Copper, manganese, zinc, nickel, cadmium and lead in human foetal tissues

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1. Concentrations of copper, manganese, zinc, nickel, cadmium and lead were measured in samples of liver, kidney, brain, heart, lung, skeletal muscle and vertebral bone from forty foetuses of 22–43 weeks gestation.

2. Cu concentrations in the liver were up to 100 times those in other tissues, but only those in the brain showed a significant increase with gestational age.

3. Mn concentrations were similar in all tissues; the over-all range was 0·35–9·27 μg/g dry matter (DM).

4. Concentrations of Zn in the liver were much higher than in other tissues and decreased with gestational age, whereas levels in skeletal muscle increased.

5. In all tissues Ni concentrations were within the range 0·04–2·8 μg/g DM and levels in kidney and muscle decreased significantly with age.

6. Cd was detected in most of the tissue samples and concentrations were within the range 0·01–0·58 μg/g DM.

7. Concentrations of Pb, where it was detected, varied from 0·1 to 2·4 μg/g DM in the soft tissues and from 0·4 to 4·3 μg/g DM in the bone samples.

In animals, deficiencies of copper, manganese and zinc (Hurley, 1976) during gestation can have severe consequences on the normal growth and development of the foetus and neonate. Faulty trace element nutrition during gestation has not yet been linked to disorders in the human foetus but may influence the development of deficiency syndromes after birth. Nutritional deficienceis of Cu and Zn have been reported in undernourished children (Cordano, Baertl & Graham, 1964; Sandstead, 1973) and in infants who were otherwise well-fed (Al-Rashid & Spangler, 1971; Hambidge, 1974).

Although it has long been known that Cu, Mn and Zn are essential for man (Underwood, 1971), nickel has only recently been found to be an essential trace element for mammals (Nielsen & Higgs, 1970). Very little work has been done on either the metabolism of this element or on its role in human nutrition (Nielsen, 1974).

At present, both cadmium and lead are regarded as toxic trace elements. Chronic maternal ingestion of excessive amounts of both elements results in foetal abnormalities and death in animals (Chernoff, 1973; Ferm, 1972), and possibly also in man (Friberg, Piscator & Nordberg, 1971; Scanlon, 1972).

Cu deficiencies have been reported in farm animals in New Zealand (Anon, 1975), and agricultural problems may also arise with Zn (Towers, 1977) and Mn (Grace, 1973). New Zealand is not heavily industrialized so high environmental levels of Cd and Pb are not expected, but several cases of over-exposure to Pb in young children living in older houses have been reported (Shellshear, Jordan, Hogan & Shannon, 1975). Thus the concentrations of these five elements and of Ni were investigated in tissues from New Zealand foetuses and compared with values obtained overseas for foetuses and adults.

Table 1. *Coefficients of variations and recoveries of trace elements in stock tissue (a mixture of sheep's liver, heart and brain)*

	Coefficient of variation in eighty ash samples	Recovery of added metals (*n* 30) (%)	
		Mean	SD
Copper	7	95	4
Manganese	8	92	4
Zinc	7	97	3
Nickel	42	98	15
Cadmium	15	100	11
Lead	43	92	17

EXPERIMENTAL

Material

Organs were collected at autopsy from forty foetuses of 22–43 weeks gestation. Twenty-three were stillborn and seventeen died within 24 h of birth. Gestational age was calculated from the last menstrual period and body-weights varied from 440 to 3750 g. Infants with major congenital abnormalities were excluded. (Full details of all cases appear elsewhere; Casey, 1976.)

Whole organs were obtained for liver, kidney, brain, heart and lung and 5–50 g skeletal muscle (diaphragm) and bone (vertebrae). Not all tissues were obtained from every case. Samples were frozen in polyethylene containers until analysed.

Analytical procedure

Tissue samples were thawed, obvious fat was trimmed and bones were scraped with a stainless-steel scalpel blade to remove adhering muscle. Samples were rinsed thoroughly with de-ionized water to remove as much blood as possible, patted dry with paper towelling and dried under infra-red lamps. Dried samples were ashed in a muffle furnace at 450° for 48 h and the ash dissolved in concentrated hydrochloric acid (Sp. G. 1·19) (Baker Analysed Reagent, J. T. Baker Chemical Co., Phillipsburg, NJ 08865, USA). Concentrations of Cu, Mn, Zn, Ni, Cd and Pb were determined by atomic absorption flame spectrophotometry (AA-5; Varian Techtron Pty Ltd, Springvale, Australia). Non-atomic absorbance in the determination of Ni, Cd and Pb was corrected by using a hydrogen continuum lamp (Varian Techtron Pty Ltd).

Samples of stock tissue (a mixture of sheep's liver, heart and brain) were ashed regularly and the coefficients of variation obtained in eighty ash samples are given in Table 1, along with the recoveries of metal added to the stock tissue before ashing. Nine ash samples of bovine liver (standard reference material 1577, National Bureau of Standards, US Department of Commerce, Washington DC, USA) were also carried out and the concentrations obtained for Mn, Zn and Cd were not different from the certificate values. Cu (181 ± 4 μg/g) was significantly lower ($P < 0{\cdot}01$) than the certificate value (193 ± 10 μg/g); the Pb concentration ($0{\cdot}47 \pm 0{\cdot}60$ μg/g) was significantly higher ($P < 0{\cdot}01$) than the certificate value ($0{\cdot}34 \pm 0{\cdot}08$ μg/g). A reference value was not given for Ni; the value obtained in this study was $0{\cdot}37 \pm 0{\cdot}08$ μg/g.

Table 2. *Water (g/g) and copper, manganese, zinc and nickel (μg/g dry matter) in human foetal tissues*†

(Mean values and standard deviations; ranges in parentheses)

	No. of foetuses	Water Mean SD	Cu Mean SD	Mn Mean SD	Zn Mean SD	Ni Mean SD
Liver	39	0·78 0·04 (0·69–0·84)	276 106 (91–566)	4·26 1·57 (1·78–9·27)	*** (267–1730)	0·66 0·54 (0·04–2·8)
Kidney	21	0·81 0·03 (0·75–0·84)	8·67 5·52 (4·76–14·1)	2·36 0·52 (1·77–3·52)	100 18 (55–137	* (0·14–1·7)
Brain	28	0·90 0·01 (0·88–0·92)	** (5·06–17·1)	1·58 0·23 (1·18–2·01)	57·7 5·8 (43·6–76·7)	0·38 0·26 (0·08–0·90)
Heart	19	0·79 0·05 (0·68–0·85)	11·6 4·8 (7·0–28·4)	1·29 0·34 (0·72–2·30)	120 13 (91–147)	0·69 0·60 (0·13–2·5)
Lung	24	0·82 0·04 (0·72–0·86)	15·0 13·1 (1·9–64·7)	1·24 0·43 (0·64–2·65)	82 19 (56–115)	0·38 0·27 (0·12–1·1)
Skeletal muscle	22	0·80 0·03 (0·75–0·84)	4·01 1·46 (2·06–8·74)	0·67 0·16 (0·35–1·07)	*** (99–190)	0·24 0·16 (0·08–0·68)
Bone	31	0·61 0·08 (0·43–0·74)	5·58 1·24 (2·24–7·44)	2·25 0·54 (1·41–4·09)	122 21 (87–163)	0·37 0·24 (0·07–1·1)

Significant change with gestational age: $*P < 0·01$, $**P < 0·001$, $***P < 0·0001$.
† For details, see p. 640.

RESULTS

The concentrations of Cu, Mn, Zn and Ni and of water in all tissues are given in Table 2. Where the concentration of an element was found to vary significantly with gestational age only the range has been given. Cadmium and Pb were not detected in all samples analysed, the limits of detection being 0·01 μg Cd/g DM and 0·1 μg Pb/g DM. Table 3 gives the results for Cd and Pb in all tissues for the number of the samples in which they were detected.

The results were examined for any relationships between the concentration of trace elements and gestational age using linear and quadratic regression analysis. Where a significant relationship was found, there was no difference in the contribution of gestational age to the variation in concentration as determined by either method. The F values and levels of significance are reported for linear regression.

Liver

The highest concentrations of Mn, Zn and Cu were found in the liver. Levels of Zn decreased significantly with gestational age (F 24·80, $P < 0·0001$) from a mean of 1020 μg/g DM for the eight cases in the 22–25 week age-group to a mean of 377 μg/g DM for the five cases at 38–41 weeks.

Kidney

Except for Cu and Zn, levels of the trace elements were of the same order as in the liver; values for Cu were up to 100 times lower. Ni concentrations decreased significantly (F 8·33, $P < 0·01$), from 0·86 μg/g DM at 22–25 weeks to 0·36 μg/g DM at term.

Brain

The lowest concentrations of Zn were found in the brain. Values for Cu increased with gestational age (F 18·03, $P < 0·001$) from a mean of 6·27 μg/g DM at 22–25 weeks to 12·2 μg/g at term.

Table 3. *Cadmium and lead (μg/g dry matter* DM*) in human foetal tissues*†

(Mean values and standard deviations for foetuses in which Cd or Pb were detected; range in parentheses)

	Total no. of foetuses	Cd		Pb	
		No. of foetuses containing‡	Mean SD	No. of foetuses containing§	Mean SD
Liver	39	33	0·07 0·06 (0·02–0·28)	29	0·9 0·5 (0·3–2·4)
Kidney	21	15	0·08 0·05 (0·02–0·21)	9	0·5 0·3 (0·3–1·0)
Brain	28	25	0·11 0·06 (0·02–0·28)	16	0·5 0·4 (0·1–1·4)
Heart	19	12	0·07 0·04 (0·03–0·14)	10	0·9 0·7 (0·1–2·4)
Lung	24	20	0·12 0·15 (0·02–0·58)	11‖	0·4 0·3 (0·1–0·9)
Skeletal muscle	22	19	0·03 0·01 (0·01–0·06)	13	0·3–0·1 (0·1–0·5)
Bone	31	25	0·08 0·04 (0·02–0·23)	28	2·2 0·9 (0·4–4·3)

† For details, see p. 640.
‡ Limit of detection 0·01 μg Cd/g DM.
§ Limit of detection 0·1 μg Pb/g DM.
‖ Only fourteen lung samples were analysed for Pb.

Heart

Concentrations of Ni in the heart samples were similar to those in liver and slightly higher than those in the other tissues.

Lung

The highest concentrations of Cd in individual tissues were found in the lung, but the mean concentration was not significantly higher than in the brain.

Skeletal muscle

Zn values showed a highly significant increase (F 27·21, $P < 0·0001$) from 110 μg/g DM at 22–25 weeks to 160 μg/g at term. The lowest concentrations of Mn, Ni, Cd and Pb were found in the muscle.

Bone

The ranges of element concentration in vertebral bone were similar to those in the other tissues, except for Pb levels which were slightly higher.

DISCUSSION

Cu

The concentration range found in the livers was similar to those reported by other workers for foetuses (Romhanyil, Fazekas & Rengei, 1962; Widdowson, Chan, Harrison & Milner, 1972), and very much higher than levels found in New Zealand adults (McKenzie, 1974). The large amount of Cu accumulated in the liver during gestation, up to 85% of the total body content by term (Priev, 1964), constitutes a store which can be utilized after birth. In a

full-term healthy infant such a store of approximately 10 mg Cu is sufficient to provide for the Cu requirement until weaning (Ashkenazi, Levin, Djaldetti, Fishel & Benvenisti, 1973). Levels of Cu in other tissues were much lower than in the liver. Values reported elsewhere for foetal (Brückmann & Zondek, 1939; Parr & Taylor, 1964) and for adult (McKenzie, 1974) kidneys were similar to this study, as were levels in adult heart and muscle (Tipton & Cook, 1963), lung (McKenzie, 1974) and bone (Janes, McCall & Elveback, 1972). This suggested there was little change in Cu concentration in these tissues throughout life. The increase in Cu concentration with gestational age in the brain appears to continue for some time after birth and might be associated with the requirement for Cu in phospholipid synthesis, particularly in the myelin sheaths (DiPaulo & Newberne, 1972). Völkl, Berlet & Ule (1974) found levels increased from birth to 11 years when adult concentrations, 15–24 μg Cu/g DM (Smeyers-Verbeke, Defrise-Gussenhoven, Ebinger, Löwenthal & Massart, 1974) were reached.

Mn

Although the range of Mn concentrations in the livers, 1·78–9·27 μg/g DM, was higher than the over-all range in the other tissues, 0·35–4·09 μg/g, the difference was not as great as found for Cu or Zn. Values were similar to those reported for foetuses by Widdowson *et al.* (1972) who suggested that the liver does not store Mn. This is supported by the results of the present study: foetal levels were similar to concentrations found in livers from New Zealand adults (Johnson, 1976) and the liver at term would contain only a small proportion of the total body content. Unlike Cu and Fe, there is no known special storage form of Mn. In the other tissues Mn levels did not change significantly during gestation but after birth slight increases may occur. Reported values for adult kidney (Plantin, 1973) and bone (Janes *et al.* 1972) were slightly higher. Concentrations in other tissues were within the ranges found in adults in brain (Gooddy, Hamilton & Williams, 1975), heart (Plantin, 1973), lung and muscle (Tipton & Cook, 1963). Mn is required for the proper development of bone and cartilage (Burch, Hahn & Sullivan, 1975) and the most striking symptoms of deficiency in animals have been observed in the foetus and newborn. A naturally-occurring deficiency has never been reported in man and the similarity of foetal and adult tissue levels suggests the human foetus is adequately supplied with Mn.

Zn

Zn showed a pattern of accumulation in foetal livers quite different from that of Cu and Mn: the concentration decreased significantly with gestational age. Other workers have found a similar trend (Widdowson *et al.* 1972; Kanabrocki, 1973). Livers from New Zealand adults contained slightly lower concentrations (McKenzie, 1974; Johnson, 1976). Other workers reported similar values for Zn in foetal kidneys (Parr & Taylor, 1964) but very much higher values in adult kidneys, 237 ± 107 μg/g DM (McKenzie, 1974). Zn is accumulated in the kidney cortex after birth (Livingstone, 1972) where it may be associated in part with metallothionein, but the concentration in the medulla increases only slightly with age (Syversen, 1975). Values in this study were similar to those reported elsewhere for foetal (Chaube, Nishimura & Swinyard, 1973) and adult (Smeyers-Verbeke *et al.* 1974) brain, foetal and adult heart (Kanabroki, 1973) and adult bone (McKenzie, 1974). In skeletal muscle Zn levels increased with gestational age but this trend did not appear to continue after birth as adult concentrations (McBean, Dove, Halstead & Smith, 1972) were similar to those in the full-term foetuses. None of the tissues appeared to store Zn, so all Zn available to the foetus would be utilized and any restriction in supply could have immediate consequences on growth and health. The offspring of Zn-deficient rats show congenital abnormalities, retarded growth and impaired brain development (Hurley & Shrader, 1972). Such effects

have not yet been observed in the human foetus but Zn depletion has been found in otherwise well-nourished infants (Walravens & Hambidge, 1976), so care must be taken to ensure the pregnant mother receives enough dietary Zn to provide for her foetus.

Ni

Concentrations of Ni found in the foetal tissues were of the same order as values which have been reported for adult liver (Johnson, 1976), brain (Nielsen, 1971), lung (Bernstein, Kneip, Kleinman, Riddick & Eisenbud, 1974), heart and muscle (Tipton & Cook, 1963). A decrease in kidney levels with gestational age was also found by Mikosha (1959). Adult kidney levels were above the foetal range (Bernstein *et al.* 1974), possibly from normal environmental exposure (Nomoto & Sunderman, 1970). Reported values for Ni in adult bone were higher than the range in the foetuses; Janes *et al.* (1972) found 51 ± 52 μg/g DM in forty adult samples. Ni has only recently been recognized as an essential trace element for mammals (Nielsen & Higgs, 1970) and, as yet, little is known of its metabolism. Since most foetal tissues contained levels similar to those in adults, Ni must cross the placenta readily, and the supply to the foetus would depend on Ni status of the mother.

Cd

Cd, regarded at present as a non-essential trace element, has a long biological half-life in the tissues and thus accumulates with age in the body (Friberg *et al.* 1971). Concentrations of Cd, where it was detected, were low and close to the analytical limit. Although much attention has been given to techniques of measuring Cd in biological materials there is still a wide divergence of results between methods and laboratories (Friberg, Kjellström, Nordberg & Piscator, 1975). Thus caution must be exercised in comparing one study with another, particularly studies done by atomic absorption spectrophotometry without correction for non-atomic absorption. Values for Cd in adult heart (Plantin, 1973), muscle and bone (Friberg, Piscator, Nordberg & Kjellström, 1974), found by neutron activation, were similar to values in the foetuses suggesting little change in these tissues after birth. Much higher concentrations have been reported for Cd in adult liver (Plantin, 1973; Johnson, 1976) and lung (Kanabrocki, 1973). Henke, Sachs & Bohn (1970) reported a range in foetal kidney similar to the present study (0·02–0·21 μg Cd/g DM), but levels in adult kidney were very much higher in both cortex, 83 ± 15 μg/g DM, and medulla, 49 ± 19 μg/g (Syversen, 1975). Like Zn, Cd is accumulated in the kidney after birth and appears to be associated partly with metallothionein (Nordberg, 1972). In the adult, liver and kidney contain approximately 50% of the total body burden of Cd (Friberg *et al.* 1974). However, because the concentrations in all foetal tissues analysed were similar, the contribution of the liver and kidney to the total body burden in the foetus at term would be only approximately 5%, the percentage of the total body-weight which these two organs comprise.

Pb

Like Cd, Pb was undetected in many samples, and most measurable concentrations were near the analytical limit of 0·1 μg/g DM. Levels in brain, heart and muscle were similar to those reported by other workers for foetuses (Barltrop, 1969) and children and adults (Barry, 1975). Concentrations in liver, kidney, lung and bone were also similar to values reported elsewhere for foetuses (Horiuchi, Horiguchi & Suekane, 1959) and infants (Barry, 1975), but were very much less than adult values (Hammer, Calocci, Hasselbad, Williams & Pinkerton, 1973; Barry, 1975) suggesting much of the Pb entering the body after birth accumulates in these tissues. Patterson (1965) proposed the concept of the natural body burden of Pb, that is the tissue concentration which prevailed during the evolution of physiological responses, before man disturbed the environmental distribution of Pb. He estimated

this natural level to be approximately 30 μg/kg body-weight. In the present study the over-all range of concentrations in the soft tissues was 0·1–2·4 μg/g DM or 10–500 μg/kg wet weight. Thus most foetal tissues already contain much more than the natural level by the third trimester and Pb accumulation appears to start very early in life.

Concentrations of trace elements found in the foetal tissues in this study were similar to values reported elsewhere for foetuses. Levels of the essential elements Cu, Mn, Zn and Ni in most tissues were of the same order as have been reported in adults; liver Cu levels were very much higher in the foetuses than in adults. New Zealand soils are generally low in trace elements (New Zealand Soil Bureau, 1962) and this has given rise to agricultural problems in plants and animals (Underwood, 1971). However, nutritional probelms have not been reported in humans and the New Zealand foetus appears to be as well-endowed with the essential elements as his foreign counterpart. He also appears to be adequately protected from excessive exposure to the non-essential elements Cd and Pb. It is of interest that levels of these elements in heart and muscle were similar in the foetus and the adult, although levels in liver, kidney and lung increased greatly with the increased exposure after birth.

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