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Studies of Trace-Metal Levels in Human Tissues—VI. Concerning the Estimation of Lead Levels in Human Lung and Vertebra, with Particular Reference to Formalin Fixation

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The recent report by OPHUS and MYLIUS (1977) of lead concentrations in the lungs of 250 Norwegian residents is potentially a most useful contribution to the study of levels of toxic metals in human tissues. Such studies are essential to establish "normal" metal levels for human tissues in different countries, and to provide baseline data for future biological monitoring of these metals. The data also allow estimates to be made of the biological half-life of the metals in different organs (ELINDER et al. 1976). The lung is particularly relevant in this regard because it is an important point of entry into the body for both cadmium and lead, with a considerably higher absorption factor [up to 40-50% for cadmium (FRIBERG et al. 1974), uncertain but probably comparable for lead (WHO 1977)] than, for example, the gastrointestinal tract [ca 6% for cadmium (FRIBERG et al. 1974), 10% or less for lead (WHO 1977)].

Unfortunately, there are three grounds on which the data of OPHUS and MYLIUS (1977) are open to criticism. First, the data are presented as lead levels in dry tissue, but "dry" is not defined, although a common meaning is 24h drying, or drying to constant weight, at 110°C. In addition, dry weight is probably the least usual basis for expressing metal concentrations in human tissues, and this increases the difficulty of comparing the present results with data already in the literature (see also below). Secondly, the selection of all samples from the apex of the (right) lung may not give results which are representative of the lung(s) as a whole, since there is evidence (GIBBS and BOGDANOVIC 1974) that apical concentrations of several metals (including lead) are higher (on an ash weight basis) than in other lung segments, and also that the ash content of "dry" lung varies between segments in no obviously systematic way.

The third criticism is probably the most serious, and relates to the analysis of tissues which have been fixed and/or stored in formalin. With such samples, leaching out of the metal(s) of interest is a distinct possibility and, as shown in Table 1, the present data are lower than those from several of the major studies in

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TABLE 1

MEAN LEAD LEVELS IN HUMAN LUNG

NOTE: Numbers in block type are based on data in the reference indicated; numbers in *italics* have been calculated using respective mean ash contents of wet and dry lung of 1.1% and 4.9% from TIPTON and SHAFER (1964). These figures are consistent with unpublished data from the laboratory at Waterloo; viz. 0.68-1.21% ash of wet lung, 4.47-6.05% ash of dry lung.

.....Reference.....	Sex	No. of Donors	µg/g wet	µg/g dry	µg/g ash
BARRY (1975)	M	59	0.22	*0.98*	*20*
	F	36	0.22	*0.98*	*20*
GIBBS and BOGDANOVIC (1974)	?[1]	16	0.50[2]	3.05[2]	50[2]
	?[3]	4	0.36[2]	2.16[2]	36[2]
GROSS et al. (1975)	M[4]	42	0.36	*1.17-1.60*[5]	23.9
HORIUCHI et al. (1959)	M,F[6]	47	0.30	*1.34*	*27*
OPHUS and MYLIUS (1977)	M[7]	146	*0.10*	0.45	*9.2*
	F[7]	98	*0.10*	0.43	*8.8*
SCHROEDER and TIPTON (1968)	M,F	150	0.39	*1.74*	*35*[8]
STRINGER et al. (1974)	M	41	*0.23*	1.04[9]	*21.2*
	F	25	*0.13*	0.57[9]	*11.6*
TIPTON and SHAFER (1964)	M,F	141	0.74	3.28	67[8]

[1]right lungs only, 8 segments of each analysed separately

[2]median level

[3]left lungs only, 8 segments of each analysed separately

[4]all segments of both lungs sampled

[5]figures based on levels in ashed and in wet tissue

[6]multiple samples, anatomical location not specified

[7]tissues fixed in formalin; 10 samples from right apex

[8]the corresponding median level is 47 µg/g ash

[9]samples lyophilised to constant weight

this area. In particular, the mean levels (particularly for males) found by STRINGER et al. (1974) are higher than those of OPHUS and MYLIUS (1977), although they are cited as agreeing because of the similar range of lead levels. It should be noted that, in Table 1, because of the different weight bases used by different authors to

express lead concentrations, the conversions to other bases are only approximate. In addition, the data may not be strictly comparable because of probable differences in the characteristics of the groups of tissue donors in the different studies, with respect to factors such as sex, age, smoking history, occupation, cause(s) of death and anatomical sampling schemes. However, there is evidence that two factors which are strongly associated with lead levels in bone -- sex and age -- are, at most, weakly associated with lead levels in lung (BARRY 1975, GIBBS and BOGDANOVIC 1974, GROSS et al. 1975). Despite these uncertainties, the data of OPHUS and MYLIUS (1977) are consistent with the assumption of significant loss of lead by leaching, although it is possible that residents of the Trondheim area of Norway have lower pulmonary lead levels than North Americans and Japanese. It is also possible that if OPHUS and MYLIUS' (1977) drying conditions are less rigorous than 110°C, a concentration basis that is still partly "wet" may have contributed to the apparently lower lead levels. A definitive separation of these possibilities must await further studies.

A second case of a significant data set where there is a strong presumption of leaching of lead by formalin is in the vertebral lead levels reported by GRANDJEAN (1975). As shown in Table 2, the mean lead concentration in vertebra samples from 81 males and 38 females is less than one-fifth that reported in several other studies. GRANDJEAN'S (1975) concentration basis is "dry" vertebra, described as transfer (from the formalin) to concentrated alcohol, rinsing with ether and drying to constant weight in a desiccator. This procedure is presumably less rigorous than 110°C, so that the relative contributions of the weight basis and leaching to the decreased lead levels are again unclear. However, it may be significant that the two studies reporting lower lead levels refer to the residents of Scandinavian countries (Norway and Denmark, respectively).

CONCLUSIONS AND RECOMMENDATIONS

The difficulties arising from the lead levels published by OPHUS and MYLIUS (1977) in lung and by GRANDJEAN (1975) in vertebra raise a number of matters relevant to all studies of trace element concentrations in human tissues. First, the fixation of tissues in formalin, prior to trace element analysis, should be avoided because of the likelihood of loss by leaching. Secondly, meaningful comparisons among different studies are difficult, if not impossible, to make unless standard conditions for the weight basis of expression of the results are used in preparing the tissues. In particu-

TABLE 2

MEAN LEAD LEVELS IN HUMAN VERTEBRA

NOTE: Numbers in block type are based on data in the reference indicated; numbers in *italics* have been calculated using respective mean ash contents of wet and dry vertebra of 18.92% and 35.07% for males, and of 17.31% and 33.22% for females. These figures are based on vertebral samples from 122 males and 61 females analysed in the laboratory at Waterloo (cf. Table 1 in FORBES et al. 1976).

.....Reference.....	Sex	No. of Donors	µg/g wet	µg/g dry	µg/g ash
GRANDJEAN (1975)	M	81	*0.88*	1.63	*4.65*
	F	38	*0.69*	1.32	*3.97*
GROSS et al. (1975)	M	45	4.42	*8.2-10.2*[1]	29.1
HORIUCHI et al. (1959)	M,F	36	5.28	*10.0*	*29.1*
SCHROEDER and TIPTON (1968)	M,F	53	?	?	51[2]
WATERLOO (to be published)	M	122	6.56	12.1	35.0
	F	61	5.00	9.6	29.2

[1]figures based on levels in wet and in ashed tissue

[2]median level

lar, a dry weight basis appears, in practice, to be the most sensitive to the conditions used; e.g., lyophilising (STRINGER et al. 1974), desiccating (GRANDJEAN 1975) or heating at 110°C (GIBBS and BOGDANOVIC 1974). Hence, because reliable trace element levels in human tissues are expensive to generate (CHERRY et al. 1978), it is clearly important to produce data which allow quantitative comparisons to be made. It is therefore recommended that future (lead and cadmium) data be given on all three weight bases -- wet, dry and ash -- where drying is carried out to constant weight at 110°C; our experience for human rib and vertebra indicates that 24h drying yields essentially constant weights for sections of these two bones (cf. CHERRY et al. 1975). GIBBS and BOGDANOVIC (1974) used 1 to 4 days at 110°C for drying lung to constant weight. Ashing the dry tissues is customarily carried out in a muffle furnace at temperatures around 450°C for metals of suitably low volatility, such as lead and cadmium.

Thirdly, if results are expressed as median metal concentrations, the mean concentration should also be given. The median concentration, as used by SCHROEDER and

TIPTON (1968) and by GIBBS and BOGDANOVIC (1974), for example, has the advantage of being a more stable measure of central tendency than the mean, particularly for data sets with the skewness which characterizes tissue levels of various non-essential elements. However, a major disadvantage is that the medians for two or more data sets cannot necessarily be combined to yield a single value for the whole data set, whereas numbers of observations and means can be manipulated in this way. This operation has been necessary to produce the limited degree of comparability between different studies achieved in Tables 1 and 2, and it is essential for proper comparisons of existing data. It is therefore recommended that future studies give, as a minimum, both mean and median metal levels for human tissues, together with the range of values.

Fourthly, authors must indicate the steps they have taken to ensure both the precision and accuracy of their data. Precision is usually checked by the analysis of independent replicate samples, and accuracy can be assessed by the analysis of standard reference materials. Analyses of these materials on an on-going basis can also provide a check for contamination and for base-line drift if analyses are carried out over a relatively long period. Unfortunately, the majority of existing studies are deficient in one or both of these respects. For example, OPHUS and MYLIUS (1977) make no explicit mention of precision or accuracy checks, and GRANDJEAN (1975) describes only the consistency of absorbance measurements on the same MIBK extract; this would not normally be considered an independent replicate and has no bearing on accuracy. However, STRINGER et al. (1974) consider both precision (replicate analyses, two analytical methods) and accuracy (recovery experiments). With standard reference materials available from organizations like NBS, there is little justification for recent studies not including such materials in their analytical scheme. It is also good practice for each laboratory to maintain its own internal standard(s) of the same tissue(s) as those being analysed. In our laboratory, these standards are five 100+ g samples of carefully ground composite ash, from a number of donors, of rib, vertebra, kidney, liver and lung. Each composite is analysed routinely with samples of the same tissue type, and samples have been sent to other laboratories for independent analyses, preferably by a different technique from that used in the laboratory at Waterloo. The use of internal and certified external standards is therefore recommended for all laboratories analysing human tissues on an on-going basis.

Fifthly, it is desirable that there should be more consistency between different studies in the age groups used to show the relation (if any) between age and tissue metal levels. Trace element studies have usually used decades (0-9,10-19, etc.) or combinations of these, whereas

the 10-year age groups commonly used in epidemiology, vital statistics and demography are 5-14,15-24, etc. Because of the need to link data from these different sources in studies of the human health effects of trace elements, it is desirable to use the same (or compatible) age ranges, and in view of the large amounts of data of long-standing in the groups 5-14,15-24, etc., it is recommended that trace-metal studies also adopt these groups. To retain comparability to existing trace element data, it is also desirable to give decade mean concentrations. In view of their relatively large number of tissue donors, OPHUS and MYLIUS' (1977) data would be more useful to other workers if they were subdivided in 10-year age groups. Users of the data can always combine age groups if more stable estimates for means are required, but cannot subdivide data given only in broad age ranges. The care needed in interpreting trends in mean levels for different age groups is illustrated by the data of GROSS et al. (1975), shown in Table 3. If the mean lead levels in wet rib or in rib ash for 45 individuals are calculated in decades, an unstable pattern is seen above ages 40-49, but the same raw data in age groups 15-24,25-34, etc. exhibit a steady increase in mean lead levels with age. To avoid the somewhat arbitrary grouping by age, suitable regression models relating trace element levels to age can be used to summarize the relationship (see also below).

Sixthly, the value of a data set involving trace-metal levels in human tissues is enhanced if information on the tissue donors includes their smoking history. The contribution of cigarette smoking to renal cadmium levels is now well established (e.g., FRIBERG et al. 1974; LeBARON et al. 1977; LEWIS et al. 1972), but except for the study

TABLE 3

AGE DEPENDENCE OF LEAD LEVELS IN HUMAN RIB

NOTE: The Table shows mean lead levels, on wet and ash weight bases, in two sets of 10-year age groups, for 45 observations obtained from GROSS et al. (1975).

Age Group	No. of Donors	µg/g wet	µg/g ash	Age Group	No. of Donors	µg/g wet	µg/g ash
20-29	6	4.03	14.4	15-24	2	4.08	13.0
30-39	4	5.56	18.5	25-34	5	4.24	15.5
40-49	8	8.09	31.6	35-44	8	6.26	22.7
50-59	9	4.91	19.5	45-54	7	7.22	28.4
60-69	11	9.82	40.6	55-64	10	7.92	33.4
70-79	6	9.42	42.0	65-74	10	8.44	36.6
80-89	1	3.20	15.0	75-84	3	9.85	39.3
All	45	7.18	28.9	All	45	7.18	28.9

of NUSBAUM et al. (1965), smoking has been ignored as a significant source of lead in hard and soft tissues. It is of interest that lead levels in the lung generally appear to be similar for males and females in the absence of any adjustment for differences in smoking habits (e.g., see Table 1), whereas data to be published from our laboratory show that the male-female differences in lead levels in rib and vertebra (cf. Table 2) largely disappear if only non-smokers are considered. Further, using data for 39 male tissue donors, it has been found (KRAILO et al. 1976) that over 71% of the variation in the logarithm of the lead concentration in rib section B (cf. CHERRY et al. 1975) can be accounted for by a mathematical model involving the age, the square of the age, and the pack years of cigarette smoking. In addition, the coefficient of each term in this model was significant (at least at the 1% level) after adjusting for the effects of the other two variables. It is therefore recommended that every effort be made in future to obtain smoking histories when information about the tissue donors is collected.

The final matter concerns the section(s) of a particular tissue which are analysed. It is rare to find an explicit description of the anatomical sites within the organ which are sampled, and OPHUS and MYLIUS (1977) are to be commended in this regard. The practical test of any publication is whether another author can repeat the work, on his set of tissue samples, from the description given. A second consideration in tissue sampling is whether the site(s) analysed give values that are representative of the organ as a whole; such values are usually the quantity that is being sought. In the light of GIBBS and BOGDANOVIC'S (1974) work, there is some concern with the representativeness of OPHUS and MYLIUS' (1977) results for lung, although the effect is probably not large, and may be negligible in relation to the effect of leaching and/or non-standard drying conditions. A more extreme example is in the measurement of cadmium levels in lung by LEWIS et al. (1972); the only description of the sample is "4.0 g of lung", and with an organ for which the normal weight is ca 400 g, the possibility of an unrepresentative sample is obvious. Unfortunately, inhomogeneous intra-organ trace element distribution appears to be the rule rather than the exception -- for example, for cadmium in kidney (LeBARON et al. 1977), for lead in lung (GIBBS and BOGDANOVIC 1974) and in rib (CHERRY et al. 1975), and for magnesium in the heart (ANDERSON 1978). It is therefore recommended that future studies should not only describe explicitly the anatomical sampling scheme and use comparable tissue samples from each donor, but should also carry out sufficient work to be able to define the trace element levels in their samples in relation to those in other regions of the same organ. MYLIUS and OPHUS (1977) have attempted to do this for lung, but their results are unfortunately open to many of the same criticisms as their later report (OPHUS and MYLIUS 1977).

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