KOMMISSION DER
EUROPÄISCHEN GEMEINSCHAFTEN

COMMISSION DES
COMMUNAUTÉS EUROPÉENNES

UNITED STATES ENVIRONMENTAL
PROTECTION AGENCY

32 General Review 1849-

INTERNATIONAL SYMPOSIUM

Environmental health aspects of lead

Die gesundheitlichen Aspekte der Umweltverschmutzung durch Blei

Les problèmes sanitaires posés par le plomb présent dans l'environnement

THE EXCESSIVE INGESTION OF LEAD AND OTHER METALS BY CHILDREN

DELVES H., BICKNELL J., CLAYTON B.

Hospital for Sick Children
LONDON, ENGLAND

and

Botleys Park Hospital
CHERTSEY, ENGLAND

Amsterdam, October 2 - 6, 1972

Summary

Samples of whole blood taken from a control group of children and from a group of children suspected of having lead poisoning have been analysed, by atomic absorption spectroscopy, for twelve metals: lead, copper, zinc, cadmium, manganese, strontium, chromium, iron, bismuth, lithium, nickel and cobalt. Many samples taken from the patients were found to contain significantly high concentrations, relative to the control group, of the first seven of the metals listed; whereas other samples from the patients contained significantly low concentrations of iron, copper and zinc. These results indicate that at least seven metals in addition to lead should be investigated in children in whom lead poisoning is suspected, particularly in those children with pica.

Introduction

Numerous reports have shown that excessive ingestion of lead is a serious health hazard for children, particularly for those who have pica (Greenberg et al. 1958; Bicknell et al. 1968). Materials such as paints, plastics and rubbers which are likely to be mouthed and chewed by children, either as a means of environmental exploration or as a result of pica contain many metals, including lead (Browning, 1961). It is possible therefore for children to ingest a number of different metals. The present study was undertaken to determine whether any of eleven other metals in addition to lead were increased in the blood of children, in whom the physician was considering a possible diagnosis of lead poisoning.

Tipton (1960), Butt et al. (1964) and Stitch (1957) have shown that twenty metals are consistently present in human tissues although only eight of these are known to be essential to man. The choice of the metals to be investigated in our study was determined by the available analytical techniques. Only the prohibitively expensive spark-source mass spectrometry was capable of determining all the metals of interest (Table I) in the small samples of blood (2 - 5 ml) available from the children. Atomic absorption spectroscopy was relatively cheap and capable of determining most of the metals of interest: iron, copper, zinc, cobalt, nickel, manganese, bismuth, lithium, strontium, cadmium, lead and chromium.

Patients and Methods

Control children   51 boys and 31 girls aged 4 days to 15 years were in-patients at the Hospital for Sick Children and a further 4 boys and 5 girls aged 18 months to 13 years were in-patients at Poole Hospital. The ages for this group followed a normal distribution pattern. The blood samples were obtained when venepuncture was being performed for other purposes frequently whilst the child was anaesthetised. Control children had a wide variety of disorders, but children with the following features were excluded: mental retardation, history of excessive pica, and X-ray involving use of a contrast medium during the previous year, congenital heart disease, leukaemia or bleeding disorder, or convulsions.

The Patients   Blood samples were obtained from 128 boys and 61 girls aged 2 months to 15 years. Although this age range was the same as that for the control children, a higher proportion of patients were in the 3 to 6 yr age range. Seventeen of the patients had more than one blood sample taken for analysis. The interval between taking samples from the patients ranged from a few days to 2 years. Most of the patients were under the care of the Hospital for Sick Children, Great Ormond Street; Queen Mary's Hospital, Carshalton, and Poole General Hospital, Dorset, and their clinical case notes were reviewed in retrospect.

Sixteen other children were patients from a large number of different hospitals and their case notes were not reviewed.

Collection of Samples Whole blood was collected by venepuncture into disposable plastic syringes using 0.1 ml of heparin solution (sodium or lithium salt) as anticoagulant. The samples were sent to the laboratory (by post when necessar either in the syringe with the needle covered, or after transf to Staynes heparin tubes (Staynes Laboratories Ltd., Marlow, Bucks). Specimens were stored at -9°C in the laboratory prior to analysis. Since certain preparations of heparin may contain manganese, the analysis of blood samples was restricte to those which had been collected into heparin known to be relatively free from manganese and other trace metals, i.e. concentrations of 1,000 i.u./ml and 5,000 i.u./ml, (but not higher concentrations) supplied by Paines & Byrne Ltd., Greenford, Middlesex; Evans Medical Supplies Ltd., Liverpool; Weddel Pharmaceutical Co.Ltd., London; and Boots Pure Drug Co., Nottingham. A detailed account of trace metal contamination from heparin has been reported (Delves, 1970).

Chemical Analysis

Duplicate 2 ml portions of the blood samples were oxidised with a mixture of nitric, perchloric and sulphuric acids, and the residues were dissolved in 2N hydrochloric acid. Chromium was determined directly in this solution by atomic absorption spectroscopy. The other eleven metals were determined in 1 ml portions of the hydrochloric acid solutions by a procedure involving sequential solvent extraction and atomic absorption (Delves, Edwards, Shepherd, 1971).

Results

Mathematical treatment of results The blood concentrations of manganese, strontium, cadmium, lithium and cobalt were not normally distributed in the control group of children but followed a skew distribution. It was necessary to apply a censored or truncated distribution analysis (Cohen, 1957) to establish the upper and lower concentration limits for these metals. Zinc tended to follow a log normal distribution and iron, lead and copper were normally distributed in this group. The upper and lower concentration limits for zinc, iron and lead together with nickel, chromium and bismuth, which did not conform to a truncated distribution, were established by using the percentile points evaluated by computer calculation. The blood concentrations of copper were dependent upon age and it was therefore necessary to calculate the limits for this metal from the standard deviation about the regression line for concentration with age. The lower and upper blood-metal concentration limits for the control group were established by taking the 2.5 percentile and 97.5 percentile points for those metals for which a significantly large ($p<0.01$) number of abnormal results were observed in

the patient group; and the 1 percentile and 99 percentile points in all other cases. In this way the number of abnormal values that would be observed due to chance fluctuations in sampling were minimised.

The mean concentrations and observed ranges for the twelve metals are given, for both groups, in Table II. Because of the limited sample size, and technical difficulties in the earlier stages of the investigation it was not possible to determine all twelve metals in every sample. Consequently the number of tests shown in Table II are not the same for each metal that was investigated. However, 75% of normals had 9 or more metals studied and of patients 90% had 9 or more metals studied. Generally bismuth, chromium or lithium were not studied in every patient.

The differences between the mean blood metal concentrations for both groups as a whole, were significant only for lead, $p<0.001$; zinc, $p<0.005$; manganese, $p<0.02$ and nickel, $p<0.005$. The mean concentrations were also calculated for the boys and girls within each group and there was no significant difference.

The correlation of concentrations with age was investigated for male and female children within each group. Only copper showed any significant correlation ($p<0.05$) with age for the control group of children. The copper concentration decreased as the child became older. This negative correlation has been taken into consideration in calculating the upper and lower limits for the control group. In the patients copper again showed a negative correlation with age and this was also observed for manganese and strontium. Only zinc showed a positive correlation with age, i.e. the blood concentration increased as the child became older.

Table III shows the upper and lower limits of concentrations in blood for the control group of children and the number of results outside these limits which were observed for each group of children. A more detailed analyses of these abnormal values revealed that whereas no children from the control group showed more than one abnormal result, amongst the patients there were 37 children with 2 abnormal results, 10 with 3 abnormal results; 10 children with 4 abnormal results, and two children with 5 abnormal results. No children had more than 5 abnormal results.

The interrelationship between any pair of metals studied was investigated for both groups of children within the age ranges 0 - 36 months, 37-72 months and 73-180 months. In the control group there were significant ($p<0.05$) correlations for seven pairs of metals only, although not for all the age groups. In the patients there were significant correlations for fourteen pairs of metals. The data are given, in detail, in Table IV.

Discussion

The determination of concentrations of metals in blood as a method for studying their ingestion by children has the obvious limitation that it comprises only one facet of the total body content and distribution of the metal throughout body tissues. Furthermore concentration in blood may change in response to causes other than ingestion of the metal itself. Those factors that may result in changes in concentration in blood are:

i) excessive ingestion of a given metal resulting in an increased concentration of that metal, e.g. lead and pica (Bicknell, Clayton, Delves, 1968).

ii) excessive ingestion of one metal resulting in an increase or decrease in the concentration in blood of another metal, e.g. the anaemia that is observed in lead poisoning leads to a reduction in the level of iron in whole blood.

iii) disease states resulting in increases or decreases of metal concentrations but not in any way related to ingestion of the metals, e.g. the increase in plasma copper and decrease in plasma zinc levels observed in leukaemia (Delves, Alexander and Lay, 1972) and in other diseases (Fell, 1972).

This present study was limited to an investigation of metal concentrations in blood because blood samples can easily be collected from large numbers of children without the risk of contamination with metals, which is not the case for the collection of urine samples.

The most frequently observed abnormal concentrations in blood were, as expected, those of lead (Table III). Eighty-one samples from the patients, but only one sample from the control children had concentrations of lead in their blood greater than 30 µg/100ml. The highest value observed for the control children was only 32 µg/100ml but values of up to 140 µg/100ml were observed for the patients. These results agree with earlier findings and confirm the relationship between excessive lead ingestion and pica (Greenberg et al. 1958; Bicknell et al. 1968). Significant correlations were observed between the concentrations of lead in blood and the concentrations of iron, chromium, manganese and strontium (Table IV). The correlation between lead and iron was negative for patients aged over 6 years, but not for younger patients. It is possible that this indicates an increased resistance to lead by the haemopoietic system in the younger child, since the children most likely to get lead poisoning are aged between 3 and 5 years. Furthermore biochemical tests of inhibition of haem biosynthesis as indicators of lead poisoning that are useful for monitoring lead absorption in adults, are of limited value for children. (Moncrieff et al. 1964).

The positive correlations between the concentrations of lead and those of chromium, manganese and strontium suggest a similar relationship between pica and the excessive ingestion of these metals as is the case for lead. It is also possible that these metals may be associated with lead in the materials that are chewed by the children; for example lead chromate is used in the manufacture of paints and the ingestion of this material would result in the ingestion of both lead and chromium.

The abnormally high levels of cadmium in the patients must be a result of excessive ingestion of this metal since this metal is not essential and is present in normal body tissues at very low concentrations. The absence of a positive correlation between lead and cadmium unlike that observed between lead and chromium, manganese and strontium is probably due to the much lower concentration range observed for cadmium than for these other metals (Table II).

The abnormal concentrations of copper that were observed (six high results from the patients and two high results from the controls) may be a result of excessive ingestion of other metals. There is a significant negative correlation between chromium and copper for the patients. The negative correlation between iron and copper observed for the control group may be due to the decrease in the concentration of copper in blood with age since there is a positive correlation between iron and age for boys in the control group. The significant negative correlation between iron and lead for the patients would have masked these observations.

The abnormally low concentrations of zinc that were observed for the patients (Table III) may be due to the ingestion of other metals, but there was no evidence of a correlation between zinc and any other metal for the patient group. One might have expected a correlation between zinc and cadmium but this was not observed. The high zinc values are probably due to some metabolic disturbance. A positive correlation between lead and zinc which would have implicated pica was not observed.

The negative correlation that was observed between nickel and iron, for both groups of children, is difficult to explain. There were no abnormally high values for nickel for either group of children, ruling out pica.

A retrospective examination of the clinical histories of children with abnormal blood-metal concentrations has not indicated any obvious relationship between these concentrations and the clinical data. Our results have shown, however, that of the twelve metals chosen for investigation seven of them, lead, zinc, copper, manganese, strontium, cadmium and chromium can be present at abnormally high concentrations in the blood of children with suspected lead poisoning (many of whom had pica) and that iron, zinc and copper can be present at abnormally low concentrations in the blood of these children. These observations suggest that more detailed studies of these eight metals should be investigated in children with pica.

Acknowledgments

We thank Mrs.P.Edwards and Miss G.Shepherd for technical assistance with the analysis of the samples and Mr.L.Friedman and Dr.B.Carter for help with the statistical analysis.

Financial assistance from the Wellcome Trust is gratefully acknowledged.

References

Bicknell, J., Clayton, B.E. and Delves, H.T. J.ment.Defic. Res. 12, 282, 1968.

Browning, E. "Toxicity of industrial metals." Butterworth's Scientific Publications, London, 1961.

Butt, E.M., Nusbaum, R.E., Gilmour, T.C., Didio, S.L. and Sister Mariano. Arch.Environ.Health, 8, 60, 1964.

Cohen, A.C. Biometrika, 44, 225, 1957.

Delves, H.T. Ph.D.Thesis, University of London, 1970.

Delves, H.T., Alexander, F.W. and Lay, H. "Changes in the plasma concentrations of copper and zinc in childhood leukaemia." Submitted for publication.

Delves, H.T., Shepherd, G. and Vinter P. Analyst, 96, 260, 1971

Fell, G.S., Canning, E., Husain, S.L. and Scott, R., Symposium on Trace Substances in Environmental Health, University of Missouri, Columbia, 1972.

Greenberg, M., Jacobziner, H., McLaughlin, M.C., Fuerst, H.T. and Pellitteri, O. Pediatrics, 22, 756, 1958.

Moncrieff, A.A., Koumides, O.P., Clayton, B.E., Patrick A.D., Renwick, A.G.C., and Roberts, G.E., Arch.Dis.Childh., 39, 1, 1964.

Stitch, S.R. Biochem. J. 67, 97, 1957.

Tipton, I.H., in Seven, M.J. and Johnson, L.A. Editors, "Metal binding in medicine," J.B.Lippincott Co.Philadelphia, Montreal, 1960, pp27-42.

TABLE I - Trace metals consistently present in human tissues and their potential sources for children with pica

Metal	Paints	Plastics or Rubbers	Paper and Printing Inks	Oils and Greases
		POTENTIAL SOURCE		
Aluminium	+	+	+	+
Barium	+	+	+	+
Bismuth				
Cadmium	+	+	+	
Chromium	+	+	+	+
Cobalt	+		+	
Copper			+	
Gold				
Iron	+		+	
Lead	+	+	+	+
Manganese	+		+	
Molybdenum	+		+	+
Nickel	+		+	
Rubidium				
Silver				
Strontium	+	+	+	
Tin	+	+		+
Titanium	+		+	
Vanadium	+		+	
Zinc	+	+	+	+

TABLE II - The concentration of twelve metals in the blood of children

Metal	CONTROL GROUP OF CHILDREN Mean Concentration	Observed range	No. of tests	PATIENTS Mean Concentration	Observed range	No. of tests
Iron	386.0	290- 525	91	373.4	200- 520	206
Zinc	510.0	319-1040	84	617.9	210-2284	192
Copper	97.9	55-147	84	102.2	44-162	183
Lead	11.7	1-32	44	32.3	1-140	203
Manganese	1.2	0-5.7	90	1.8	0-15.0	206
Cadmium	0.49	0-1.9	89	0.57	0- 7.9	204
Strontium	2.8	0-8.9	77	3.0	0-27.4	182
Chromium	3.5	0-9.7	50	4.1	0-15.0	85
Cobalt	0.46	0-2.1	67	0.32	0- 5.0	167
Nickel	2.3	0-9.3	69	1.37	0- 8.4	167
Lithium	0.38	0-2.1	69	0.36	0- 3.0	146
Bismuth	0.67	0-6.5	48	0.93	0- 6.5	131

Concentrations are µg/100 ml for all metals except iron, in which the units are µg/ml

TABLE III - Concentration limits for 12 metals in blood and the numbers of observed values outside these limits

Metal	Control Group: Concentration limits μg/100ml	Control Group: Percentile*	Blood samples with values outside normal ranges: Control Group: No.	Control Group: (Total No. tested)	Patients: No.	Patients: (Total No. tested)
Iron Lower	29,500	2.5	3	(91)	29	(206)
Iron Upper	52,500	99.0	0	(91)	0	(206)
Zinc Lower	320	2.5	2	(84)	15	(192)
Zinc Upper	886	97.5	2	(84)	27	(192)
Copper**		2.5	0	(84)	6	(183)
		97.5	2	(84)	6	(183)
Lead Upper only *	30	97.5	1	(44)	81	(203)
Manganese *	3.5	97.5	3	(90)	20	(206)
Cadmium *	1.5	97.5	4	(89)	15	(204)
Strontium *	9.9	97.5	0	(77)	8	(182)
Chromium *	9.7	99.0	2	(50)	6	(85)
Cobalt *	2.7	99.0	0	(67)	2	(167)
Nickel *	10.0	99.0	0	(69)	0	(167)
Lithium *	2.5	99.0	0	(69)	0	(146)
Bismuth *	7.2	99.0	0	(48)	0	(131)

* 2.5 percentile = 97.5% of control group are above this figure
97.5 " = 97.5% " " below " "
99 " = 99% " " " " "

** The concentration of copper in blood varies with age. The range (mean ± 2 S.D. for the control group was from 76 - 158 μg/100 ml at less than one month, to 41 - 124 μg/100ml at 15 years.

TABLE IV - Significant interrelationships between pairs of metals

CONTROL GROUP: Age range (years)	Correlation**	PATIENTS: Metals	Age range (years)	Correlation	Metals	Age range (years)	Correlation
>3	-	Cr, Cu	>3	-	Ni, Sr	>3	+
>6	-	Cr, Ni	>6	-	Ni, Li	>3, <6	+
>3	-	Pb, Fe	>6	-	Sr, Li	>6	+
>6	+	Fe, Ni	>6	-	Pb, Mn	>3, <6	+
>6	+	Fe, Co	>6	+	Pb, Sr	>6	+
>3, <6	+	Cd, Ni	>3	+	Pb, Cr	>3	+
>3	+	Cd, Li	>6	+	Co, Ni	>6	+

* p < 0.05

** - indicates as the first metal increases, the second decreases
+ " " " " " " " increases