LEAD TOXICOLOGY AND NEUROTOXICOLOGY

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INTRODUCTION

A controversy has continued over many years in both popular, scientific and medical press around the presentation of the case against environmental lead exposure to the general population. This has awakened the scientific and medical community as well as the general public to the importance of this subject. That is not to say that the medical, scientific community was unaware of the nature, extent or seriousness of the problem but rather that they were struggling to adequately define its provenance.

Recognition of a neurotoxin usually begins with the identification of often dramatically high dose effects. As knowledge of the biochemical toxicity of the toxin broadens, more sensitive measures of toxicity are developed and the threshold for effect is revised accordingly downwards. Vulnerable segments of the population are then identified and the question of whether ordinary community exposure is toxic is raised. Epidemiological and experimental studies are then conducted to define the extent of the problem and to elucidate the potential toxic mechanism(s). This is exactly the course of events that has occurred with lead. effects are certainly not in dispute. Knowledge of such an illness of the nervous system, caused by lead extends historically to antiquity. Indeed, Dioscorides in the first century A.D. foreshadowed us – "the mind gives way, the limbs are paralysed". The second category of chronic lead poisoning is more of a problem today. It occurs as a result of industrial exposure to relatively high concentrations of lead over a lengthy period, and results in insidious but discernable health effects such as changes in neurological function, kidney damage and changes in cardiovascular function. Until relatively recently it was thought that lead poisoning was almost exclusively a disease of industrial exposure. Presently, great interest is being shown in the possibility of insidious effects resulting from long-term exposure to environmental levels of lead. The profound effects of overt lead poisoning are not in dispute but great controversy exists in the literature as to the effects of lesser exposure and indeed at what level of exposure they begin to occur. A vast literature of data has been compiled over the years on the toxic effects of lead, using animal models of exposure. The first generation of studies on the subject revolve around the gross effects of very high levels of lead exposure in animals (Table 1). These extended to a

second generation of studies in which more subtle deficits in humans were studied at much lower levels of exposure and, at the current time tertiary emphasis clearly lies upon the prospective and retrospective study of such exposure. This third generation of studies has palpably been unable to present any convincing new results on the subject, rather they have reiterated the findings of older work and in many cases only added further to the confusion which revolves around the exact relationship between over-exposure to lead and neurological deficit.

LEAD AND THE NERVOUS SYSTEM

The tissue most prone to lead toxicity is the brain and central nervous system (CNS), the tissue least capable of repair. As a result, of all of these effects of lead the one which has attracted the most attention are the effects upon the nervous system and therefore upon cognitive function. Numerous studies, stretching back into the last century, have shown that lead can clearly have an effect on learning potential. The effect on the nervous system which can be observed both biochemically and by histopathological techniques, might be considered to have two components. Firstly, an effect on nervous system development and growth, and thus upon nervous system morphology /1/ an effect which is almost certainly largely irreversible and will result in permanent neurological changes especially where exposure occurs in early life; and secondly, an action on various biochemical systems to alter the concentration of essential neuroactive metabolites. This action, in contrast to the first one, is probably reversible on removal of the toxin.

This type of neurological effect is most important in the youngest members of the community. The newborn, or indeed unborn, child is most prone to insult from excessive exposure to lead and in these children, the greatest social and economic consequences can be seen in the long term. Lead has been long known to have abortifacient properties. In this respect it is evident that the toxin lead is well capable of acting in utero, the very time that the human body is probably at its most vulnerable with respect to development of its organs especially the nervous system. The blood-brain barrier which is permeable to lead, is not as well developed in the young as in later life. Bryce-Smith and his colleagues /2/ have demonstrated that stillbirths contained between five and ten times the lead that was observed in live newborn, while Wibberly's group /3/ have also demonstrated a significant increase in placental lead in those infants who failed to survive both birth and the neonatal period. An interesting study with possible detrimental implications with regard to the foetus was carried out by Alexander & Delves /4/. These workers noted that maternal blood lead fell during pregnancy, the magnitude of the fall being greater than would be expected by the dilutional effect of the increased plasma volume normally observed in pregnancy. The authors put forward several possible explanations for the fall:

1. A displacement of the equilibrium which exists between the maternal blood and soft tissues or skeletal tissue
2. Transfer of lead from the mother to the placenta or foetal tissue
3. Enhanced maternal excretion by way of the urine
4. Altered lead exposure, for example as a result of moving from the work to the home environment or a change in smoking or dietary habits.

Obviously if the second explanation were true the foetus may well be exposed in utero to relatively high concentrations of lead presumably if the mother has herself a significant lead burden.

One aspect of the scientific problem is to define the exact relationship between exposure and the bodily accumulation of lead. This in part has been answered by the studies carried out in Great Britain on pulmonary exposure to lead /5/ and in the duplicate diet studies in the West of Scotland /6/. In these studies there is clear evidence that increase in blood lead, and therefore bodily lead concentrations, does not follow a linear relationship with exposure but rather a curvilinear one. The most commonly applied equation is where the blood lead varies as the cube root of the exposure vector although various other mathematical formulations can equally well be applied /7-10/. The various studies of neurological and psychological function carried out to date have been unable to define a level of exposure to lead below which no neurological deficit can be found. However, studies of the neurochemistry of lead would suggest that it can potentially influence nervous system function at levels of environmental exposure which are less than the lowest ones found in the world today. Controversy exists concerning the definition of elevated lead exposure. In the United States of America, an upper limit of acceptability and action level of 1.5 uM of blood lead (30 μg/100 ml) has been set by the Centre for Disease control /11/ for the prevention of lead poisoning in children. It has, however, been quite impossible in all practical studies to disentangle those components of effect that can be unequivocally attributed to lead and not to other factors disadvantageous to mental development such as social background, malnutrition and a host

of other factors which may be found in abundance in socially deprived areas.

METHODOLOGICAL CONSIDERATIONS OF A STUDY

The only satisfactory method of assessing body lead burden is blood lead concentrations. Other measures, such as urinary lead concentration, concentrations of lead in hair and teeth, or secondary measures of lead exposure all have marked disadvantages. Blood lead also has disadvantages but these, in general, are rather less than the problems associated with the use of other indices of exposure. Although blood lead gives a good marker of current exposure to this toxin, it does not generally show any indication of cumulative body lead burden, for which purpose lead concentrations in bones or teeth are required.

The most important factors to determine the effects of lead on the nervous system include the duration and intensity of exposure to the metal and the age of the subject at which exposure commenced. Most evidence would show that the younger a subject is when exposed to lead, the more likelihood there is of subsequent neurological damage and, in consequence, children, especially newly born children, must be considered to be a group at special risk. In epidemiological studies, a number of particular problems may be experienced associated not only with choice of subjects but also with the accuracy and precision of lead measurement. Subject choice has lead to a major level of criticism in a number of studies where there was inadequate control of compounding co-variables. Biological variability must be considered: that is at similar levels of exposure, some subjects may manifest central nervous system symptoms, while other may not. The confidence with which performance deficits, if observed, can be attributed to past lead exposure, depends on whether the study has successfully addressed methodologic issues. There are four well recognised basic methodologic problems which have been identified and considered to varying degrees in various studies /12/.

1. *Inadequate Markers of Exposure*

"Low level lead" is a vague and ambiguous phrase and if exposure is not assessed adequately then it is most unlikely that an existing effect will be identified. Blood lead measurements reflect recent lead exposure. When investigating children, they may well have acquired a considerable lead burden as infants and toddlers which will not be reflected in a blood lead measurement. Similarly a single blood lead estimation can reflect a temporary and perhaps unimportant rise. It is a very serious drawback that blood lead measurements are not able to distinguish between short-term and long-term lead intoxication, since it may well be that only chronic lead exposure is damaging. More appropriate, is the use of serial blood measurements or alternatively, tooth lead estimations. Data has shown that primary dentine of deciduous teeth is a useful index of lead exposure in the six months in utero until tooth eruption. Following eruption until it is shed lead exposure is better reflected in circumpulpal dentine /13, 14/. Thus tooth lead content reflects cumulative exposure to the metal. There is a limitation in using deciduous tooth lead as an index in that one is confined to those children who are at the age of shedding such teeth.

2. *Insensitive Measures of Performance*

At present only the grossest changes in functioning of central nervous system can be reliably detected. Attributes which have long been regarded as associated with central dysfunctions such as attention span, distractability and poor learning cannot be readily measured and certainly not by rating scales. Where tests of cognitive development and function are available, many are standardised for children older than three to five years and are therefore not entirely suitable for this age group. It is of course the younger child and toddler who is at greatest risk to the adverse health effects of lead.

3. *Biased ascertainment of subjects*

Selection of individuals from a relatively restricted source may lead to a reduction in the population to which findings may be generalised. In addition restriction to a population most at risk for the target condition, in this case lead exposure, increases the possibility of establishing a true effect. Subject selection may be biased if factors leading to non-participation are related both to lead level and to other factors, such as lack of parental interest or unwillingness to co-operate within the structures of the study protocol: subjects who enter a study may differ in a systematic fashion from those who reject participation. The study must recruit a large

enough sample size in order to be able to detect subtle defects.

4. *Inadequate Identification and Consideration of Non-Lead Related Confounding Variables that Effect Development*

Poor educational attainment is clearly related to a wide range of indices of social disadvantage. Measures such as these also appear to be related to higher lead exposure, and thus it is necessary to demonstrate statistically that lead and attainment are still related after the influence of mediating social factors are taken into account. Most hard to control are the genetic factors as estimated from parental I.Q., socioeconomic status, and early parent-child interactions all of which greatly influence the child's cognitive development. Deficiencies in the general home environment during the vital first two years of life can aggravate the risk of increased lead absorption. Studies have shown that children of lower socioeconomic status tend to have higher blood lead concentrations than those from a more privileged background /15/. There are a great many medical and social conditions which can cause or contribute to the deficit of measured intelligence. A carefully controlled study must incorporate appropriate methods of statistical analysis to assess the relative contributions made by the various factors. It must also be considered that there are medical conditions which may have an adverse effect perhaps only transitory on a child's response to an I.Q. test. Although most studies exclude permanently brain damaged children, a minor degree of illness such as infection, anaemia, or poor nutrition can cause a temporary lack of responsiveness and most psychological tests are performed on a single occasion. Although it is impossible for an epidemiological study to achieve complete control of an infinite number of variables that contribute to outcome, it is vital to take into account as many variables as possible. Where differences are small as they are in studies reporting positive effects of low level lead exposure, biases are critical. An additional problem is that even where confounding variables are identified and measured as reliably as possible, there are no fully satisfactory statistical methods for their control. Commonly employed techniques, such as analysis of covariance, multiple regression or matched pairing on all known confounding variables incompletely corrects. It has also been pointed out that there is a subtle but distinct difference between ""backwardness" and "underachievement" which must be observed.

5. *Multiple Comparisons*

By far the most important factors are the issues of confounding variables and assessment of lead exposure. However, it should be noted that although it is desirable to employ several measures of behaviour and intelligence, in any large number of independent statistical comparisons, a specified proportion will reach statistical significance in the absence of a true effect. A significance level of 5% implies that 1 to 20 of the differences will be a chance finding.

CLINICAL MANIFESTATIONS OF LEAD POISONING

Lead poisoning will result in severe neurotoxic effects and the features of such poisoning have been well described. Within 1-2 weeks of high levels of exposure a number of indications of neurological change including dullness, restlessness, irritability, poor attention span, headache, tremor, hallucination and loss of memory, which can progress to delirium, mania, convulsions, paralysis, coma and death can be observed. In general this kind of presentation will not be found in adults until blood lead concentrations are in excess of 6 μmol/l. although in some cases acute features of exposure have been observed as low as 4 μmol/l. In addition to central effects on the nervous system, a number of peripheral effects may also be found relating particularly to changes in nerve conduction and to segmental demyelination and axonal degeneration. Such effects are due to the influence of lead on the Schwann cells and it has been shown that where lead exposure is terminated, especially in animal studies, these cells can carry out remyelination repair processes. By observing such changes, it is clear there is a large level of biological variation. Whilst most workers have not found changes in nervous function below 4 μmol/l a few workers have found some clinical signs and symptoms that may be seen as low as 2 μmol/l. But it must be remembered that in most cases lead measurement only reflects a point measurement of exposure at the time of examination whilst changes in nervous tissue may have accrued over a considerable period of time and may not be directly correlatable with the current level of blood lead in circulation. In general, therefore, acute lead poisoning may be evidenced by a number of neurological signs and symptoms which will affect both the central and peripheral nervous systems. These effects may be observed at blood lead concentrations as low as 2 μmol/l. but generally are only found at very much higher levels

consistent with much greater degrees of exposure to the metal. The signs and symptoms of lead poisoning may persist for many years after cessation of exposure.

In chronic lead exposure associated with relatively lower exposures that might be found in acute poisoning, there are reports of changes in nerve conduction velocity and in psychological performance. These studies have generally been carried out in workers in lead-using industry and usually relate to blood lead concentrations lying in the range of 2.5-4.0 μmol/l, although changes in nerve conduction velocity are reported to have been found as low as 1.5 μmol/l. Again there is some controversy about the levels of effects. Whilst some workers have found little difference in psychometric performance tests others have shown changes in visual motor performance, I.Q., hand dexterity, mood, nervousness and coping. In general however, the most convincing evidence of change at these levels of exposure relate to changes in nerve conduction velocity. Whether such changes represent any clear change in motor function is, however, still open to discussion.

The one area of investigation which has received attention beyond all others is the effect of relatively low levels of lead exposure in children. At high levels of exposure where lead poisoning has occurred, the effects in children are identical to those seen in adults, although in the child the quantity of lead, especially blood lead, associated with such effect is usually much lower than that observed in the adult. Where encephalopathy occurs there is a highly significant mortality rate which has been reported as 5-40% and as high as 65% before chelation therapy was introduced. At the current time, chelation therapy of choice would be using calcium disodium ethylenediaminatetraacetic acid although oral treatment with penicillamine has also been shown to be useful. The neuropathological findings in children are essentially the same as for adults with cerebral oedema, perivascular glial proliferation and capillary permeability. The damage, however, is variable and is due in part to anoxia and cellular ischaemic change. As part of the syndrome of acute lead poisoning in children, neurological sequelae have been observed following lead exposure.

HISTORICAL BACKGROUND TO CHILDHOOD STUDIES

Research began at the turn of the century when the risks for children especially became apparent. Gibson in Australia described a lead-induced optic neuritis in a child and identified lead paint as the source /16/. This observation was rapidly followed by a case noted by Ruddock in 1924 /17/ when he described lead poisoning in a child the cause of which was pica. For many years it was generally assumed that upon recovery from the acute phase of intoxication, children were left without significant residual deficit in central brain function. The classic paper of Byers & Lord /18/ put an end to these beliefs. Randolph Byers demonstrated that children who were assumed to be asymptomatic following an episode of lead intoxication were, in fact, profoundly impaired. Following through the early school years of twenty children who had previously sustained episodes of clinical lead poisoning as evidence by clinical symptoms such as encephalopathy and peripheral neuritis with lead lines on x-ray, nineteen subsequently exhibited impaired cognitive function and altered behaviour; shortened attention span, antisocial behaviour, impaired visual-motor coordination. Considering this data, it is reasonable therefore to look for lesser impairments after a less extreme exposure to lead.

Spurred on by Byer's classic paper, a great wealth of data on the postnatal neurobehavioural and developmental effects of lead exposure have appeared during the 1970's and 1980's. During the 1960's screening programmes were set up to determine how widespread the problem of "elevated" lead exposure amongst children was, if such a definition of elevated could be decided on. In 1972, an article in the New England Journal of Medicine summed up the dilemma that confronted health workers and researchers of that decade, and indeed this one, as follows:

> "a critical question...is whether lead can damage the central nervous system of young children in the absence of overt signs and symptoms referable to that system"
>
> Lin-Fu 1972 /19/

The observations of Byers and Lord have been confirmed in a number of other studies in which lead poisoning has occurred without any evidence of life-threatening encephalopathy. Following such over-exposure to lead, chelation therapy has been demonstrated to ameliorate the likely results of such over-exposure. It does not, however, guarantee return to absolute normality. Where encephalopathy has occurred there is increased incidence of permanent neurological and psychological dysfunction. The morphological effects in these cases are essentially the same as those for adults. Examination of the brain shows cerebral oedema and perivascular glial proliferation. The neurotoxic effects described so far relate primarily to the very evident cases of lead poisoning, intoxications

where blood lead concentrations are in all cases well above the ranges of limits of exposure that have been proposed throughout the world. At the current time, attention has concentrated upon the effects of much lower levels of exposure to lead but it is this type of exposure that has generated the greatest amount of controversy over the past two decades. Indeed at the present time, research in this area has not defined, or been able to define, either a level of lead below which no neurotoxic results can occur or whether the point of exposure to such levels of lead with subsequent treatment have any long term sequelae.

The many studies performed in adults and children can be separated into two types; those investigating the neurotoxic effect of lead on the peripheral nervous system and those studying the central effects. Several lengthy reviews of available data prior to 1980 have been published /12, 20, 21/ each attempting to draw conclusions from the wealth of confusing data published. Effects of lead on the central and peripheral nervous systems will be considered separately.

EFFECTS ON THE CENTRAL NERVOUS SYSTEM

Multiple approaches have been taken to investigate the central neurotoxic effects of lead. Two basic approaches have been undertaken:

1. The indentification of a population with minimally increased lead levels and search for a central nervous system dysfunction, or
2. The converse, that is the investigation of a population with central dysfunction in which to establish lead levels.

This type of study diminishes dramatically the sample size required to examine effects. The net information that accrues from them is generally the same no matter the reasons or means of exposure or the levels of investigation applied thereafter.

Within the second approach, many studies have shown significantly increased lead in the blood of mentally retarded children /22-27/. Many of these studies have selected three groups:

1. Normal subjects
2. Mildly retarded subjects whose retardation is of unknown aetiology
3 Retarded subjects in whom the origin of abnormality is known.

The incorporation of this third group is vital since these studies are subject to a cause or effect problem. The presence of increased blood lead in retarded compared to normal children does not prove an aetiological relationship between mental retardation and raised but non-encephalophathic blood lead levels. Critics suggest that the mentally retarded children may well ingest lead more readily than their normal peers and are generally predisposed to an increased lead burden. However, it is noted in these studies that there is no significant difference in blood lead in the mentally subnormal children of known aetiology by comparison with control normal children /25, 29/. A supportive observation to the hypothesis that lead is involved in the aetiology of the retardation in these children, is that these subjects exhibited hyperactivity, a feature said to occur as a result of lead exposure. The data presented by David et al /25/ has however been criticised /29/ for several reasons. Firstly, the authors failed to consider any confounding variables such as parental factors and social class, which may be related to the retardation. Additionally, it should be noted that the blood lead levels observed in this group of "unknown aetiology" mentally retarded children were not extremely high with the mean being 1.2 μM (25 μg/100 ml) and the highest value being in the region of 2.6 μM (54 μg/100 ml). A slightly different approach has been taken in the retrospective study of Moore et al /26/. This group obtained phenylketonuria cards containing blood spots obtained a few days after birth from mentally retarded children, again of unknown aetiology. A clear correlation between mental subnormality and blood lead was observed. Beattie et al /23/ reported a significant correlation between high levels of lead in household water supplies used during pregnancy and the first year of life and mental retardation in children born to mothers using such water. The case that lead is a causal factor in this impaired intellectual development is more strongly supported in this study since the children's lead exposure cannot be explained by their behaviour. The doubt does remain, however, as to the fine distinction that exists between unknown and known aetiology with regard to subnormal mentality. Other studies have obtained similar data. Youroukos et al /27/ also observed that the mean blood lead of mentally retarded children exceeded that observed in either a retarded group but of known aetiology or a control normal group. A group in Wales however have noted no difference in water lead content of educationally subnormal children compared to normals /30/ and have sewn doubts on the influence of lead on mental retardation. These doubts however only stand if a significant proportion of the lead these children are exposed to is derived from water. The problem with this type of study is that it is extremely difficult, if not impossible, to prove that the mental

retardation is a function of over-exposure to lead. Although studies have shown that lead exposure is associated with mental retardation, whether or not this is because the children are mentally retarded and thus unknowingly expose themselves to greater concentrations of environmental lead or whether the environmental lead has led to the mental retardation is far from clear.

The other traditional approach which regrettably requires very large population numbers, are the various forms of general population studies especially in areas where lead exposure is high. Children with known lead exposure have been identified and resultant deficits in brain function sought. The primary requirement of these studies is the identification of a properly matched control group of children for comparison. The means of assessment of overall exposure to lead has varied. The classical studies of Needleman and co-workers concentrated on tooth lead analysis. Other workers have used blood lead analysis and a few, erythrocyte protoporphyrin levels. In all of these studies the actual source of exposure to lead is of little relevance. The main point is that appropriate psychometric testing be applied to the children. The tests that have been most properly applied in this respect are the Bayley scores although Stanford-Binet, Wechshler, McCarthy, Peabody, Bender-Gesalt and various other tests of educational ability, adaptive functioning and general psychological performance have been used.

De la Burde & Choate /31, 32/ performed a longitudinal study on children at the ages of four and seven, who as toddlers, had blood lead concentrations in excess of 1.9 μM (40 μg/100 ml) or lead lines in the long bones of radiography. Psychological testing at school age indicated deficits in behavioural tests, I.Q., visual and fine motor coordination. Not surprisingly, there was no significant difference in blood lead concentrations between the previously lead exposed children and a lesser exposed group, since blood lead reflects the recent exposure of the individual. Mean tooth lead contents, a more cumulative and long-term exposure index, were however divergent. In a study of black preschool age children in New York, who were divided into a low lead group whose blood lead was less than 1.4 μM (30 μg/100 ml) or a high group if the blood lead exceeded 1.9 μM (40 μg/100 ml), psychological testing indicated impaired cognitive and perceptual performance in the high lead group. This study controlled for covariants such as parental intelligence, age and birth weight /33/. Other studies have investigated children with known lead exposure. Albert et al /34/ observed New York children in which, not surprisingly, those who previously had exhibited symptoms of encephalopathy and lead exposure had a deficit in I.Q. However, those who were asymptomatic but whose blood lead exceeded 2.9 μM (60 μg/100 ml) did not differ significantly from controls with respect to I.Q. although they had more attention and concentration difficulties. No lead related deficiency in cognitive tests were observed in children whose blood lead exceeded 2.4 μM (50 μg/100 ml) compared to those with a lead level less than 1.9 μM (40 μg/100 ml) – Baloh et al /35/. A greater percentage of the "high" lead group however were considered hyperactive by either the parents or teachers.

A great many studies have selected a group of children residing close to a lead source such as a smelter, and compared them to a similar group living further away, and therefore presumably less exposed. Whereas some studies have demonstrated detrimental effects of lead, others have not. One much quoted study performed in the east end of London and which failed to demonstrate a detrimental effect of lead, is that of Lansdown et al /36/. After measuring I.Q., and classroom behaviour in two groups of children, living either close or more distant from a lead smelter, no relationship was found between I.Q. and either distance from the smelter or blood lead. However, grave doubt is placed on the results of this study as selection of the "control" group was performed rather inappropriately. The group of children residing further from the lead source exhibited an increased proportion of disturbed children, and as a result lower levels of intelligence and higher rates of disturbance were more related to social factors than lead exposure. In the vicinity of a lead smelter in El-Paso, Texas, Landrigan et al /37, 38/ divided a batch of asymptomatic children into two matched groups exhibiting a blood lead of either less than 1.9 μM (40 μg/100 ml) or greater (observed range 1.9-3.9 μM (40-80 μg/100 ml). The groups were matched for socio-economic status, sex, length of residence and proximity to the smelter and the language spoken at home. Using both a parental questionnaire and psychological testing performed by a physician, subtle but significant impairments in non-verbal cognitive and perceptual-motor skills as measured by using the Wechsler Intelligence scale, were noted. Performance I.Q. was impaired by 8 points but there were no significant differences in verbal I.Q., behaviour or hyperactivity between the groups. In the same location, an independent study conducted by McNeil and his colleagues /39/ dividing asymptomatic children on the basis of proximity to the smelter, failed to detect any lead related defects by means of physical, neurological, psychometric or school performance

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measures. The Werry-Weiss-Peters scale for hyperactivity also did not detect any difference as a result of lead exposure. The control group in this study lived in another area of El-Paso. These results may not be inconsistent with the data obtained by Landrigan and co-workers since they employed a different research strategy. Following the publication of the Lawther report /21/ two members of the committee carried out a study near a leadworks in London (Landsdown et al, 1981; Yule et al, 1981), the results of which generally contradicted the conclusions of their earlier study /36/. This later study reported a 7 point I.Q. difference related to blood lead, a deficit which remained after subtraction of the component of I.Q. related to a varying degree of social class. The range of blood lead values observed was in the range 0.3-1.6 μM (3-33 μg/100 ml) with mean 0.7 μM (14 μg/100 ml). Blood lead was significantly related to attainment scores on tests of reading, spelling and intelligence but not mathematics, even after social class effects had been partialled out. One comment on the paper however, was that the blood lead measurements which reflect recent exposure, were taken 9 to 12 months prior to psychological testing. Teachers were asked to complete three behavioural rating scales, including the eleven item forced-choice scale employed by Needleman et al /40/ on each child. The pattern of results obtained in this British study was similar to that observed by Needleman's group. A battery factory in Birmingham provided the lead source for a study performed by Hebel and colleagues /41/. The data suggest a tendency, though not statistically significant, for children living closer to the factory to score 1-2 points lower on verbal reasoning. In another study a non-significant reduction in I.Q. of 108 in a "moderate" lead group (mean 1.4 μM, range 0.9-1.7 μM; 28 μg/100 ml, 18-35 μg/100 ml) compared to 102 in a 'high' group (mean 2.1 μM, range 1.7-3.1 μM; 44 μg/100 ml, 36-64 μg/100 ml) was observed in children again residing close to a battery works in Manchester (Ratcliffe, 1977). The lead estimations were however performed at the age of two whereas psychological testing did not take place until school age.

Much of the remaining data available relating to the central neurotoxic action of lead is obtained from general population studies, some of which have been performed in the United Kingdom. The possibility therefore exists that these studies have been performed in areas where social and educational conditions differ from Britain. It is noticeable however that the general trend in all of these studies whether statistically different or not is towards decreased I.Q. in conditions of high lead exposure.

Undoubtedly the largest population study was carried out in two primarily white working class towns adjacent to Boston, Massachusetts /40, 43/. This was a well designed study which attempted as best as is possible to take into consideration confounding non-lead related variables. Considering other methodological problems the first design advantage over many other previous studies was the use of an alternate means to blood lead in order to assess lead status of the children. In this study more than 2,000 asymptomatic children were recruited and body lead burden assessed by way of the lead content of shed deciduous tooth dentine, a reflection of cumulative past exposure to the metal /44/. From these levels the extremes were selected, the highest and lowest tenth percentiles. The high lead group had a tooth dentine lead level of 20 μg/g or more, while in the low group the lead content did not exceed 10 μg/g. For the purpose of comparison with other studies, blood lead levels in these groups were measured, and observed to be 0.9-2.6 μM, with mean 1.7 μM (18-54 μg/100 ml; 35.5 μg/100 ml) in the high group and 0.6-1.8 μM with mean 1.2 μM (12-37 μg/100 ml; 23.8 μg/100 ml) in the low group. These figures indicate quite clearly that blood lead cannot be interchanged with tooth lead levels for the purposes of selecting "high" and "low" lead groups. Each child included in the study of these extreme groups with regard to body lead burden, underwent a 4 hour neuropsychological examination. In addition, the mother of the child completed a lengthy questionnaire designed to test 39 non-lead confounding covariates. Comparison was considered for physical, medical, socioeconomic, family variables and also parental attitude, namely aspirations for the child, home learning environment, attitudes to school and the child, and restrictiveness. Teachers provided crude rating measures of behaviour by way of an 11 question forced choice questionnaire covering distractability, non persistent, disorganised, hyperactive, impulsive, easily frustrated, day dreamer, doesn't follow a sequence of directions and low overall functioning.

Needleman paid great attention to the problem of confounding non-lead variables. The groups were broadly similar on background variables, but the high lead group were slightly older at the time of testing, slightly more socially disadvantaged and had parents with a slightly lower I.Q. As a result, 5 covariates were included in the analysis. The battery of tests of neuropsychological function were analysed by analysis of covariance, and

the outcome demonstrated that the two groups differed significantly on several tests including verbal intelligence, verbal and auditory processing, attention span and full-scale I.Q., the deficit being 4.5 points as measured by the Weschler Intelligence Scale for Children (WISC). The high lead group had a corrected mean full-scale WISC-R I.Q. of 102.1 compared to 106.6 in the low lead group, a difference which was highly significant. The high lead group also scored significantly lower on 9 of the 11 indices of classroom performance rated by teachers. Although the teachers reports showed increased distractability and prevalence of daydreaming, and lack of persistence and an inability to follow a series of directions, all features said to constitute "hyperactivity", this feature was not specifically reported to be elevated. This increase in frequency of detrimental classroom behaviour was reported to be dose related. Statistical analysis demonstrated that none of the non-lead confounding variables could account for the observed difference in I.Q. Although the difference in I.Q. of 4.5 points is statistically significant one should consider the difference in another light. It must be pondered over as to whether this difference loses significance when compared with the conventional standard deviation of 15 points employed on the WISC-R scale. Another point to note is that the occurrence of pica was three times as common in the high lead group as in the low group and yet was not included as a covariate; 30% of the high group exhibited pica in comparison to 11% in the lower group. Is then, pica not a marker for pre-existent behavioural deviation, and hence if this were the case, the increased lead burden would be an effect rather than a cause of the deficit? Data however indicates there is no relation between the pica and the teachers ratings and therefore this is an unlikely proposition /43/. It would have been interesting to know whether the intermediate group was also intermediate psychologically since if it were not, then it would weaken the argument that the association represented a causal influence of lead.

Subsequent to this study, supportive data of altered central nervous system functioning as a result of chronic low lead exposure in these children was obtained by Burchfiel et al /45/. A random subset of the same group of children was selected for quantitative electro-encephalographic (EEG) pattern analysis. The data in this study shows an overall altered pattern of alpha and delta waves. Measurement of brainwave energy over four wavebands, delta (0.5-3.5 Hz), theta (4-7.5 Hz), alpha (8-12 Hz), and beta (12.5-91.5 Hz) indicated a significant increase in low frequency delta waves over the central parietal and occipital cortices and a reduction of alpha waves over the parietal and occipital regions. Re-analysis of the same data obtained by Needleman et al /40/ to investigate a possible deficit in the child's I.Q. from the expected I.Q. as calculated from maternal I.Q. was performed by Bellinger & Needleman /46/. A regression line for the maternal I.Q. and child's I.Q. was calculated for the sample as a whole, that is high and low lead groups together, and also for each group separately. Then, if the complete sample regression line is used to obtain an estimate of the child's expected I.Q., the difference between the child's observed I.Q. and this expected I.Q. can be calculated. This difference differed significantly between the two groups. The observed I.Q. was on average 3.94 points (± 11.57) greater than expected. The next question to be asked in this study was if the difference varied with the child's dentine lead. Within the low lead group the correlation was essentially zero. In the high group however, the difference was significantly correlated with dentine lead. In summary, for every part per million rise in dentine lead over 20, the observed I.Q. fell 0.42 below that expected on the basis of maternal I.Q.

If it could be shown that a reduction in body lead was followed by intellectual gains and/or behavioural improvement, powerful evidence would be provided for a causal link between lead and behaviour. Chelation studies theoretically provide a suitable model, but such studies to date suffer from methodological drawbacks. Pueschel et al /47/ reported a rise in I.Q. of 8 points amongst children who had previously had raised blood lead and many of whom had symptoms of exposure, and who subsequently had chelation therapy. Hyperkinetic children with blood lead in excess of 1.2 μM (25 μg/100 ml) were studied following chelation therapy /24/. The authors concluded that those children whose hyperactivity was of unknown cause showed improved behaviour following treatment while those whose abnormal behaviour was of a known origin, failed to improve.

Although the vast majority of data relating to the central neurotoxic action of lead has been obtained in studies of young children since these are the most vulnerable members of the population, a handful of studies have relatively recently been performed in adults.

In a Danish battery plant, a study of males exhibiting blood lead concentrations in the range 2.8-4.0 μM (58-82 μg/100 ml) having neuropsychological testing, showed that they lacked concentration and memory: I.Q. however was normal or above average /48/. At a slightly lower level of exposure, in a foundry, a study by Baker et al /49/ demonstrated an increased rate of

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5-YEAR FOLLOW-UP STUDY

BRAINSTEM AUDITORY EVOKED POTENTIAL COMPONENT v LATENCY ADJUSTED FOR H.O.M.E. SCORE

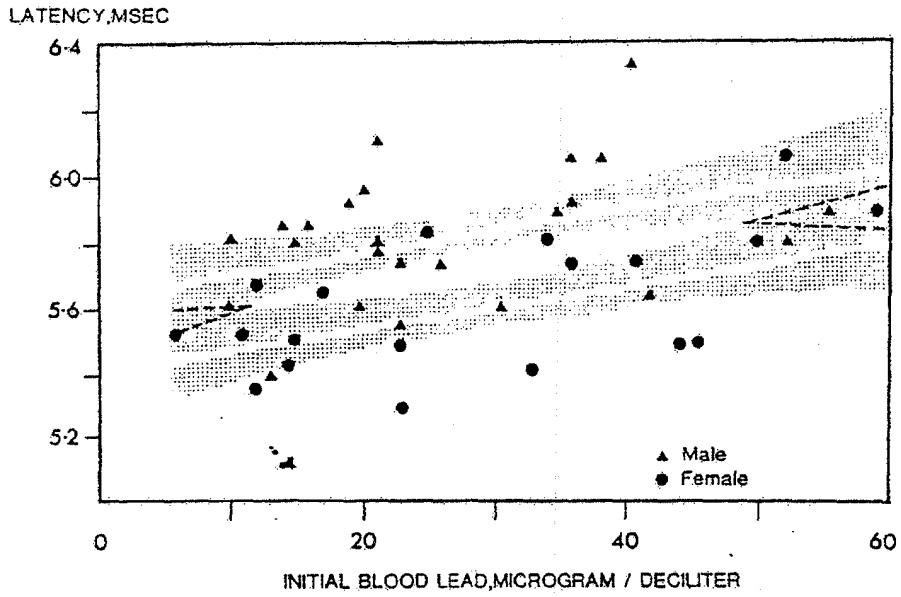

Fig. 1: The influence of lead on auditory evoked potentials in children. (Diagram by courtesy of Dr. D. Otto).

non-specific symptoms such as depression, confusion, anger, fatigue and tension. Those workers who had a blood lead between 1.9-2.9 μM (40-60 μg/100 ml), also exhibited impairment of other aspects of neuro-behavioural functioning including verbal concept formation, memory and visual-motor performance. Zimmerman et al /50/ divided workers at an electric storage battery plant into a "low" group who had a blood lead of less than 1.7 μM (35 μg/100 ml) or a "high" group whose blood lead fell within the range 2.2-2.9 μM (45-60 μg/100 ml). An additional group of "non-exposed" persons who were locally employed male nurses were also included in the study. Each volunteer was asked to complete a questionnaire of psychological and physical symptoms. The "high" lead group reported a significantly greater number of symptoms especially neurological than either the "low" lead group or the control group which did not differ from each other. This study attempted to control the bias which may exist when asking lead workers to self-report symptoms when they are very aware of the dangers of lead exposure. The inclusion of an additional "low" lead group alleviated these problems. Further electrophysiological evidence of central nervous system dysfunction as a result of lead exposure comes from Otto and coworkers who have performed 3 successive evaluations of a single group of children, comprising an initial assessment followed by 2 follow-up studies at 2 and 5 years /51-54/ (Figure 1).

In conjunction with these studies, an independent group of children was studied /55, 56/. The children involved in this study by Otto were aged 1-6 years in the initial evaluation, 3-4 years at 2-year follow-up and 6-12 years at 5-year follow-up, whilst those in the replication group were aged 3-7 years. The children were from low income black families exposed to lead from a variety of sources. Contamination of dust derived from the clothing of workers from a battery manufacturing plant, and the use of leaded paint in older housing featured prominently in the range of lead sources.

Lead status was performed by blood lead concentration before electrophysiological data was accrued. The data obtained showed an alteration of EEG potentials which were changed in a linear fashion with blood lead over the observed range 0.3-2.7 μM (6-55 μg/100 ml).

Thus there may well be a continuum of effects of lead on the central nervous system either with no or a very low threshold level of exposure. These changes in brain function appeared to remain over the two year interval between the studies despite a fall in blood lead.

The data obtained at 5 years follow-up is uncertain, as to the effect of lead on electrophysiological processes. Such effects on the brain observed at the 2-year follow-up and initial assessment, were not noted in these older children. It is not certain, however, whether this indicates a lack of effect of lead in older children or alternatively, a reduction in lead exposure.

It is most probable that there are differing effects of lead on the various nerve types. Data obtained in the rat would suggest that there is a differential vulnerability to the neurotoxic effect of lead within the different nerve fibres /37/.

As with all studies, there is a need for independent replication. Winneke et al. /59/ performed a similar population study to that of Needleman et al /40/ but on a smaller scale. 458 school age children (aged 7-10 years) in Duisburg, Germany, were selected and segregated into "low" and "elevated" lead exposure groups by way of incisor tooth lead content. The low group had a whole incisor tooth lead content less than 3 parts per million (mean 2.4 ppm), while if the lead content exceeded 7 parts per million the child was classified as "elevated exposure" (mean of group being 9.2 ppm). The elevated group represented approximately the upper 15% of the distribution which covered the range 1.4-12.7 μg/g. Non-lead variables were accounted for by pair matching the children for age, sex and parental occupation.

Several psychological tests were performed and the data indicated that two of the tests of perceptual motor integrity were significantly different between the groups. A deficit of 5-7 points in I.Q. was however not significant. These results supported the hypothesis of an association between increased lead exposure and disturbances of neurophysiological development. However, they were not unequivocal and pronounced enough to prove a relationship between the lead burden and observed neuropsychological deficit. A second study was performed by the same group /59, 60/ near a smelter at Aachen. Tooth lead contents were significantly higher than those observed in Duisberg. Three groups were identified, "low" (< 4 ppm), "moderate" (< 4-10 ppm) and "high" (< 10 ppm). The study revealed no detrimental effects of lead on verbal I.Q., performance I.Q. or full scale I.Q. and the same was the case for several other neuropsychological parameters. However, there was a significant association between tooth lead content and perceptual-motor integration.

Again using tooth lead as an index of cumulative exposure, Maracek et al /61/ studied a group of black children in urban Philadelphia. The two groups selected exhibited median tooth leads of 5 and 59.8 μg/g with the groups being selected from a skewed distribution. A battery of neuropsychological tests was applied and overall exposure to lead was associated with a decrement in performance. Statistical significance was reached most often on tests of visual-motor functioning and perceptual integration. Motor functioning was however not affected. With regard to non-lead variables, the authors concluded that socioeconomic factors were not related to lead exposure.

The final population study to date, again performed in the United States /62/ selected urban black children living in New York. Here multiple markers of exposure were employed, namely preschool blood lead, school age blood lead, free erythrocyte protoporphyrin, and deciduous tooth dentine lead. A battery of tests was performed - McCarthy scales, reading tests, teachers assessments, and the results suggested impairment being associated with lead exposure. With no control of parental I.Q. one could conclude from the data that lead may be associated with deficiencies in general cognition, verbal skills, motor performance and reading test performance. However, when a brief measure of parental I.Q. was considered, the statistical deficits were nulled out, and the authors concluded that lead had if any very minimal effects on central nervous system dysfunction. Rutter /12/ for one, suggests that the authors are over emphasising their non-significant results. There was a significant association between blood lead and I.Q. even after controlling for confounding variables, even though not significant, although a significant proportion of the association was due to confounding variables rather than lead per se.

DISCUSSION ON STUDIES

In most cases these studies have shown that changes can be found where differences in lead concentrations between test and control groups are sufficiently large. In the few cases where lead has been thought to have little effect these are usually because either insufficient range has been included in the difference between the exposed children and the non-exposed children or because covariants such as parental I.Q. and age have not been

explicitly controlled. In other studies where the level of exposure to lead was less, other controlling factors such as social class and parental intelligence have been found to be very much more important as covariants of I.Q. than lead and in some cases these studies have shown that lead is not contributing at all to the drop in I.Q. But even in those studies there are suggestive downward trends in I.Q. and behaviour in the lead exposed children. Thus these studies provide little evidence that any form of large neuropsychological deficit is associated with relatively modest levels of lead exposure. Overall, however, the studies can be interpreted as providing acceptable evidence that full-scale I.Q. deficits of about 4 points can be found where blood lead values lie in the range of 1.5 to 2.5 μmol/l. An important assessment of the likely impact of lead is being further examined in a number of prospective studies currently underway in various parts of the world. Such studies have concentrated upon accurate longitudinal assessment of lead exposure in children from birth and in some cases prenatally. Amongst these studies some have shown that at 6 months of age Bayley scores in the mental development index are lower in the exposed children than in unexposed children. The results from such studies awaits the full development of the programmes of research over a number of years. Reliable results are unlikely to be available in the near future. There is however no doubt that lead is a toxic substance which in sufficient quantities can cause encephalopathy which may result in permanent brain damage to babies and children. The response of a particular child exposed from any source depends upon so intricate a network of interrelated factors that a simple relationship between dosage and degree of injury which would allow the risks to be calculated has not, and possibly cannot be established. As with all biological phenomena, the sensitivity of any individual child to lead exposure will vary; nevertheless it is recognised that once a child shows signs of lead encephalopathy, there is a danger that permanent damage may result.

None of the types of study discussed here provides definitive proof that lead causes, or is a primary contributing cause of, impaired intellectual development. Significant methodological issues discussed previously limit the inferences that can be drawn from any one approach. The inferences drawn from published work differ between reviews, with the Lawther Committee /21/ and the Conservation Society /63/ lying at the two extremes, and Rutter /12/ taking a middle line with respect to these two groups.

What is clear is that certain sections of the population are potentially much more vulnerable to the neurotoxic effects of this metal. The neurobehavioural findings agree with morphological and biochemical studies of function. From these one would conclude that the young are the most susceptible and that females are more susceptible than males. The reasons for this are not hard to find. The rapid development of the nervous system makes it vulnerable to toxic influences not only to the metal itself but also other biochemical consequences of over-exposure to lead such as alteration in the availability of adequate nutrition. It is not at the present time clear why females are more vulnerable than males. The prospective studies at present being carried out throughout the world will hopefully answer questions relating to another imponderable-reversibility of this process. Current evidence would suggest that as well as being biochemically reversible, that is biochemical events due to lead being reversed when the lead is withdrawn, there is also a strong probability that morphological repair can also occur. However such morphological repair is seldom complete as is evidenced by numerous studies in primates other than man. In particular there are permanent changes in synaptogenesis and in development and myelination.

EFFECTS ON THE PERIPHERAL NERVOUS SYSTEM

In contrast to the infant and young child, the neurotoxic effects on lead in the adult, apear in the peripheral nervous system. Radial palsy in the past was a classical sign of lead neurophathy. Within the peripheral nervous system, a spectrum of lead related deficits can appear, from paresis to slight functional defects only capable of detection by sensitive electrophysiological techniques. Although peripheral paresis is well known, the occurrence of palsy in severe lead poisoning is now rare. Many studies have selected a group of men exposed to lead in the working environment, but who are neurologically asymptomatic and have demonstrated impaired motor conduction nerve velocities /64-69/. Seppalainen, Hernberg & Kock /70/ measured a battery of functions of the peripheral nervous system; maximum motor conduction velocity of the median and tibial nerves, motor conduction velocity of the slow fibres of the ulnar nerve, sensory conduction velocity of the forearm region of the median and tibial nerves, distal sensory conduction velocity of the median nerve from the finger to wrist and the motor distal latency of the median nerve. All these parameters correlated with the

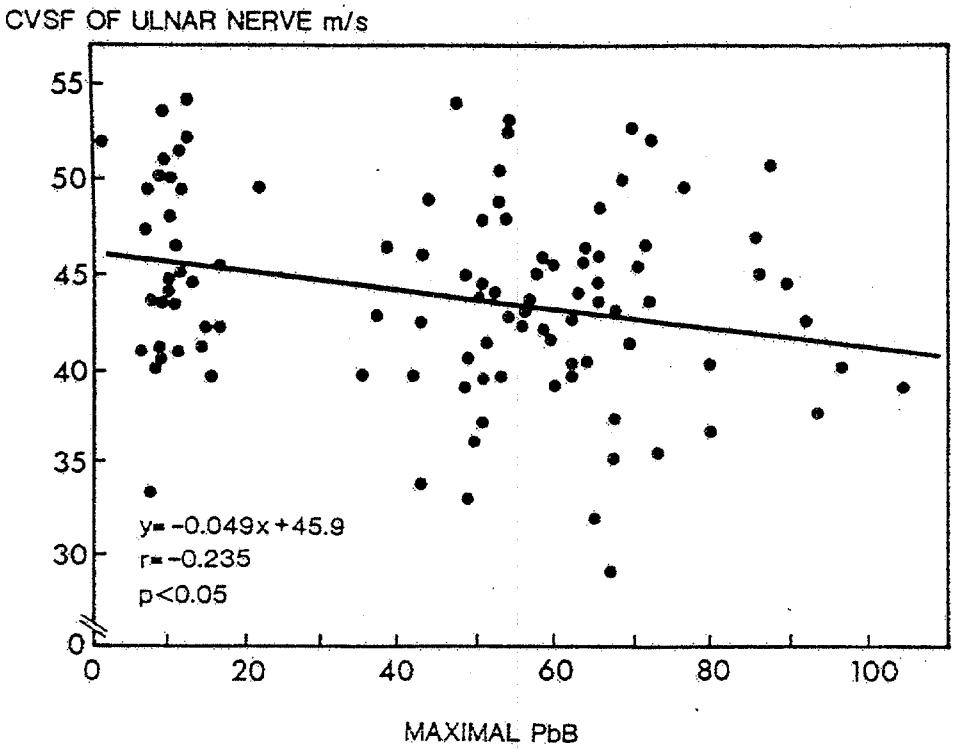

Fig. 2: Relationship between blood lead concentration and conduction velocity of the slow fibres of the ulnar nerve.

maximum blood lead during the entire period of occupational lead exposure, with a time weighted average blood lead, and with the actual blood lead at the time of testing. It would appear that the most sensitive indices of the peripheral neurotoxic action of lead are the slowing of the conduction velocity of the slower motor fibres of the ulnar nerve and the motor latency of the median nerve /65, 66/ (Figure 2). In order to investigate the time span between the start of lead exposure and the onset of these manifestations, the same laboratory /71/ selected a group of newly exposed lead workers. After one year exposure to the metal, these workers exhibited significantly longer motor distal sensory conduction velocities of the median nerve. The data in this study suggested that lead has detrimental effects on the peripheral nervous system in these lead workers at a blood lead level of less than 1.9 μM (40 μg/100 ml).

Electromyographic analysis has demonstrated abnormalities such as fibrillation and a diminished number of motor units in maximal contraction /66/.

In their study of children exposed to chronic low level lead from a nearby smelter, Landrigan et al /38/ noted a lead related slowing of wrist-tapping which may be an indication of low grade motor neuropathy. The slowing of nerve conduction velocities described above will precede the development of signs and symptoms of peripheral neuropathy. The asymptomic slowing of sensory nerve conduction velocities preceeds the development of motor nerve effects /72/. The general conclusion from most of these studies is that electrophysiological effects of lead occur at concentrations well below those currently considered to be safe. Whether such neurochemical effects have functional sequelae remains, however, to be proven.

ANIMAL STUDIES

Similarly to the data available in man, the studies performed in animals, mainly mice and rats, have provided data which is inconsistent and contradictory. Early behavioural studies in animals tended to study locomotor activity in response to the observations of David et al /28/ indicating a relationship between hyperkinesis and lead exposure in children. Although

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research in the early 1970's suggested that lead produced hyperactivity /73-77/ an observation subsequently supported by other laboratories /78-79/, this observation is not universally supported. Other groups have noted either no effect of lead on activity /80, 81/ or even hypoactivity /82-84/. Such discrepancies may be due to methodological issues such as different experimental protocols, time period of lead administration, age of testing or perhaps the specific test(s) employed. A study measuring activity levels in mice has demonstrated that the effects of lead are not invariant and are influenced by factors such as the time of day the testing is performed /79/. Several investigators have examined a number of behavioural paradigms including mazes, discrimination tasks and shock-avoidance situations. The available data demonstrates a lead associated impaired performance in certain types of learning tasks /85-87/ although it has been suggested by one group that learning may well be improved by lead /83/; altered social behaviour including changes in aggressiveness, reduced social interaction and stereotyped behaviour /74, 88-90/. It has been generally consistently noted that where lead affects learning tasks, the deficiency is greatest where the tasks are of a more complex nature. Studies involving the use of learning tasks of different complexity, such as orientation, which is relatively easy, and size discrimination, a more demanding task, have demonstrated no deficits on easy task learning /85/. However since performance in a learning task is complex and based on a number of functional processes such as motor functions, motivation, sensory functions, cognitive functions namely memory and/or learning processes, it is not easy to pin point which function is being affected, although cognitive function is thought not to be the deficit /59, 91/. It must be realised however that neurobehavioural studies on learning and memory in animals are relatively poor models of human cognitive function. It has been suggested that the deficit is associated with hippocampal damage /92/. The influence of lead on classical (Pavlovian) conditioning has been investigated. Adult rats exposed to lead in their chow showed enhanced conditioned emotional response; that is they showed an increased frequency of lever pressing in response to a cue representing the occurrence of an electric shock. However, these same animals were slower to learn this task than non-exposed animals /93/.

The question arises when investigating the behavioural and learning effects of lead in animals as to the age of animals which should be employed in the study. The main problem of lead exposure in man peaks at age 1-3 years /94/, a stage of neural and metabolic maturation more advanced than that of infant rodents. The effects of lead on adult rodents by comparison to younger animals are uncertain, with some authors claiming older rodents are insensitive to lead effects /20, 92, 95/ while other studies have demonstrated quite clear effects in older animals /96, 97/. However, it is not easy to separate the effects of lead pre- and post-weaning, since the feeding dam will impart lead to the offspring via the milk if she was exposed to lead while carrying her young, even if not exposed after parturition. A study by Dolinsky et al /98/ suggests that the effects of lead administered postnatally is greater than those effects resulting from in utero exposure. Cross-fostering experiments however would suggest a largely prenatal effect of lead in causing a delay in development of exploratory and locomotor activity /99/. The level of exposure of the rodents is important since at high levels of exposure the observed effects are confounded by undernutrition. Nursing dams will consume significantly reduced quantities of food and water when exposed to lead.

The question arises as to the reversibility of lead related behavioural changes. Krass et al /100/ studied the degree of recovery from lead-induced neurobehavioural deficits in rats after the blood lead concentration had fallen to pre-exposure levels. Following an exposure-free period of four months, during which time the blood lead had fallen, persistence of neurobehavioural symptoms occurred and so the question of irreversibility arises.

NEUROCHEMICAL EFFECTS

As much as neurobehavioural effects are difficult to define and quantify, neurochemical effects are exceedingly easy to define and to quantify but the interpretation of such results remain elusive. Within the cell lead can be shown to have numerous biochemical sequelae all of which could potentially alter nerve function. As a heavy metal poison it inhibits the activity of a number of enzymes such as those involved in the synthesis of catecholamines and their cofactors, those involved in cellular respiration and many of the enzymes for haem biosynthesis. It can also have an effect on other biochemical systems such as pyrimidine 5'-nucleotidase /101-103/ and in the processes synthesising the various forms of vitamin D, both of which are not readily associated with neurochemical events. Within various systems synthesising the neurotransmitters, those ones involved in the synthesis of the catecholamines are most clearly affected by lead. In general, however, the

correlation between these neurochemical events and behavioural effects of lead in animals have in the past been both contradictory and inadequate.

CATECHOLAMINES

Since early reports of the possible involvement of brain catecholamines in the lead-induced hyperactivity commonly observed in animals /74, 77/, attempts been made to correlate the observed hyperactivity with changes in the content of established and some putative neurotransmitters. Catecholamines, especially dopamine, appear to be involved in the central regulation of motor activity /104, 105/. The nucleus accumbens would appear to be the primary locus of this dopamine-mediated locomotor activity. Evidence supporting these observations comes from studies demonstrating increased locomotor activity following apomorphine, a dopamine agonist /106/ and following amphetamine administration, which induces catecholamine release /107/.

Early research concentrated on the effects of lead on endogenous levels of the neurotransmitters, mainly noradrenaline and dopamine but also tyrosine to a limited degree. Studies have investigated these parameters in either rats or mice exposed to lead during the neonatal period or as young adults. Several studies have investigated the levels of catecholamines in the brain as a whole. These studies initially indicated no change in noradrenaline concentrations in the rat /74, 75/ and mouse /108, 109/ but subsequent data obtained by the same group of workers showed increased noradrenaline levels in the brain /77, 110/, an effect also confirmed by Silbergeld /111/. These same studies initially indicated that dopamine levels were reduced /74, 75/ but later they reported no alteration to the level of this catecholamine /77, 110/, an observation in agreement with that of Silbergeld and Goldberg /112/ in the mouse. Regional analysis of the brain however demonstrates a slightly clearer picture. Rat forebrain, midbrain and brainstem exhibited an increased concentration of noradrenaline as a result of lead exposure /112, 115/. Other studies however, failed to demonstrate any effect in any region. No change in noradrenaline was observed in the cortex, brainstem, cerebellum, hypothalamus, striatum or diencephalon /80, 113, 116, 118/. Even more data shows a fall in noradrenaline in the hypothalamus, striatum and brainstem /115, 119/. There would appear therefore to be no consistency in the data obtained concerning the effect of lead on brain noradrenaline concentrations even within the same brain region.

Turning now to dopamine levels in discrete regions of brain, significant reductions in this catecholamine were observed in the striatum /120/ and cortex, midbrain, hypothalamus /114/, other studies have shown no change in various regions; cortex, brainstem, hypothalamus, striatrum and forebrain /80, 108, 109, 116-118, 120/.

Indirect measurements of dopamine levels were obtained in a study which quantitated the level of prolactin. Lead exposure causes an increase in prolactin levels /121/. Since prolactin secretion is controlled mainly under hypothalmic dopaminergic inhibitory control this data implies a reduction in dopaminergic activity in this brain region. The study of Dubas & Hrdina /114/ cited above showing a reduced concentration of dopamine in the midbrain and hypothalamus is interesting since this same laboratory in another paper quoted an increase in dopamine in these same regions /115/.

There exist only two papers from the same group of researchers which have quantitated endogenous levels of tyrosine. Data in these papers indicates that lead does not effect the level of this amino acid at least in whole brain /108, 109/.

A series of studies by Meredith and his coworkers have investigated the effects of lead on brain noradrenaline, dopamine and adrenaline in certain very small discrete regions, following both acute and chronic exposure to the metal. Acute exposure consisted of the administration of either 5 or 20 μmol/kg intraperitoneally in rats for 14 days while chronic exposure was achieved by supplying a 2mM lead acetate drinking solution for a period of 8 to 26 weeks. Following acute exposure, noradrenaline was increased in both the anterior and posterior hypothalamus and adrenaline was increased in the anterior pituitary but only in the 20 μmol/kg group. Additionally, there was a slight rise in dopamine in this same group of animals, again in the anterior hypothalamus. No significant changes in either catecholamine were observed in other brain regions examined – cortex, pons-medulla or hippocampus. In the chronic study, however, there were significant reductions in both noradrenaline and dopamine after 26 weeks of exposure. After the shorter exposure period of 8 weeks, the level of catecholamines was increased but this failed to reach statistical significance /122, 123/. These studies suggest that there may be a biphasic response to lead depending on the length of exposure and/or exposure level.

The effect of lead on both noradrenaline and dopamine catabolism has been investigated. Turnover rates have been studied by looking at levels of metabolites present in the tissues.

Concerning noradrenaline catabolism, Silbergeld & Chisholm /124/ demonstrated a significant increase in the noradrenaline metabolite, vanillylmandelic acid (VMA), both in the brain and urine of mice exposed to 5g lead/l of drinking water from birth. Urinary metabolites most probably are derived from, and hence reflect peripheral catecholamine metabolism, and therefore both a central and peripheral action of lead is implicated from this data. Silbergeld & Goldberg /112/ also observed an increase in brain VMA which was associated with an enhanced MAO activity, a feature which could account for the increased metabolite levels. Taking another approach Michaelson et al /110/ observed an increased rate of decline of noradrenaline, in rat wholebrain following α-methyltyrosine. However, although this data would indicate an enhanced utilisation of noradrenaline as a result of lead exposure, Schumann et al /109/ have failed to demonstrate an alteration in the rate of noradrenaline synthesis from tritiated tyrosine in the mouse brain.

More data exists concerning dopamine catabolism. In mouse forebrain, a significant increase in homovanyllic acid (HVA) was demonstrated following lead exposure indicating enhanced dopamine turnover. Concordant with this tissue rise, there was also an increase in the urine /124/. However, following labelled tyrosine administration, no change in dopamine synthesis was observed in the mouse brain /109/ or rat forebrain /125/. On the other hand, other studies of dopamine metabolism indicate that the metabolism is altered, with the direction of change being regionally specific. 3,4-dihydroxyphenylacetic acid (DOPAC) considered a reliable indirect indicator of the functional ability of dopaminergic neurones, was first demonstrated to be significantly reduced in the striatum of rats exposed to 2.5 g lead/l in water throughout life /121/. Confirming this observation and extending their data, the same laboratory demonstrated a significant increase in DOPAC in the rat nucleus accumbens and frontal cortex with no change in the substantia nigra. Once again, there was a reduced DOPAC level in the striatum /126, 127/. In both these studies, the animals exhibited an incresed locomotor activity. Supportive data was also obtained in a study by Memo et al /128/. In the rat, DOPAC was diminished in the striatum and enhanced in the nucleus accumbens, effects which appeared to be reversible since they had returned to normal 30 days after cessation of lead treatment.

Using a different approach of following the decline in DOPAC following α-methyltyrosine administration, data is obtained which is consistent with the previously discussed results and with an increased dopamine turnover. A reduction in DOPAC decline following α-methyltyrosine administration was observed in the striatum /121, 126, 127/ whilst an enhancement was seen in the nucleus accumbens and frontal cortex /126, 128/. Again in the striatum, Jason & Kellog /120/ observed a reduction in dopamine turnover following lead exposure which was based on a decline in dopamine levels following α-methyltyrosine treatment. Although the above cited studies were performed in either the mouse or rat, a study of children provides data which is generally in agreement with these animals studies. Similar to the data in the mouse, where Silbergeld & Chisholm /124/ observed an increase in VMA and HVA in the brain and urine, the same authors noted these metabolites to be also increased in the urine of children who were significantly exposed to lead; that is they had blood lead levels in the range 2.8-3.3 μM (59-68 μg/100ml). The data would thus suggest increased peripheral catabolism of catecholamines in these exposed children. The regional alterations are probably opposite in nature and thus are cancelling each other out. Caution must be taken when interpreting turnover studies in which an enzyme of the catecholamine synthetic pathway, often tyrosine hydroxylase, is inhibited. If the accumulation of intermediates is measured, and lead were to alter the activity of a synthetic enzyme then no change would be identified by these studies.

Most studies investigating the uptake and release of catecholamines explore on the high affinity uptake of dopamine by synaptosomes. A single paper has reported data on noradrenaline uptake. Silbergeld & Goldberg /112/ observed no alteration in high affinity noradrenaline uptake by mouse forebrain synaptosomes. The same authors also observed a significant reduction in dopamine uptake following in vivo exposure, and in addition, an enhancement in uptake of tyrosine. Other groups performing similar uptake studies following in vivo lead exposure failed to exhibit alterations in dopamine uptake in rat forebrain slices, striatal synaptosomes or striatal minces /125, 129/. One group even observed enhanced dopamine uptake into rat synaptosomes /130/. The report of enhanced tyrosine uptake quoted above /112/ is consistent with an increase in dopamine turnover in the same brain regions. As with dopamine

uptake studies, data relating to dopamine release is also conflicting. Following a load of tritiated dopamine, Wince and coworkers /125/ observed a fall in potassium stimulated release from brain slices, data in conflict with results from Jason & Kellog /131/ who observed no effects on potassium stimulated release from striatal minces. Ket et al /132/ measured the noradrenaline release from the cortex, hippocampus, the release of dopamine from the striatum, substantia nigra, and of both transmitters from the nucleus-o-tubercle and hypothalamus. No difference was observed between the spontaneous, potassium-stimulated or amphetamine-stimulated release of catecholamines from tissues of lead treated compared to control rats. The addition of lead in vitro resulted in a concentration dependant diminished high affinity dopamine uptake in rat striatal synaptosomes /111/ an observation not confirmed by either Bondy et al /133/ in mouse wholebrain synaptosomes or by Ramsay et al /129/ using striatal synaptosomes. The same picture is seen when looking at dopamine release following in vitro exposure to lead. While Silbergeld /111/ and Komulainen & Tuomisto /134/ reported no effect, two papers reported stimulated release /129, 133, 135/. Data relating to the activity of individual enzymes of the biosynthetic pathway is relatively sparse (Figure 3). Although evidence of one group /130/ suggests that tyrosine hydroxylase activity is stimulated as a result of lead exposure, a study by Deskin, Bursian & Edens /136/ suggests this enzyme is unaltered in two specific brain regions at least – striatum and hypothalamus. Yet another series of studies by Meredith and colleagues /122, 123/ have demonstrated a lead-induced fall in tyrosine hydroxylase activity both following acute exposure and chronic exposure in the hypothalamus. No alterations were noted in other regions; cortex, pons-medulla or hippocampus.

In vitro experiments have shown a pregressive irreversible inhibition of phenylethanolamine-N-methyl transferase (PNMT) by lead /137/ an effect which the author suggests is due to an action of lead on sulphydryl groups on the enzyme. Studies involving in vivo lead exposure have not however demonstrated an alteration to the activity of this enzyme /122, 123/.

Finally, the possibility of an alteration in receptor populations must be considered, and in this respect only the dopamine receptor has been studied extensively. The dopamine D_1 receptor which is linked to adenylate cyclase can be assessed for functional ability using dopamine stimulation. Reports indicate either no change in dopamine stimulated activity in rat striatum and accumbens /126, 138/ or alternatively, Wince et al /125/ reported a significant lead-induced reduction in cyclase

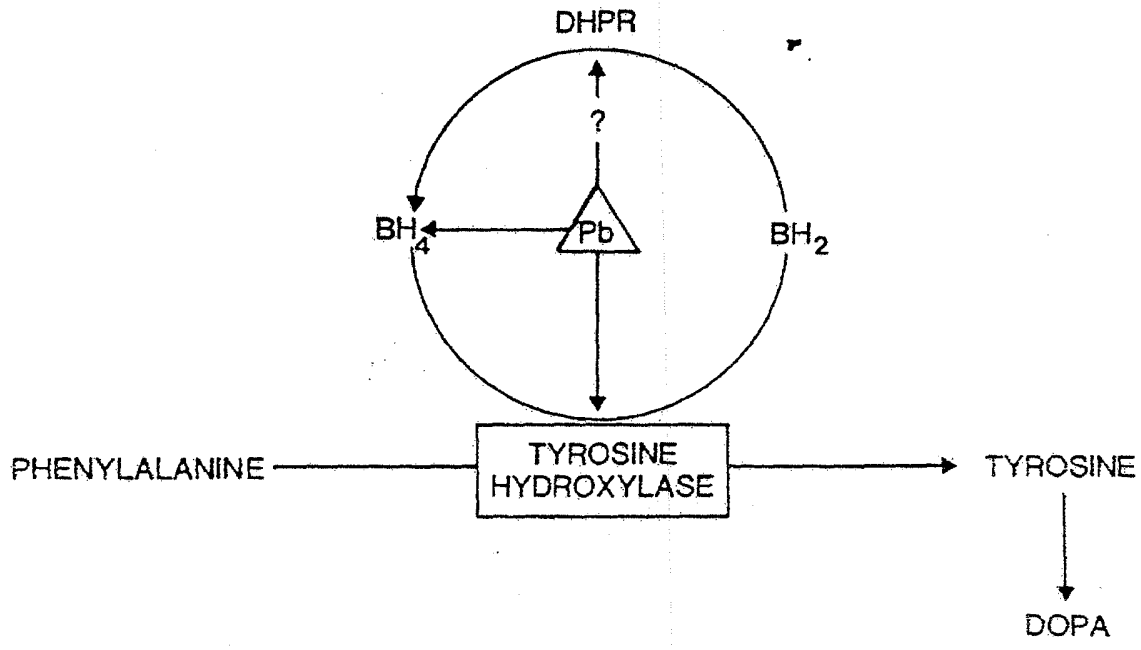

Fig. 3: The proposed effects of lead on the tetrahydrobiopterins and the catecholaminergic systems (see text).

activity and a marked inhibition of apomorphine-induced stimulation. These workers employed rat forebrain synaptosomes as tissue. As far as the dopamine D_2 receptor is concerned, conflicting results emerge depending on the ligand selected for binding. While H-spiroperidol binding is not altered /126, 138/ in rat striatum and accumbens following neonatal lead exposure, increased striatal binding and decreased binding in the nucleus accumbens was reported when H-sulpiride, which is reputed to be more specific for the D_2 receptor, was employed /138/. These findings concerning the dopamine D_2 receptor are in the opposite direction to alterations of DOPAC levels /121, 126/ and are thus in agreement; that is increased dopamine synthesis as a result of a decrease in receptor sensitivity and vice versa.

Many reports have demonstrated a lead-induced block in post-synaptic adenylate cyclase activity associated with both the noradrenaline and adrenaline receptor, at relatively low lead levels /139, 140/ although another group have failed to confirm this effect /141/. However, it is difficult to relate these in vitro effects on enzymatic activity to the effects of lead on the intact nervous system.

EFFECTS ON TETRAHYDROBIOPTERIN METABOLISM

Related to these changes in catecholamine metabolism, are lead related changes in the pteridine, 5,6,7,8-tetrahydrobiopterin. 5,6,7,8-tetrahydrobiopterin (BH_4) functions as the cofactor for the phenylalanine hydroxylase catalysed conversion of phenylalanine to tyrosine and for the rate controlling enzyme of catecholamine synthesis, tyrosine hydroxylase, which catalyses the further hydroxylation of tyrosine to dihydroxyphenylalanine (Dopa). In addition, it has a role in the hydroxylation of tryptophan to 5-hydroxy-tryptophan. The concentration of tetrahydrobiopterin is believed to determine the level of tyrosine hydroxylase activity /142/ and therefore the rate of catecholamine synthesis. Any reduction in the level of such an important cofactor may have serious neuropathologic consequences. The cellular concentration of tetrahydro-biopterin is maintained by two mechanisms (Figure 1). There is firstly de novo synthesis from guanosine triphosphate through a number of poorly characterized intermediates /143, 144/. During the hydroxylation reactions in which tetrahydrobiopterin acts as a cofactor, the pteridine is converted to a labile species quinonoid dihydrobiopterin (qBH_2) (Figure 1). A salvage pathway catalysed by dihydropteridine reductase (DHPR) is then responsible for reconverting the qBH_2 back into BH_4. This forms the second mechanism involved in the maintenance of cellular BH_4 levels. Any qBH_2 which is not recycled to active biopterin re-arranges into the more stable 7,8-dihydrobiopterin (BH_2) which is lost from the cell.

Tetrahydrobiopterin concentration can be altered by substances interfering with the de novo pathway of BH_4 synthesis at either one or several intermediate sites; or, alternatively, interference may occur in salvage pathway. Lead has been implicated as an environmental agent that can act at both of these sites. Purdy et al /145/ demonstrated inhibition of BH_4 synthesis in a rat brain homogenate system in vitro and DHPR activity from both rat brain and liver was irreversibly inhibited. In addition, a significantly decreased level of serum biopterins has been observed in subjects with clinically expressed lead poisoning /146/. A positive relationship was found between plasma biopterins and blood lead levels in environmentally exposed man /147, 148/. This observation would indicate an inhibitory effect of lead on the salvage pathway. However, studies involving rats indicated other inhibitory effects on the de novo path /148/. Acute exposure to lead in rats (0.3 mg/kg body wt injected i.p. every 48 hr) has been shown to produce a significant fall in serum biopterins after 2, 4, and 6 days /149/.

Studies in rats have demonstrated selective alterations in the concentration of the cofactor and activity of DHPR in the diencephalon of the brain with no apparent alterations in other regions studied: cerebellum, midbrain and telencephalon /150/. The data from this study suggested a rise in cofactor concentration in this discrete brain region. Increased levels of tetrahydrobiopterin could be caused by:

a. Increased de novo synthesis (Figure 4)
b. Increased activity of dihydropteridine reductase
c. Decreased activity of hydroxylation enzymes.

The third possibility is unlikely to explain the rise in the level of the cofactor since the cofactor concentration is thought to determine the activity of tyrosine hydroxy-lase activity /142/. An increase in the activity of the salvage pathway enzyme is a possibility supported by the data obtained in this study. The possibility cannot be excluded, however, that lead has an action on the de novo synthesis pathway of tetrahydrobiopterin metabolism.

This possibility must, however, be judged in the knowledge that lead inhibits pyrimidine 5'-nucleotidase /101-103/. This will clearly alter the balance between

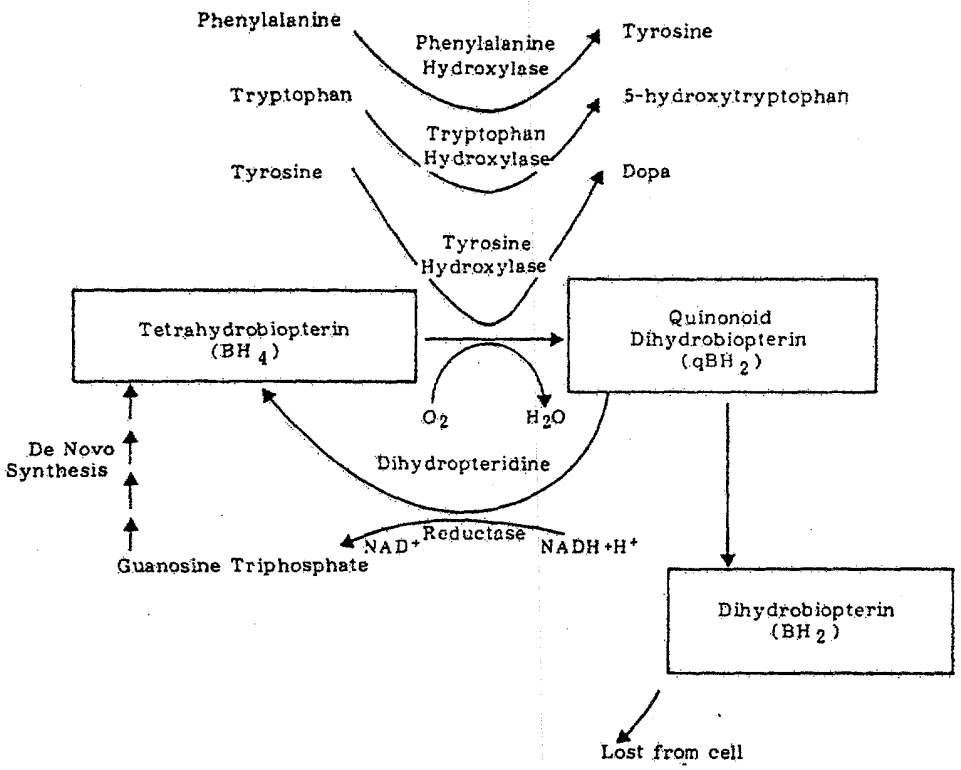

Fig. 4: The pathway of tetrahydrobiopterin metabolism showing de novo and salvage pathways of synthesis.

pyrimidine and purine bases within the cell and consequently the availability of GTP for pteridine synthesis.

CHOLINERGIC EFFECTS OF LEAD

Various aspects of cholinergic function have been investigated with regard to the effects of lead, and the data is complex and varied /151-155/.

The effects of lead on cholinergic neurotransmitters have been investigated because of the long history of clinical data describing peripheral neuropathies of lead poisoning. The peripheral neuromuscular pathology of lead, clinically described as lead palsy, may result at least in part from the action of lead on prejunctional sites of the peripheral cholinergic nervous system. Increased exposure to lead has been shown to result in a reduction in mean nerve conduction velocity /156/ and an impairment in fine motor coordination /31/ in children. It must be remembered however, that these peripheral pathologies may well not ensue as a result of a specific effect of lead on cholinergic function but as a consequence of the segmental demyelination and axonal degeneration of peripheral nerves known to occur in lead exposure /157, 158/. Studies to date have concentrated on the investigation of the action of lead both in vitro and in vivo in the rat and mouse.

The observations recorded on cholinergic function by various groups as a result of lead exposure, are not consistent. Such variability is probably due to differences in approaches used in the various laboratories to expose the animals to lead, as well as methodological techniques used to kill the animals, and to analyse the tissue for acetylcholine and choline content. In particular, the method of sacrifice is extremely significant because there are rapid post mortem changes in the levels of both substances. Ideally one should be able to inactivate instantaneously in situ all post mortem degradative changes in order to obtain a reflection of the levels of choline and acetylcholine in the brain at the time of death.

The effects of lead on the cholinergic nervous system have been studied both peripherally and centrally. The data relating to the peripheral effects are less equivocal. Although early work in frogs attempted to locate the site of action to a lesion within the muscle itself,

showing changes in inorganic phosphorous and creatine phosphate /159/ it soon became evident that the lesion was of pre-synaptic nature /160-164/. In contrast to the effect of lead on the response to the evoked action potential, in vitro exposure to the metal results in an increase in spontaneous acetylcholine release as evidenced by an increase in frequency of miniature endplate potentials /162/. It would thus appear that the blockage by lead is of a synaptic nature and very little action is of a curariform nature, that is lead does not interact significantly with motor endplate receptors /162, 165/ a hypothesis which was earlier proposed /166/. There is strong evidence to suggest that the action of lead on the peripheral cholinergic nervous system may in part be modulated through calcium. The presence of lead in the bathing medium around an isolated muscle preparation leads to a fall in acetylcholine release in response to nervous stimulation which is similar to the effect of reduction in the calcium concentration of the medium /163, 164, 167/. In addition, increasing the calcium concentration can partly overcome the block caused by lead /164, 167/. using radiolabelled ^{45}Ca, these authors have demonstrated a lead induced reduction in calcium uptake by preganglionic nerve terminals in the frog; in other words lead is preventing acetylcholine release in response to nerve stimulation by preventing calcium entry. Manalis et al (1984) have put forward a hypothesis based on calcium interactions to explain the reason why the blockage of evoked release caused by lead precedes the increase in spontaneous release. Blockage by lead of external calcium receptors on the nerve terminal results in an inhibition of evoked release while there is a delay as the metal enters the axon to disrupt one or more of the calcium buffering systems and thereby raise the intracellular calcium concentration and hence miniature endplate potential frequency.

Calcium and lead can bind the same protein and probably interact with carboxyl groups /168/. Indirect evidence suggests lead can enter the nerve terminal via calcium channels. The rise in miniature endplate potential frequency is prevented by the presence of cadmium /169/ which can specifically block inward calcium currents /170/. Lead has been demonstrated to be associated with the mitochondrion /171/ and to reduce calcium uptake by these organelles /172/. If this hypothesis were true, lead would not be alone amongst the heavy metals in reducing evoked acetylcholine release via a competitive action with calcium /165, 167, 173/ as cobalt, manganese and cadmium /174-176/ have a similar action. This hypothesis is not however supported universally. Silbergeld & Adler /177/ for example, proposed that lead does not enter the peripheral synapse but that its actions are mediated by competition with calcium at the receptor site situated externally on the neuronal axon.

Less well clearly defined are the neurotoxic effects of lead on the central cholinergic system. In vivo studies involving exposure of rodents to lead at an early age either via their drinking water or via exposure of the nursing dams have shown in general no alteration or an increase in acetylcholine concentration in specific brain regions. The levels of the precursor choline are unaltered except in one study where they were reduced in rat midbrain /151, 178/. Both choline and acetylcholine were unaltered by lead exposure in mouse forebrain /112, 179/ rat cerebellum, hippocampus, midbrain, pons-medulla, cortex and striatum /178, 180, 181/. The rat diencephalon however, exhibited a small but significant rise in acetylcholine content /119, 180, 181/. However, this study by Modak and his colleagues employed a high dose of lead (10,900 ppm – 1%) and as a result, the animals were significantly stunted in growth with respect to non-exposed control rats. Hrdina et al /119/ also observed a significant increase in rat cortex as a result of lead exposure.

More recently the use of microwave irradiation to sacrifice the animals has led to the observed transmitter levels being closer to the in vivo situation, and a regional diminution of brain acetylcholine as a result of lead exposure has since been observed; reductions were observed in the cerebellum, medulla, diencephalon, cerebrum, striatum, midbrain and indeed whole brain following 30 days lead exposure /182/. However, exposure for a longer period resulted in the levels returning to normal in whole brain /182/ midbrain, hippocampus, striatum and cortex /178, 183/.

Perhaps the least contradictory data existing concerning the effect of lead on the central cholinergic nervous system, involves turnover studies which indicate a downgrading of cholinergic metabolism as a result of lead exposure /178, 183/. Similar to the data observed in the periphery, in vivo lead treatment results in a reduction in potassium induced acetylcholine release and also of choline in mouse cortical minces /179/. Spontaneous release of acetylcholine was also significantly increased but spontaneous release of choline was unaltered.

The high affinity transport of choline has been shown to be inhibited by lead using synaptosomes prepared from mouse forebrain tissue /112/; low affinity transport

being unaffected. Other groups of workers have however failed to demonstrate any alteration in choline transport in mouse cortical minces /129, 179/.

Various groups have measured the activities of enzymes involved in cholinergic metabolism, namely, acetylcholine esterase, choline acetyltransferase or choline phosphokinase in the mouse /179/. These findings, observed mainly in rats but also in mice, are in accord with data obtained in man /185/. In this study, serum acetylcholine esterase activity was significantly reduced in man exposed to high concentrations of lead in the environment and in patients with lead poisoning. Acute exposure of rats to lead (7.5 mg/kg lead acetate intra-peritoneally from birth for 10 days) has shown a significantly decreased activity of acetylcholine esterase in the hippocampus and the medulla-oblongata /1/. No changes were however seen in other brain regions – cerebellum, cerebrum, midbrain and striatum. The activity of butyrylcholine esterase, an enzyme involved in acetylcholine metabolism, although of uncertain physiological significance, has been investigated following lead exposure. Sobotka et al /116/ have observed a significant reduction in the enzyme in the cortex and brainstream with no change or a slight increase in the cerebellum following exposure of rats via gavage which resulted in a slight state of undernutrition in the animals. Similarly, acute intravenous exposure of rats to lead for 15-20 days after birth resulted in a significant reduction in activity in selected brain regions, namely cerebrum, hippocampus and midbrain.

OTHER PUTATIVE NEUROTRANSMITTERS

One area where gamma amino butyric acid may have a role to play is in its relationship to delta aminolaevulinic acid which is greatly increased in lead exposure. 5-aminolaevulinic acid and the putative amino acid neurotransmitters, glutamic acid and γ-amino butyric acid are structural analogues with ALA and GABA differing by only one carbon. Although ALA does not affect GABA synthesis or metabolism as demonstrated in rabbits /186/ evidence suggests that ALA is capable of acting as a false transmitter at the GABA receptor /187, 188/. Data obtained in synaptosomes prepared from rabbit cerebral cortex suggests that in vitro at least, ALA is capable of causing inappropriate release of GABA /189/. It has been postulated that the pharmacological neurological effects of ALA may be related to these features of GABA metabolism.

The levels of the indoleamines 5-hydroxytryptamine and its metabolite 5-hydroxyindolacetic acid have been reported as decreased in brain areas following lead exposure /114, 115/. Of these neurotransmitter amino acids glutamate and aspartate have been reported as reduced in a number of brain preparations /190/. This was thought to be as a result of depression in the metabolism of glucose and in the flux of these metabolites through the processes of intermediary metabolism.

Generally the effects of lead upon intermediary metabolism probably provide a rational explanation of the effects of this metal on neurochemical and neurological systems. There is, in addition, evidence that one of the toxic effects of this metal results in diminished transport through capillaries. Brain tissue is particularly susceptible to this factor because of the continuous nature of the endothelial cells in these capillaries. Thus larger molecules, such as proteins, typically ones being horseradish peroxidase, cannot enter the brain whilst smaller molecules and water soluble components like glucose, can enter the brain either through active or passive transport mechanisms. The active transport system is maintained through the availability of adenosine triphosphate, and, as will be shown later, lead will influence the synthesis of this compound through its effects on intermediary electron transport mechanisms. Other forms of transport, such as carrier-mediated transport of glucose, could also be affected by heavy metal poisoning but current evidence would suggest that the threshold of effects of lead on this mechanism is greater than levels that might rationally be found during human lead intoxication. During lead exposure in animals, respiration using nicotinamide adenine dinucleotide linked substrates is inhibited in cerebellar mitochondria. Similar effects can be found in isolated brain mitochondria exposed to lead in vitro. The direct effects of lead on cellular aerobic energy metabolism are very important in the pathogenesis of lead encephalopathy. Within the processes of terminal oxidation, electron transport is coupled to the formation of ATP and provides most of the energy from the aerobic breakdown of glucose. The limiting features of this pathway are controlled, not only by the availability of oxygen and inorganic phosphate, but also by the functional integrity of this transport chain. Topographically succinate dehydrogenase and the cytochromes are located on the inner membrane of the mitochondrion, whilst the dehydrogenases lie within the mitochondrial matrix. Lead has been shown to inhibit NAD linked cytochrome dehydrogenases but has also been shown to limit the synthesis of haemoproteins such as the cytochromes. In animal studies respiration is diminished

with oxidation of glutamate and malate, whilst in isolated mitochondria, systems utilising NAD linked mitochondrial respiration, are inhibited by lead. There are two potential reasons for these changes. The first is that there is direct inhibition of dehydrogenase activity within the mitochondrion /191/. Second is that the availability of haem is compromised through the effect of lead on the haem biosynthetic pathway and consequently synthesis of mitochondrial cytochromes is also diminished. In addition to this, haem requiring enzymes within the mitochondrion, such as cytochrome oxidase, would be expected to have lowered activity. This has been suggested in human lead poisoning /192/ and there is also good evidence in humans, through extended antipyrine half-lives, that cytochrome P450 requiring metabolic systems are also compromised /193/.

The anaemia of lead poisoning is well known and numerous studies have adequately demonstrated altered haem metabolism in lead exposed subjects. This factor of the effects of lead upon haem-biosynthesis is of particular importance. There are numerous similarities between lead poisoning and acute porphyria, both of which have in common neuropsychiatric syndromes and alterations in the processes of haem biosynthesis. Control of haem biosynthesis through ALA synthase is achieved by feedback inhibition; the enzyme is represented by haem or a haem-like compound and hence if the rate of haem production is reduced, ALA synthase (ALAS) becomes de-repressed and increased amounts of ALA are formed (Figure 5). The pathway is well controlled via ALA synthetase, producing only sufficient amounts of intermediates and ultimately haem to service the requirements for haemoglobin and other haemoproteins such as cytochrome P450. However under certain pathological conditions this coordination falls down. Lead has been demonstrated to upset haem metabolism significantly. In lead poisoning there are early rises in urinary ALA and coproporphyrin with rises in PBG in severe cases. In the blood, erythrocyte protoporphyrin is increased. Studies performed both in the rat and man on the individual enzymes of the path indicate two factors are responsible for the accumulation

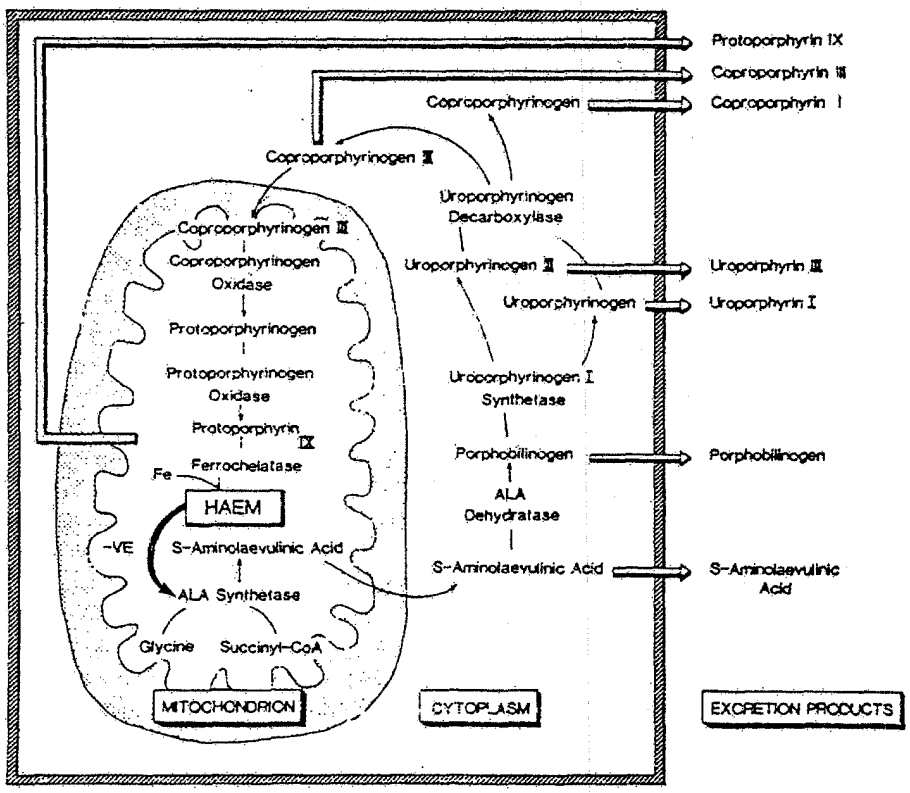

Fig. 5: The haem biosynthetic pathway showing the cellular compartmentisation of the various steps. Control of the pathway is exerted by negative feedback by haem.

of ALA by lead, namely a reduction in the activity of ALA D and an enhancement in the activity of ALA S /194-200/ although an in vitro study by Morrow and colleagues /201/ suggested an inhibition of ALA S by lead although at much higher concentrations. Additionally lead is responsible for the depression in activity of coproporphyrinogen oxidase and ferrochelatse /196/ in human leucocytes. The inhibition of these enzymes combined with enhanced haem degradation also caused by lead exposure, leads to a diminished concentration of free haem and hence a feedback induced increased activity of the rate limiting enzyme, ALA S /193, 202/. A similar observation of increased ALA has also been shown to be the case in acute porphyria as a consequence of diminished activity of porphobilinogen deaminase. From the foregoing description of the effects of lead on the haem biosynthetic pathway, it is evident that there is an overproduction of ALA during lead exposure. It has relatively recently been suggested that this haem precursor may be responsible for at least some of the neurological features of lead exposure. Unlike PBG and the porphyrins which are pharmacologically inactive, ALA has activity. ALA can penetrate into many tissues such as heart, liver, kidneys, spleen, gut and fat in which it appears to be bound or maintained in tissues by active mechanisms /203/ and it is capable of crossing the blood-brain barrier /204, 205/ the relationship between blood ALA concentration and that in brain being of the familiar curvilinear nature /205/.

Both in vitro and in vivo experiments have demonstrated the pharmacological potency of ALA in a variety of tissue preparations and species. In 1968 Feldman and his colleagues /206/ demonstrated the presynaptic neuromuscular inhibition of potassium augmented miniature end plate potential (mepps) frequency. The response of the frog sciatic gastrocnemius preparation to electrical or nervous stimulation was also inhibited by ALA /207/ as was evolked release of acetylcholine in low calcium-high magnesium bathing fluid /208/. Again in the frog, ALA caused depolarisation of the sartorius muscle /209/ and caused inhibition of both the spinal cord ventral and dorsal root responses /187, 210/. Both spontaneous and acetylcholine induced contractions of rabbit duodenum were inhibited by ALA in a dose dependent manner /211/.

Studies in small rodents suggest ALA is capable of altering activity. Intra-peritoneal injection of ALA into mice resulted in increased spontaneous activity /212/. The same group of workers later demonstrated a differential effect of ALA depending on the mode of exposure. Acute exposure in mice resulted in an initial depression of spontaneous locomotor activity followed by a period of hyperactivity, while mice exposed chronically exhibited a reduction in spontaneous activity and reduced excitability /213/. Cutler, Moore & Ewart /214/ studied the social behaviour of male mice 30-40 minutes following an injection of ALA. These rodents explored the cage and scanned less frequently than saline injection controls and also showed longer periods of immobilisation. A hypotensive action of ALA has been demonstrated in the anaesthetised and pithed rat /215/. The mode of action of ALA which is responsible for the actions on nerve-muscle function are uncertain but possibly the action of ALA to inhibit the sodium-potassium dependent ATPase, and thus the associated increase in capillary permeability is important. The significant reduction in resting membrane potential observed in the frog sartorius muscle preparation by Becker et al /209/ is consistent with an action of ALA on the ATPase. The data of Cutler et al /214/ also indicates an effect of ALA on ion transport when they demonstrated the presence of polyphasic action potentials again in the frog neuromuscular preparation following application of ALA. The evidence that ALA produced in excess has pharmacological activities is strong but in general the quantities of ALA required to produce such pharmacological changes are much greater than normally found in vivo.

Evidence for the haem based hypothesis is in some ways stronger. It has consistently been shown that in circumstances where haem biosynthesis is compromised, as in the experimental porphyrias, or lead poisoning, there is diminution in haem availability as assessed by the activity of tryptophan pyrrolase or through measurement of cytochrome P450 levels. If the quantities of one cytochrome, or activity of at least one haemoprotein enzyme, is diminished by lack of availability of haem, then it would seem equally likely that the haemoproteins involved in the terminal stages of oxidative metabolism would also be affected and this appears to be the case. As yet evidence for direct effects on cytochrome oxidase have only been shown in muscle tissue but it would seem reasonable to hypothesise that similar effects would be found in nervous tissue.

There is some evidence that resistance to lead toxicity occurs in nervous tissue and that this is associated with lead localisation in the astrocyte and in particular in nucleolysosomes and cytoplasm inclusions. This is important because this cell appears to accumulate lead and localise it within the cell in areas where it can do no harm. Another important feature about this localisation

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is that it would protect other cells, such as small neurones, from lead exposure and resultant toxicity. Similar mechanisms can be seen in the capillary in the endothelial cell. It is not clear how the astrocyte localises the lead, but it is possible that it occurs by mechanisms similar to the processes of calcium uptake. The inhibition of pyrimidine 5'-nucleotidase activity is another areas of particular interest. This is because of its role in not only the synthesis of RNA but also in the consequences of such changes to erythrocyte stability and function. This enzyme mediates the dephosphorylation of the pyrimidine nucleotides in the maturing erythrocyte and has been shown to be inhibited in children at values as low as 0.5 μM. When inhibition of this enzyme occurs, erythrocyte stability is compromised and there is evidence of diminished survival of the erythrocytes. On top of this, protein synthesis will inevitably be altered because of the effects upon messenger RNA, consequently there will be changes which will clearly be observed in the synthesis of globins.

Amongst the other potential neurotransmitter effects, those on gamma aminobutyric acid are of interest. In most cases workers have found little change in GABA levels /74/. The exception to this is that there are significant postnatal development changes in cerebellar GABA /216/. However, this is not reflected in changes in the synthetic processes of GABA metabolism and there is no change in the turnover of GABA in the cerebellum. If there is a decrease in GABA concentrations this would provide some explanation for increased central nervous system excitability since GABA is a major inhibitory neurotransmitter. But again, the evidence for major change in GABA is weak. In consequence any effect of lead on GABAergic neurotransmission would be expected to produce an increase in the central nervous system excitability or inhibition of the neurochemical pathways that mediate inhibition of the central nervous system. Animal studies have shown that there are variable effects of lead on GABA metabolism: some authors showing decrease in the GABA levels in the cerebellum whilst other finding no change. In a functional sense other work has shown that lead exposure sensitises rats to the behavioural effects of convulsant agents and to several aspects of GABAergic function including the uptake of this amino acid, changes in GABA transaminase, glutamic acid decarboxylase and increases in the rate of GABA synthesis /217/. However, Govoni and co-workers /127/ found no changes in at least two of these parameters at lower levels of lead exposure.

A number of other amino acids have been considered in examination of the neurochemical effects of lead especially those that have been considered to be neurotransmitters. The normal developmental increase of glutamate, aspartate and glutamine have been found to be decreased /190/. It has been suggested from these studies that the alteration in amino acid levels are a function of a generalised depression of glucose metabolism and in particular in the transfer of metabolites through the Krebs cycle. The uptake into the forebrain of many other amino acids are generally unchanged /112/.

5-hydroxytryptamine metabolism has not yet been studied in depth but the data that has accumulated up to the present time again shows discrepancies between different studies with some showing little change in 5-hydroxytryptamine and 5-hydroxyindoleacetic acid content and uptake /112/. However, the findings of Dubas and colleagues /114, 115/ showed increases in the endogenous levels of 5-hydroxytryptamine in the cortex, midbrain and hypothalamus. However, in these studies the brain lead levels were considerably higher than in the two studies without effect. There is some evidence, however, that such changes in tryptophan metabolism may be of importance. This importance relates to the effects of lead upon haem biosynthesis and the requirements for haem for the activity of tryptophan pyrrolase the rate-limiting enzyme of tryptophan breakdown /218/. There is little doubt that lead diminishes haem concentrations within the cell and equally, under circumstances where changes in tryptophan pyrrolase occur indicate that there are increases in not only plasma tryptophan, but also in serotonin and 5-hydroxyindoleacetic acid. Thus elevated tryptophan levels have been associated with human hepatic encephalopathy and where there is increase in the concentration of this amino acid in rats there are structural alterations to astrocytes, oligodendrocytes and neurones as well as degeneration of glial cells and axonal wasting. In addition to these morphological effects there are pharmacological and physiological effects of both tryptophan and serotonin which is mimicked by the conditions of acute prophyria /219/. In support of all this, haem infusions increase the concentrations of tryptophan pyrrolase and reverse the elevations of the tryptophan, serotonin and 5-HIAA in the brain. (Figure 6).

There is little work and evidence on other neurotransmitters. The effect of lead on opioid neurotransmitters has recently been studied. Some studies have demonstrated an increase in striatal enkephalins /127, 220/ while others have shown a fall as a result of lead

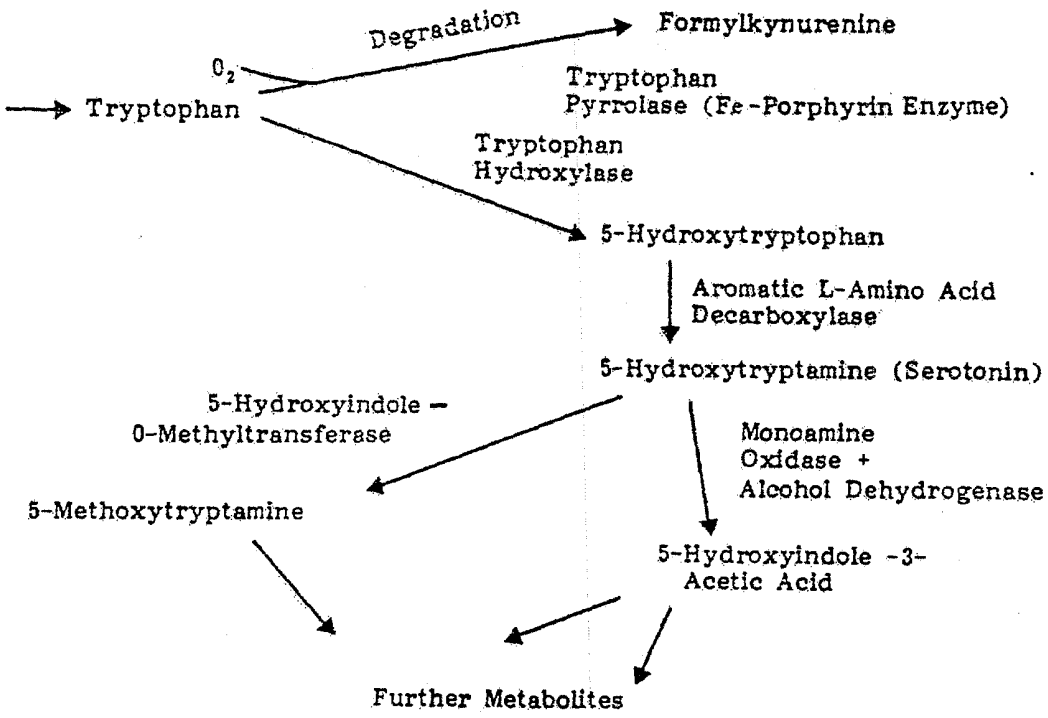

Fig 6: The role of tryptophan pyrrolase in tryptophan metabolism and in the formation of the neurotransmitter, 5-hydroxytryptamine.

exposure /221, 222/. An increase in striatal enkephalins has been observed following three months of continuous lead exposure /223, 224/. Similar effects have been found for shorter and lower levels of exposure to lead /221, 222/.

Of all the interferences in intermediary metabolism, the one most likely to be of supreme importance, especially within the nervous system, are those biochemical effects upon terminal oxidative metabolism. In vivo and in vitro studies have shown changes in mitochondrial function. At relatively high levels of exposure, it is possible to see changes in mitochondrial morphology especially within the kidney /225/. It has also been observed in heart /226, 227/ liver /228/ and in the central and peripheral nervous system /229, 230/. Both in vivo and in vitro studies have shown that these effects upon the mitochondrion will result in changes in terminal oxidative metabolism. In vitro studies have been well reviewed by Bull/191/ and showed there is uncoupling of energy and metabolism together with inhibition of cellular respiration whether using succinate or nicotinamide adenine dinucleotide linked substrates, together with alterations in the kinetics of intracellular calcium transport. In his examination of mitochondrial respiration, Holtzmann and co-workers /231/ showed alterations in cerebellar mitochondrial respiratory control. It was inhibition of phosphorylation coupled respiration for NAD linked substrates, but not for succinate and in following studies the same author showed that the cytochrome content of cerebellar and cerebral mitochondria fell. However, there were few changes in cerebral cytochrome content but these changes are again consistent with diminution in the availability of haem for the synthesis of these cytochromes and is supported by the fact that there is a reduced availability of the mono-oxygenase the cytochromes, P450 following lead exposure. These changes in the cytochrome levels are dose-dependent and are probably most important in the latter stages of oxygen transport such as at the level of cytochrome oxidase in humans /192/.

In conclusion therefore it is not unexpected that lead will be neurotoxic in view of the diverse biochemical effects it can have both in vivo and in vitro. The mode and site of action of lead upon the nervous system are not fully undestood but it is clear that there are several components to this action involving both neurochemical and cytological features. It is almost certain that all

relate eventually to the neurochemical events occasioned by over-exposure to the metal. The relative importance of these various neurochemical events is difficult to apportion but it would seem that the fundamental feature of interference with the terminal oxidative metabolism must rank high in these changes and the reasons for such changes in oxidative metabolism are due to inhibition of haem synthesis. In addition to the changes in haem synthesis, there are obviously unrelated events such as alterations in catecholaminergic function and tryptaminergic function. The first of these may eventually be shown to be linked to the availability of pteridine cofactors. Other systems such as the cholinergic or GABAergic systems are less markedly affected but, undoubtedly, especially at higher levels of exposure, contribute to such change and the same applies to the plethora of other neurotransmitters. Scientific investigation of such changes is now beyond the stage of simple indentification of effect and is proceeding steadily towards a goal of approportionment of the importance of these various effects.

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