Trace elements and human disorders: application of neutron activation analysis methods

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INTRODUCTION
The role of trace elements in human metabolism and health is an increasing field of research (1-5), not only for the analyst who is confronted with the problems of accurately detecting elemental levels in the sub-nanogram range, but also for the biochemist, nutritionist and medical community as a whole. Evaluation of these elemental concentrations plays a fundamental part of understanding the ‘balance’ of human elemental needs, relating to dietary factors, absorption, transport, utilization and storage/excretion patterns. Subsequent variations in these needs through deficiency or excess can induce physiological changes (6-8).

The application of trace element measurements for diagnostic investigations into the state of human health and disorders depends on one major consideration — the accuracy and precision of the multielement analytical method.

MEASUREMENT OF TRACE ELEMENT LEVELS BY NEUTRON ACTIVATION ANALYSIS
Neutron activation analysis (nna) is a particularly valuable analytical technique, providing simultaneous multielement analysis with a minimum of sample handling and chemical preparation, where the systematic errors caused by contamination, analyte loss or human variation are minimal. Before the development of current analytical methods such as nna, electrothermal atomic absorption spectrometry (eaas) and inductively-coupled plasma spectroscopy, the failure to detect a particular element in a particular tissue implied its absence, whereas in fact it could mean simply that the method was insufficiently sensitive. Now that sub-nanogram, picogram levels can be measured with some degree of confidence, the ‘wide variation’ of existing levels has increased and also considerable attention has been given to the attendant risks of contamination. Table 1 shows the change in blood serum values in the stillbirths. However, the most important finding is the significantly (P < 0.001) elevated zinc levels in foetal livers for the stillbirths. Therefore it is suggested that possible zinc deficiency as shown in the stillbirth foetal blood sera may be directly associated with zinc immobilisation and accumulation within the foetal liver. This adverse effect on the development of the foetus may arise simply from the operation of more than one factor in isolation, for example the interaction of such factors as alcohol, smoking, a high phytate diet, dietary deficiencies of folate and exposure to environmental pollutants. Because these matters are complex, more samples from the South Wales area are being analysed.

Placental tissue has received less attention for determining the influence of elemental (nutrients and toxins) selectively between the maternal and foetal circulations. The cadmium (Fig. 1), lead (Fig. 2), and zinc (Fig. 3) placental levels (µg per g) for 100 cases from Barnsley, South Yorkshire, with a mean (range) gestational age (281; 245-300 days), and birth weight (3387; 2100-4810 g), show clear patterns as a function of birth weight. This clearly demonstrates the implications of the foetotoxic elemental (Cd, Pb) effects on foetal development and the cause/effect relationships with zinc deficiency.

Tobacco smoking has been known to have adverse effects on maternal and foetal circulations. The cadmium (Fig. 1), lead (Fig. 2), and zinc (Fig. 3) placental levels (µg per g) for 100 cases from Barnsley, South Yorkshire, with a mean (range) gestational age (281; 245-300 days), and birth weight (3387; 2100-4810 g), show clear patterns as a function of birth weight. This clearly demonstrates the implications of the foetotoxic elemental (Cd, Pb) effects on foetal development and the cause/effect relationships with zinc deficiency.

TRACE ELEMENTS AND HUMAN DISORDERS: BIRTH AND GESTATIONAL STUDIES
Monitoring of trace element levels in neonates can identify 'normal' and 'abnormal' levels at birth and thereby give a basis for all trace element studies on human metabolism and health. Elemental variations may be caused by nutritional requirements, physiological development, environmental exposure and any underlying disturbances of body chemistry. The influence of thalidomide in Europe revealed how seemingly benevolent drugs can seriously disrupt prenatal organogenesis(12). The general role of defective nutrition in causing disorders of foetal development (e.g. spina bifida) has been related to dietary folate deficiency(13-18). The connection between the toxicological antagonists (lead, cadmium) and sterility, abortion, and stillbirths (19, 20) are well known.

In view of these connections various investigations are being undertaken to evaluate the trace element levels in various British centres: Barnsley (South Yorkshire), Liverpool, Widnes (Merseyside), South/Mid-Glamorgan (Wales), and Oxford (Oxfordshire); together with some samples from Crete (Greece) and Soweto, Transvaal (South Africa). The human tissues (maternal and neonatal hair, placenta, umbilical cord, bone and amniotic membranes) and fluids (blood, amniotic, milk and urine) along with foetal organs, (brain, heart, kidney, liver, lung, skin) are being measured with regard to 'normal' pregnancies (in order to provide a baseline), and for such disorders as pre-term low birth weight and early membrane rupture, nutritional deficiency cases, neutral tube defects and spina bifida.

A pilot study of 25 'normal' pregnancies from Oxford(21) showed that a close similarity exists between the mean maternal and neonatal whole blood values, suggesting that the placenta normally provides little or no barrier to translocation of either essential or inessential toxic elements from the maternal to the foetal circulation. Analysis of amniotic fluid from pregnancies of various gestational membrane rupture periods(22) shows that for most elements there is no substantial difference between the various gestational periods. At the low analyte levels for amniotic fluid it is difficult to establish a potential for elemental analysis of amniotic fluid to diagnose pre-natal or post-natal congenital malformations.

As a result of the high incidence of birth malformations (in particular spina bifida) in South Wales, where ratios of 1 in 30 spina bifida per birth cases are known the elemental levels in the blood serum and foetal organs/tissues of 24 stillbirths and 20 social terminations were studied(23). The results for Cu, Pb and Zn are shown in Table 2 (for blood serum, foetal, kidney and liver). There is clearly a case of increased lead and low zinc blood serum values in the stillbirths. However, the most important finding is the significantly (P < 0.001) elevated zinc levels in foetal livers for the stillbirths. Therefore it is suggested that possible zinc deficiency as shown in the stillbirth foetal blood sera may be directly associated with zinc immobilisation and accumulation within the foetal liver. This adverse effect on the development of the foetus may arise simply from the operation of more than one factor in isolation, for example the interaction of such factors as alcohol, smoking, a high phytate diet, dietary deficiencies of folate and exposure to environmental pollutants. Because these matters are complex, more samples from the South Wales area are being analysed.

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influences on foetal development (24-26). Cigarette smoke can contribute significant amounts of cadmium to the total body burden(27). An estimated 0.1 μg Cd is inhaled from the mainstream smoke of each cigarette. The sidestream smoke contains even higher cadmium levels, and it has been shown that the livers and kidneys of long-term smokers contain much higher than average amounts of cadmium(28).

Table 3 shows the cadmium levels in tissues and fluids from the Barnsley neonate study as a function of smoking. There is a significant increase (S*, P between 0.05 - 0.001) in maternal whole blood cadmium for those women who smoke, and contribute significant amounts of cadmium to the total body burden(27). An estimated 0.1 μg Cd is inhaled from the cigarette smoke, as there is a very-highly significant (S**, P <0.001) increase in Cd for unwashed maternal scalp hair of smokers which can be removed by washing. Both neonatal scalp hair and maternal pubic hair showed no differences.

CONCLUSIONS
The development of a pre-chromatography separation procedure using hydrate antimony pentoxide (HAP), and its application to the multielement analysis of biological fluids and tissues, provide an accurate neutron activation analysis method for estimating the elemental variations associated with disorders of metabolism. The role of trace element concentrations in understanding state of health, and in the diagnosis, treatment, and ultimately the prevention of disorders, is not yet determined. However, with the development of analytical methods of suitable analyte, precision and accuracy at the nanogram, sub-nanogram levels (combined with facilities to remove possible contamination sources during sample collection, preparation and analysis), more understanding can be gained of the complexity of these disorders.

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Table 1: Reported chromium concentrations for blood serum since 1956

<table>
<thead>
<tr>
<th>Year</th>
<th>Analytical Technique</th>
<th>Mean (Cr)Ng/ml</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956</td>
<td>emission spectr.</td>
<td>185</td>
<td>82 - 308</td>
</tr>
<tr>
<td></td>
<td>(Monacelli et al.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1962</td>
<td>emission spectr.</td>
<td>55</td>
<td>10 - 390</td>
</tr>
<tr>
<td></td>
<td>(Niedermeier et al.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1969</td>
<td>atomic absorption</td>
<td>17.4</td>
<td>5.1 - 40</td>
</tr>
<tr>
<td></td>
<td>(Fieldman)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1972</td>
<td>aas</td>
<td>5.07</td>
<td>3.1 - 7.2</td>
</tr>
<tr>
<td></td>
<td>(Davidson and Secret)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>naa</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Kasperek et al.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1980</td>
<td>naa</td>
<td>0.160</td>
<td>0.0382 - 0.351</td>
</tr>
</tbody>
</table>

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Fig. 1: Placental cadmium concentration: birth weight.
Fig. 2: Placental lead concentration: birth weight.
Fig. 3: Placental zinc concentration: birth weight.