from the absorption cell by the use of a desiccant tube.

Conclusion

The cold vapour technique for the determination of low levels of mercury in biological materials gives results that compare well with literature values; good recoveries of mercury are obtained and it is a fairly rapid method for use as a routine analytical procedure.

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Appendix 5

Lead concentrations in bones and soil

Reprinted from *Journal of Archaeological Science* 1979, **6**, 295–298

Lead Concentrations in Bones and Soil

H. A. Waldron,[a] Ashok Khera,[b] Gayle Walker,[b] George Wibberley[b] and Christopher J. S. Green[c]

Lead concentrations were measured in 41 rib samples from the Romano-British cemetery at Poundbury and in two sets of soil samples from the graves yielding up the bones. The levels of lead in the soil were in no way exceptional (means for the two series were 16·7 and 24·8 μg/g dry weight) and there was no correlation between soil lead and bone lead concentrations.

Since bone lead concentrations vary independently of those in the soil, it is unlikely that the bones are contaminated by lead in the soil, at least under the conditions prevailing at this particular site.

Keywords: POUNDBURY, ROMANO-BRITISH, LEAD, BONE, SOIL.

Introduction

In our studies of the concentration of lead in ancient bones (Mackie *et al.*, 1975; Waldron *et al.*, 1976), one doubt concerning the interpretation of the results has been caused by the possibility that lead present in the soil may have contributed to the lead content of the bone, especially following a stay underground of many hundreds of years. Our view has been that soil lead does not materially affect the lead levels in the bone since lead is bound relatively firmly in the soil and only moves freely under acid conditions which do not favour good bone preservation (Jurinak & Santillan-Medrano, 1974; Zimdahl & Skogerboe, 1977). We are now able to report some experimental data which support this view.

The source of the samples

The soil samples used in this study were recovered during the 1975 and 1976 excavations in the Romano-British cemetery at Poundbury on the outskirts of the Roman town of Dorchester, Dorset (Green, 1977). The cemetery, one of several serving the town, was established during the 4th century AD on open ground beside a suburban farming settlement. The subsoil is Upper Chalk, the grave fills consisting of chalk rubble and occasional lenses of grey-brown soil. The latter have proved to be from the pre-existing topsoil, the molluscan fauna they contain indicating open ground with a cover of close-cropped grass.

[a]London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.
[b]Department of Pharmacy, University of Aston in Birmingham.
[c]Dorchester Excavation Committee.

Forty-one burials were sampled out of a total of 265 excavated, the sampled graves in each case containing an extended inhumation unaccompanied by gravegoods but surrounded by iron nails from a wooden coffin. Lead-lined wooden coffins also occurred in the cemetery but in the area downhill from that covered by this survey.

The soil was sampled at one or both of two levels in the grave, at the top of the coffin, and from within the area corresponding to the abdominal cavity of the skeleton. Forty-one samples of each type of soil were available for analysis and by matching these with the necessary 50 bone samples we have been able to correlate levels of lead in the bones with one, or two soil lead concentrations.

Methods

The bones, all pieces of rib, were dried overnight at 100 °C, crushed, and wet digested in nitric acid. The soils were air dried at 40 °C, ground to a fine powder, sieved through a 2 mm mesh and then boiled for at least 30 min in an equal mixture of water and nitric acid. After filtration, the soil residue was re-boiled for a further 30 min and refiltered and the two filtrates were pooled for analysis. The lead concentrations were all determined using atomic absorption spectrophotometry and the results expressed as micrograms/gram (μg/g) dry weight.

Table 1. Lead levels (μg/g dry weight) in soil from Poundbury Romano-British Cemetery[a]

	Abdominal samples	Coffin samples
Mean	16·7	24·8
Standard deviation	9·1	30·6
Standard error	1·4	4·8
n	41	41
Range	5–50	2–103

[a]The mean concentration of lead in soil is usually considered to be *c.* 15 μg/g.

Table 2. Relationship between bone lead concentration (μg/g dry weight) and increasing soil lead concentration (μg/g dry weight)

		Abdominal sample				Coffin sample			
	Soil lead	0–9	10–19	20–29	30+	0–9	10–19	20–29	30+
	Mean	65·8	65·2	71·0	70·7	68·2	64·8	78·2	57·9
	SD	29·5	27·0	34·3	17·1	21·5	32·3	30·0	37·0
Bone	SE	10·4	6·0	10·8	9·9	7·2	7·8	12·3	12·3
Lead	*n*	8	20	10	3	9	17	6	9

Results

The levels of lead in the soil at Poundbury are in no way exceptional (Table 1), and although the samples from the top of the coffin have a higher mean value (and a wider range) than the abdominal samples, this difference is not statistically significant. The correlation between soil lead and bone lead concentrations is of a very low order; between bone concentrations and coffin soil samples, the correlation coefficient (*r*) has the value

0·09, and for bone and abdominal soil samples, $r=0·10$. From Table 2 it can be seen that bone lead levels do not differ significantly as the amount of lead in the soil increases.

In order to guard against the possibility that any relationship between soil lead and bone lead levels may not be a linear function, rank correlation coefficients (τ) were also calculated. For bone and abdominal soil samples, $\tau = 0·08$, whilst for bone and coffin samples, the value was 0·04.

Discussion

Lead poisoning is one of the few toxicological hazards which the archaeologist can study, since much the greater part of the body burden of lead is stored and fixed in the skeleton, and so it is important to be sure that analysis of lead concentrations in ancient bones is not influenced by the lead in the soil. The study reported here is the largest of its kind so far undertaken, and indicates that bone lead concentrations vary independently of those in the soil. The only other study comparable to ours has been reported on a number of occasions by Grandjean, his 1975 paper being the most comprehensive. Grandjean took a total of 22 vertebrae dating from the early Iron Age to the 18th century and compared their lead levels with those in the soil contained within the vertebral foramen. There was a significant rank correlation coefficient ($P<0·001$) between the two sets of values, but as Grandjean explains, this was not surprising since "the bones having the lowest lead content were derived from old culture layers with a low concentration of lead and the bones with a high lead content had been dug up in a big city" (Copenhagen).

Unfortunately Grandjean did not have enough samples to permit him to judge whether there was a correlation between the bone and soil levels from the same period, as we have been able to do. His overall conclusion, however, was that the exchange of lead between soil and bone is a very slow process.

Additional data from his paper, in which virtually identical lead levels were found in exhumed bones and in bones of similar anatomical type and archaeological age which had been recovered from vaults where they had rested above ground since death, indicate that the exchange between bone and soil does not occur at all to any appreciable degree. This would be the conclusion which we draw from our own results. Nevertheless, it is still important to take soil samples where possible from sites at which it is proposed to analyse lead levels in exhumed bones, since a comparison of the soil lead levels with other soils, or the presumed "natural" level may indicate the extent to which lead was used by the inhabitants. This would certainly be the case in urban soils from recently occupied sites, especially from the 18th century onwards, when lead smelting increased at a very rapid rate. In our own century, levels of lead in the soil have been increasing in many urban areas because of the use of lead as an antiknock agent in petrol.

It has to be remembered, of course, that low lead levels (as at Poundbury) do not exclude excessive exposure to lead, but if they are typical of the area as a whole, indicate that, at the time it was not an area of primary lead production or smelting nor a place where lead products were manufactured to any considerable degree. However, as noted above, lead-lined coffins were in use in the cemetery and these had presumably been manufactured in either the adjacent settlement or some workshop within the town.

There are a number of other lines of research connected with the issue of bone and soil lead which we are also following up. For example, the concentrations of lead in some of the juvenile and infant bones which have already been examined are sufficiently high to be consistent with a diagnosis of death from lead poisoning. If this is indeed the case, then we would expect to find radiological lead lines at the growing ends of the bones from some of these individuals; the presence of these lines would confirm that the lead in the bones had been absorbed during, rather than after life. It is also possible to

locate the lead within the bone structure using either histochemical methods or the electron probe. The successful application of either method will allow us to study the distribution of lead within the bone. If the uptake from the soil is minimal, then the lead should be more or less evenly distributed throughout the bone, whereas if absorption from the soil occurs at a significant degree it is likely that there will be a concentration gradient from the external surface inwards. We hope to report on some of these findings in due course.

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Printed in Great Britain by Henry Ling Ltd., at the Dorset Press, Dorchester, Dorset

Appendix 6

Cadmium and lead levels in blood and urine in a series of cardiovascular and normotensive patients.

Intern. J. Environmental Studies 1980, Vol. 14, pp. 309–312
0020–7233/80/1404–0309 $04.50/0

CADMIUM AND LEAD LEVELS IN BLOOD AND URINE IN A SERIES OF CARDIOVASCULAR AND NORMOTENSIVE PATIENTS

A. K. KHERA†, D. G. WIBBERLEY†, K. W. EDWARDS‡ and H. A. WALDRON§

(Received February 28, 1979)

The mean blood-lead, blood-cadmium, urine-lead and urine-cadmium levels of a series of male Caucasian patients suffering from cardiovascular disease were significantly higher at every age group than the corresponding levels in a control series of normotensive patients.

INTRODUCTION

Despite intensive studies, particularly over the past six years, the question as to whether the levels of lead and cadmium in the blood of hypertensive patients or those with cardiovascular disease, are higher than the corresponding levels in normal subjects remains unsolved. Even more intractable problems concerned with whether such conditions are actually caused by the presence of either of these heavy metals must await this resolution. The higher levels of cardiovascular disease in soft water districts has been cited[1] as a possible reason for implicating lead as a factor and the induction of hypertension in laboratory animals with additional cadmium in their diet as a reason for suggesting that cadmium is a factor.[2] In the case of blood-lead levels the most important contribution has come from the Glasgow team of D. G. Beevers *et al.*[3] who have shown a significant excess of cases with high blood-lead levels among hypertensives in the West of Scotland where tap-water lead levels are notoriously high. The same authors,[4] however, did not detect an appreciable difference in average blood-cadmium levels between two groups of hypertensive and control patients matched for age and sex.

In contrast, S. C. Glauser *et al.*[5] found more than a three-fold difference between average blood-cadmium levels in 17 untreated hypertensive patients and a group of ten somewhat younger normotensive controls.

One of the problems is undoubtedly the considerable difficulty of determining with accuracy and consistency the low levels of the two metals in blood. In the case of cadmium for example, in the last five years, nine different authors have claimed blood-cadmium levels in the normal population of between 0.003μ mole/1 (0.4 ng/ml)[6] and 0.33μ mole/1 (37 ng/ml).[7]

Lauwerys[8] has claimed that the mean blood-cadmium levels for the general population is 0.04μ mole/1 (4.1 ng/ml) and that it is now generally recognised[9] that the normal concentration is below 0.09μ mole/1 (10 ng/ml).

PATIENTS

For a period of 15 months from October 1976, 50 patients who attended the General Hospital Birmingham because of a moderate to severe cardiac condition and/or hypertension gave, on the same day, one sample of blood and one of urine which were analysed for cadmium and lead. During the same time similar determinations were made for 75 patients attending the hospital with no known cardiovascular symptoms.

METAL DETERMINATIONS

Blood was drawn from each patient into a heparinised metal-free container and urine collected in a glass metal-free tube. 1g samples were dissolved in soluene (5 ml) and diluted with toluene to 10 ml

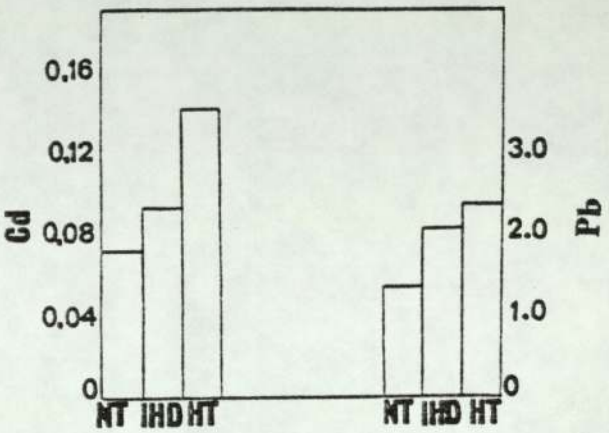
† Department of Pharmacy, University of Aston, Birmingham B4 7ET (U.K.).
‡ Technology Policy Unit, University of Aston, Birmingham B4 7ET (U.K.).
§ London School of Hygiene and Tropical Medicine, Keppel Street, London EC1E 7HT (U.K.).

and aliquots (25μl) pipetted into a H.G.A. 74 graphite furnace of a Perkin Elmer 360 A.A.S. modle.

In the case of cadmium the mixture was dried at 60°C for 30 seconds, charred at 550°C for 30 seconds and atomised at 1900°C for 10 seconds. The samples were then burnt off at 2700°C for five seconds. Controls were prepared from blood spiked with cadmium chloride to give blood-cadmium concentrations in the range of 0.009–0.04μ mole/1 (or 1ng/ml–4 ng/ml). For lead, drying, charring and burn-off temperatures and timings were the same as for cadmium. The samples were atomised at 2100°C for ten seconds. The lead standards were in the range of 0.24–2.4μ mole/1.

RESULTS

For all four analytical determinations there was an appreciable difference between the mean heavy metal values in the cardiovascular patients and in the normotensive patients (Table I). The cardiovascular patients contained small numbers of women (7) Negroid and Asian patients (7) and the normotensive patients included 14 patients less than 30 years of age. When these three groups were removed from consideration because of difficulties in matching, there remained 38 male cardiovascular patients aged over 30 and 48 matched normotensive patients. The results from these matched groups are recorded in Table II. The urine metal levels were extremely variable and other workers[10] have shown that 24 hour samples are necessary to overcome diurnal variations. The blood-cadmium and blood-lead levels are recorded in Table III for four matched age groups. There was some evidence of variations in heavy metal levels with the nature of the cardiovascular disease (Figure 1) but the small numbers in certain categories make definite correlations uncertain.



NT = Normotensives (48 subjects)
IHD = Ischaemic heart disease (25 patients)
HT = Hypertensive (13 patients) of whom three also had ischaemic heart disease.

FIGURE 1 Disease pattern in cardiovascular patients and normotensives in relation to blood-cadmium and blood lead.

TABLE I

Cadmium and lead levels (μ mole/l) in all patients

Patients	Metal level (μ mole/l)			
	Blood-Cd	Urine-Cd	Blood-Pb	Urine-Pb
Cardiovascular	0.10	0.07	2.12	0.37
Normotensive	0.07	0.06	1.35	0.29

TABLE II

Cadmium and lead levels in blood and urine in Caucasian males aged over 30

Patients	Metal level (μ mole/l)					
	Blood-Cd		Urine	Blood-Pb		Urine
	mean	range		mean	range	
Cadiovascular	0.10	0.02–0.35	0.07	2.17	0.43–4.00	0.34
Normotensive	0.07	0.01–0.18	0.05	1.4	0.58–2.2	0.27

TABLE III

Cadmium and lead levels for four matched age groups

	Age group	Average age	No. of patients	Blood-Cd (μ mole/l) mean	Blood-Cd (μ mole/l) range	Blood-Pb (μ mole/l) mean	Blood-Pb (μ mole/l) range
Cardiovascular	30–39	34	5	0.14	0.04–0.27	2.65	1.16–3.96
Normotensive	30–39	35	9	0.06	0.09–0.10	1.35	0.42–1.74
Cardiovascular	40–49	46	8	0.10	0.02–0.25	2.12	0.68–3.23
Normotensive	40–49	45	9	0.07	0.01–0.18	1.45	0.58–1.98
Cardiovascular	50–59	54	16	0.09	0.02–0.35	1.98	1.50–3.04
Normotensive	50–59	51	16	0.07	0.02–0.13	1.30	0.58–1.98
Cardiovascular	> 60	65	8	0.11	0.07–0.21	2.17	0.43–3.96
Normotensive	> 60	67	17	0.07	0.03–0.15	1.40	0.87–2.03

ilarly, smoking habits were not determined n sufficient accuracy for firm conclusions, ough overall results showed little change with oking and lead levels but cadmium levels ca 20 cent higher than normal in normotensive okers compared with non-smokers. For the diovascular patients the group of ex-smokers the highest blood-cadmium levels but for all ee groups the disease state appeared to mask smoking effect.

SCUSSION

e results of this pilot study have proved to our sfaction that average blood-lead and blood-mium levels are consistently higher in patients h a wide range of cardiovascular-disease than in tched normal subjects with no such disease nptoms. We have found that the analyses are remely sensitive to a whole range of factors and near the limits of sensitivity and reliability of instrument. The results demonstrate yet again difficulty of inter-laboratory comparisons ich must await validated control work[11] on the ne blood samples. The patients were also far m being a cohesive group having a wide range of rt disease varying from severe myocardial ction and ischaemic heart disease to moderate ertention. Most were also receiving a range of g therapies and in view of the results found with ying disease states these should be standardised future trials.

Beevers *et al.*[3] have suggested that chronic posure to unsatisfactory levels of lead in drink-water can lead to the development of hyperten-n.

Voors and Shuman[12] have similarly concluded, on the basis of high liver-cadmium levels in North Carolina residents who died of heart disease, that cadmium has a toxic effect on the cardiac conduction system. Our work on placental lead levels did not enable us to clearly implicate lead as a causative factor in stillbirths and neonatal deaths[13] and in our opinion, a similar dilemma exists in the case of cardiovascular disease. Environmental levels of cadmium and lead could be inducing cardiovascular disease in some patients or such disease may result in abnormal metabolism and consequential higher blood-cadmium and blood-lead levels.

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Appendix 7

Placental and stillbrith tissue lead concentrations in occupationally exposed women

British Journal of Industrial Medicine 1980;37:394-396

Placental and stillbirth tissue lead concentrations in occupationally exposed women

A K KHERA,[1] D G WIBBERLEY,[1] AND J G DATHAN[2]

From the Department of Pharmacy,[1] University of Aston, Gosta Green, Birmingham B4 7ET, and Wolstanton,[2] Newcastle-under-Lyme, Staffs ST5 0HB, UK

ABSTRACT The lead values in maternal and infant blood, in placental tissue, and in stillbirth liver, kidney, and rib- and skull-bones have been determined in samples from the Stoke-on-Trent area. The lead values in antenatal blood and placenta increase with occupational exposure; liver and kidney stillbirth lead values are lower than those of much older children and rib-bone lead values from stillbirths were on average three times as high as those from a control group comprised of cot deaths and early infant deaths from accidental causes.

The abortifactant properties of lead salts in moderate to high doses and the high rate of miscarriage in women working in a poorly controlled environment containing lead are well documented (see references within Waldron and Stöfen[1]), as are the teratogenic effects of lead alone, and with cadmium, in animals.[2-4] What is not certain, however, is whether low concentrations of lead have any effect on the human fetus. Some workers, on the basis of a few cases, have claimed that lead can be teratogenic[5 6] while others[7] suggest that the case is not proved. Our own work on Birmingham mothers showed that placental-lead concentrations were appreciably higher in cases of stillbirth or neonatal death, but our results did not distinguish between causative and consequential effects.[8] In the present investigation we have examined lead values in various tissues from women employed in the pottery industry in Stoke-on-Trent.

Tissue samples

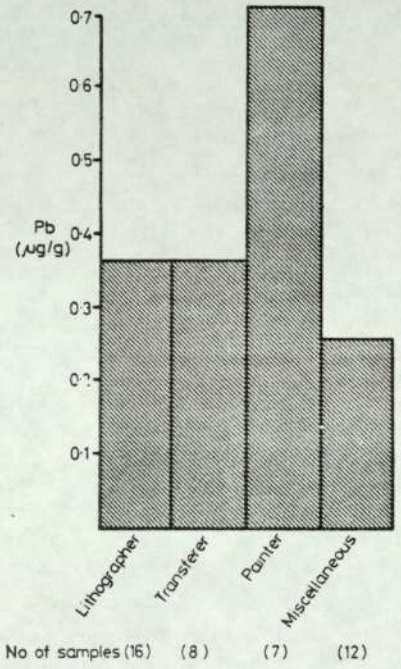
Placentae, blood, stillbirth bone, liver, and kidney were supplied by JGD (formerly of the Health and Safety Executive) from mothers employed in the pottery industry in Stoke-on-Trent. Records were available for the occupation of the women from whom blood and placental samples had been taken. Placentae and stillbirth samples were stored at $-20°C$, and the time of storage before analysis varied from 6 to 12 months. Blood samples were analysed within a few days of collection. Control samples of infant bones were supplied by Dr H A Waldron (London School of Hygiene and Tropical Medicine) and consisted of cot-deaths (age under 1 week) and accidental deaths (age under 18 weeks).

Stillbirth bone samples were wet-digested with nitric acid and diluted solutions analysed by graphite furnace atomic absorption spectrophotometry (AAS). Placental, liver, and kidney samples were solubilised with Soluene -350 and diluted solutions analysed by graphite furnace AAS using our previously reported method.[9 10] Blood samples were analysed at the medical laboratory of the Health and Safety Executive.

Placenta

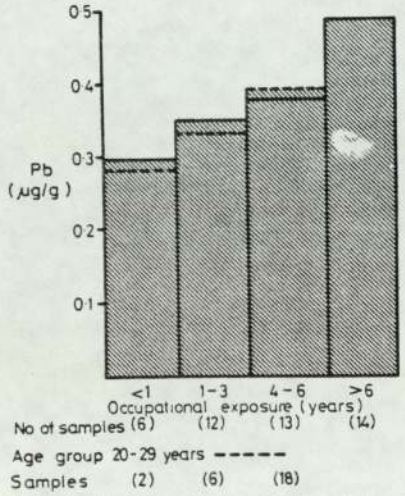
In several respects the placental-lead values gave similar results to our Birmingham investigation though with lower absolute values. Thus the placental-lead values where a stillbirth had occurred (20 cases) had a high average value ($0{\cdot}45 \pm 0{\cdot}32\ \mu g/g$) compared with that from eight mothers who had not worked in industry during the previous two years ($0{\cdot}29 \pm 0{\cdot}09\ \mu g/g$). The mean value for the nine mothers who had had a malformed live-birth was not high ($0{\cdot}32 \pm 0{\cdot}14\ \mu g/g$) but in ten cases where record sheets gave some statement concerning fetal distress, the placental-lead values ($0{\cdot}43 \pm 0{\cdot}19\ \mu g/g$) were close to those where a stillbirth had resulted. The average placental-lead value for all these Stoke-on-Trent samples ($0{\cdot}35 \pm 0{\cdot}23\ \mu g/g$) was about one-

Received 7 January 1980
Accepted 20 February 1980



1 *Relation between placental lead and type of pation (normal births).*

d that of our earlier reported values for 1971 ningham samples but appreciably higher than value we now find for fresh Birmingham pla-as (ca 0·12 μg/g). We ascribe this difference to precipitation of placental-lead with continued age at −20°C coupled with our method of sample



2 *Relation between placental lead and duration of pational exposure (normal births).*

preparation which entails the prior removal of excess fluid.

When comparing the results from the different occupational groups (fig 1) it was found that lithographers (0·34 ± 0·14 μg/g) and transferers (0·34 ± 0·16 μg/g) had the same mean placental-lead value and that this was higher than that of the women described as following miscellaneous occupations (0·25 ± 0·07 μg/g). One paintress had an exceptionally high value (1·7 μg/g), and even omitting this result, the average value for this group (0·54 ± 0·09 μg/g) was significantly greater than for lithographers or transferers ($p < 0·01$). The placental-lead burden also increased appreciably with length of occupational exposure (fig 2). Although placental lead also increases with maternal age from an average value of 0·30 ± 0·16 μg/g for age < 20, 0·35 ± 0·14 μg/g for age 20-29 to 0·51 ± 0·44 μg/g for age ⩾ 30, within a group of mothers aged 20-29 (26 samples) the placental-lead figures showed an increase with increasing job exposure (fig 2).

Blood

In common with sevaral other reports we find a highly significant positive correlation ($r = +0·40$, $p = <0·001$) between lead values in maternal and infant blood (65 samples) but an insignificant correlation between maternal blood lead and placental lead ($r = +0·16$). There was little variation between the lead values in maternal blood samples taken immediately before birth (12 ± 5 μg/100 ml) irrespective of occupation or length of employment, but when antenatal samples (mean value 17 ± 7 μg/100 ml) are grouped, an occupational variation is apparent (table).

Antenatal blood-lead concentrations in different occupational groups

	Transferer	*Lithographer*	*Paintress*
Pb (μg/100 ml)	12	19 ± 9	24 ± 9
No of samples	2	27	11

Kidney and liver

Lead values in 11 stillbirth kidney samples varied from 0·30 to 0·69 μg/g with a mean value of 0·48 ± 0·13 μg/g (wet wt). The same stillbirths had liver-lead values of 0·02-1·12 with a mean of 0·42 ± 0·30 μg/g. Previous workers have quoted liver and kidney values for stillbirths of 53 and 38 μg/g ash wt and for children aged 0-19 years of 74 and 62 μg/g ash wt respectively.[11] No samples were available for comparison using our present analytical techniques.

Bones

Lead values in 10-rib samples from stillbirths ranged from 0·73 to 3·75 μg/g with a mean value of 2·01 ± 0·97 μg/g (wet wt basis). The lead values in skull-bone samples from seven of the same births ranged from 0·36 to 2·53 with a mean of 1·28 ± 0·66 μg/g. The ratios of wet:dry weight (1·85) and wet weight: ash (3·23) were in the expected range. A series of cot-deaths (age less than 1 week) and two infant deaths (age less than 18 weeks) yielded rib-bone lead values of 0·72 ± 0·46 μg/g, which was significantly lower than the stillbirth ribs ($p < 0·001$).

Conclusion

Women working in the Stoke-on-Trent area in jobs exposed to lead have higher antenatal blood lead concentrations and higher than average placental-lead concentrations. In addition, concentrations of lead in stillbirth tissues appear to be high and, in the case of rib-bone lead where we were able to make direct comparisons, there is an accumulation of lead. It is not possible to say, however, whether the occupational lead exposure caused any of the stillbirths that were examined or whether the high lead values were consequential to the fetal death. Nevertheless, even in the absence of any direct causal evidence of the subclinical effects of lead, we would regard it as only prudent to ensure that wherever possible lead is removed from the environment of the pregnant worker. The ethical implications with respect to the continuation of employment of women in such an environment have been discussed, particularly in American reports.[12 13] A consultative document has been issued by the Health and Safety Commission[14] in Britain in which proposals are made that, "In order to safeguard the developing fetus from lead (1) pregnant women should be suspended from lead work and (2) a woman of child-bearing capacity should not be employed in lead work when her blood lead concentration exceeds 1·9 μmol/l."

We thank the staff of the North Staffordshire Maternity Hospital for their help and co-operation and Dr D L Smith and Dr Barbara Ockenden for their advice and help.

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