TRACE ELEMENTS IN MENTAL RETARDATION

by

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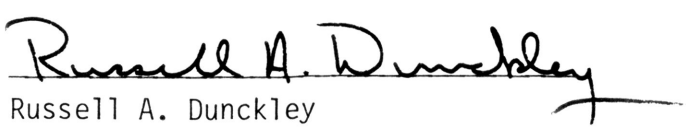

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ABSTRACT

Overt poisoning by some trace elements (Cu, Hg, Mn, Pb) is known to cause extensive central nervous system damage which can result in mental retardation. Recent evidence indicates that low, undetectable exposure to lead may have deleterious effects on mental functioning and behavior. This study took the first step in examining the hypothesis that one factor in the cause of some mental retardation is the effects of subclinical levels of lead and other toxic trace elements. The objective was to investigate the relationship between levels of trace elements and mental functioning in children with a diagnosis of mental retardation due to unspecified causes.

Nine males and fourteen females, ranging in age from three years, two months to 13 years, one month, were selected from a state-operated institution. Head hair samples from each subject are being analyzed for cadmium, copper, iron, lead, manganese, mercury, and zinc content. The effects of a wide variety of factors including age, sex, race, neurological disorders in addition to mental retardation, length of institutionalization, diet, exercise, hair color, geographic residence prior to institutionalization, family income, family size, education of parents, and occupation of parents on trace elements are being considered.

Preliminary data indicates that hair copper content for mentally retarded subjects are slightly higher than for normal children their age. IQ was not significantly correlated with copper levels. However, significant correlations between copper levels and sex ($r=0.39$), race ($r=-0.46$), and activity level ($r=-0.54$) were observed.

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CHAPTER I

INTRODUCTION

In this time of growing concern for environmental pollutants and industrial chemicals, the physiological role of trace elements in nutrition and industrial toxicology has attracted an increasing amount of attention. In spite of this, very little attention has been given to the effects of these elements on mental functioning and behavior. These influences are of concern for several reasons. The most obvious is that some trace elements such as manganese, copper, mercury, and especially lead are known to cause extensive central nervous system damage, if they are present in large amounts. More importantly, some researchers have gathered evidence which demonstrates that subclinical lead levels are associated with impaired learning, defects in perceptual and fine motor functioning, and hyperactivity. These findings are complicated by the fact that low level exposure to and absorption of trace elements (and sometimes even high level exposure, which results in acute poisoning) frequently does not produce observable symptoms. Also, the levels at which trace elements exert deleterious effects are not well defined, and they vary from one person to the next. Moreover, many of the elements are readily available from the environment.

The goal of this study was to investigate the relationship between subclinical levels of trace elements and intellectual functioning in mentally retarded children.

Citations follow the form and style used in the Journal of Abnormal Psychology.

CHAPTER II

LITERATURE REVIEW

Trace Elements

Trace elements are chemical elements which are normally found in plant and animal tissues in minute quantities. Early researchers were unable to measure their precise concentration with the primitive analytical methods then available. They were, therefore, frequently described as occurring in "traces" and the term trace elements arose to describe them. Out of the 45 trace elements occurring in human tissues, currently only 15 have been shown to be essential for normal growth and maintenance of the body (Newberne, 1976). These are iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium, and arsenic.

Essential trace elements act primarily as catalysts in enzyme systems in the cells, where they serve a variety of functions. Their roles range from producing weak ionic effects to functioning in metalloenzymes (Underwood, 1977) - a highly specific protein where the metal atoms are firmly associated with the protein molecule in a fixed ratio.

The characteristic concentrations and functional forms of essential trace elements must be maintained within narrow ranges if the functional and structural integrity of all tissues are to remain unimpaired. Continued ingestion of diets or prolonged exposure to environments that are imbalanced, deficient, or excessively high in a particular trace element will induce changes in its functional forms and concentrations in the body tissues and fluids so that they rise or fall below the normal narrow limits. In these situations, biochemical defects develop which affect

the structure and physiology of an organism in a manner dependent on the particular element involved, on the degree and duration of deficiency or toxicity, and on organismic variables such as age and sex.

The adverse effects that high level exposure to some trace metals has on the brain and behavior have been known for some time. For example, the Committee on Biologic Effects of Atmospheric Pollutants of the National Academy of Sciences (1973) found that workers in manganese ore mines and manganese alloy plants may develop psychosis followed by extensive disorders resembling either parkinsonism or Wilson's disease. Mercury poisoning can cause irritability, moodiness, depression, progressive incoordination, loss of vision and hearing, and mental deterioration, depending on age and form or forms ingested (Underwood, 1977). Frank lead poisoning is characterized by sudden onset, abnormal, excessive accumulation of serous fluid in the brain cavity, hallucinations, coma, and convulsions. More important, it is followed by long-lasting aftereffects including mental retardation, seizure disorders, severe behavioral abnormalities, and occasionally by blindness, aphasia, hemiparesis, and mortality (Perlstein and Attala, 1966).

Evidence from recent studies indicate that undetectable, low level exposure to a variety of trace elements is associated with deficits in mental functioning and behavioral abnormalities. Exposure of pregnant women to methylmercury can result in mental retardation, cerebral palsy, or death of the fetus (Kurland *et al.*, 1960; Takeuchi, 1972). Of greater importance is low level exposure to lead, the effects of which are rarely accompanied by noticeable symptoms. In fact, many researchers, such as Moncrieff *et al.* (1964) have demonstrated that the usual symptoms of lead poisoning (such as the radiographic demonstra-

tion of lead in the gut or ends of long bones and "lead lines" on the gums) may be entirely absent even in cases of overt poisoning.

Although the literature is not entirely consistent (e.g. see Kotok, 1972; Lansdown *et al.*, 1974; Baloh *et al.*, 1975), recent experimental studies, such as the one conducted by Brown (1974), indicate that slowed learning and behavioral disorders can be produced in very young animals with doses of lead insufficient to produce either clinical symptoms or abnormalities in the brain. The work of Byers and Lord (1943), Albert *et al.* (1974), and de la Burde and Choate (1974), among others, shows that these effects also occur in children. They reported that a significant number of lead-exposed children exhibited deficits in fine motor functioning, impairment of learning, and behavioral problems. David *et al.* (1974) reported an increased blood lead content in hyperactive children relative to nonhyperactive children of similar background. Perino and Ernhart (1974) found that as a child's subclinical lead level increased, his general cognitive, verbal, and perceptual abilities decreased.

Problems in Diagnosis

There are three major obstacles which make diagnosis of low, but elevated levels of trace elements in the body difficult. First, people burdened with increased trace element levels will not normally display overt symptoms. Second, there is still some confusion over what amounts of these trace elements constitute normal and dangerous levels. Moncreiff *et al.* (1964) and Perino and Ernhart (1974) suggested abandoning any previously held ideas about the concentrations of lead in blood at which toxicity can occur. This disagreement stems from the varying

responses different individuals have toward the same concentrations of lead. What constitutes a toxic dose for one person may be a harmless dose for another.

The third problem in diagnosis concerns the procedure commonly used in detecting levels of elements, especially lead, in human tissues. Tests utilizing lead concentrations in the blood for diagnosis fail, in general, to relate dose and response. Beretic (1971) concluded that the response of humans to the absorption of lead is not a simple relationship and that a particular individual's response cannot be diagnosed directly by determining the amount of lead in blood. However, modifications of this procedure (which involve determining erythrocyte protophyrin concentrations along with lead concentrations in the blood) have proved to be valuable in relating dosage to response (Chisholm, 1977).

Analyzing for trace elements in hair holds obvious advantages over the analysis for them in blood or other tissues. For example, hair can be painlessly and easily removed and readily transported, stored and analyzed. On the other hand, blood can be removed only with some degree of discomfort and only by a trained health professional. In addition, blood cannot be stored for long periods of time. Trace elements are accumulated in hair at concentrations that are generally at least ten times higher than those found present in serum or urine (Valkovic, 1977). Although hair has a fairly low metabolic activity, experiments show that it almost immediately reflects changes in trace element levels in the body (Valkovic, 1977). Furthermore, hair can be analyzed for a variety of trace elements, but the number that can be determined in blood samples is very limited. Thus hair analysis offers great possibilities in determining the interdependence of trace elements. For these reasons,

hair analysis has the potential to become a valuable diagnostic tool.

Increased Vulnerability of Children

The effects of trace metals on young children are of particular concern. Besides the fact that they are usually unaccompanied by easily recognizable symptoms, low chronic levels of trace elements in young children seem to produce damage which surfaces only in later childhood (Chisholm, 1977). Moreover, studies conducted on rats by Kostial *et al.* (1974) and Momcilovic and Kostial (1974) show that the younger the animal, the greater is the amount of lead absorbed from the intestinal tract. Furthermore, studies with experimental animals also provide evidence that dietary deficiencies of iron, copper, and calcium increase the absorption and the adverse effects of lead in the very young, but not in the mature animal. Moreover, population studies indicate that deficiencies in dietary intakes of iron and calcium are prevalent in preschool age children (Chisholm, 1977). Finally, and probably most importantly, low exposure to trace elements in infancy and early childhood may pose a major health problem because it occurs simultaneously with major developmental changes in the nervous system. Thus, experimental data indicates that the very young are potentially more susceptible to the effects of trace elements such as lead.

Research Using Hair Analysis

Pihl and Parkes (1977) reported that they could distinguish between normal children and those with learning disabilities with 98 percent accuracy by analyzing concentrations of 14 elements. Particularly important in the differentiation were increased levels of lead, cadmium, and manganese, and reduced levels of lithium and chromium. A follow-up study

of these children after two years of behavioral and nutritional therapy (Maugh, 1978) indicated that both their behavior and the trace element profile in their hair had almost returned to normal.

In hair from 67 women with Down's syndrome, Barlow and Kapel (in Maugh, 1978) observed below normal concentrations of calcium, copper, and manganese. In hair from 37 patients with schizophrenia, Barlow and Kapel found below normal concentrations of cadmium and manganese, and above normal concentrations of lead and iron. In addition, they observed below normal concentrations of manganese, iron, lead, and copper, and above normal concentrations of zinc in hair from a group of 25 "severely subnormal young people" (Maugh, 1978, p. 1273) in Denmark.

Objectives

Mental retardation can be produced by a great number of agents. It may be hypothesized that one factor in its cause in children is the effects of subclinical levels of toxic trace elements. This study attempted to take the first step towards examining this hypothesis by determining the relationship between levels of trace elements and intellectual functioning, as measured by IQ scores. Hair from children whose cause of retardation is unknown was analyzed for cadmium, copper, iron, lead, manganese, mercury, and zinc.

CHAPTER III

METHOD

Subjects

The subjects were 23 mentally retarded children, selected from a state-operated institution for a diagnosis of mental retardation due to unspecified causes and an age of 12 years or less. Upon sampling, subjects ranged from three years, two months to 13 years, one month of age, with a mean age of eight years and six months. Informed consent of each child's guardian was obtained prior to participation.

Apparatus

Hair samples were mechanically agitated during the washing procedure by a Burrell 75 wrist action shaker. Samples were dried in a Virtis 10-100 uni-trap freeze-dryer with a Sargent-Welch 1402 duo seal vacuum pump. Dry sample weight was determined with a Mettler P163 top-loading analytical balance. Heating of hair samples during digestion was effected by five Corning PC-100 hot plates. Three elements (Cu, Fe, Zn) were analyzed in digested hair samples using a Jarrell-Ash 810 dual channel atomic absorption spectrophotometer and Soltec 281 recorder. Analyses of Pb, Cd, and Mn were made using a Perkin-Elmer 306 atomic absorption spectrophotometer equipped with an HGA-2100 graphite furnace atomizer, a deuterium arc background corrector, and a Sargent-Welch SRG recorder. All digested sample and standard solution dilutions were carried out with an Ainsworth A-200 top-loading electronic balance.

Procedure

Collection of hair samples. All hair samples were taken by a cosmetologist at the institution according to a standardized procedure. Samples ranging from 0.75 to 1.50 grams were taken from the crown of the head. Each lock was snipped within a few millimeters of the scalp with a pair of stainless steel hair shears.

Collection of personal data. Access to each participant's record was gained through the informed consent of their guardian. The types of information taken from each file included date of birth, sex, age, ethnic origin, IQ test administered and IQ, diagnosis, other neurological disabilities, date of admission to present institution, dates of admission to and release from previous institutions, smoking habits, diet, amount of activity or exercise, hair color, geographic location of residence prior to institutionalization, income of family, education of parents, and occupation of parents. Knowledge of these variables was required to account for the influence they may have on trace element levels in hair.

Coding of personal data. In order to protect confidentiality, two coding systems were employed. Upon screening and selection, each subject was assigned a number for identification purposes. This number appeared on both the plastic bag containing the hair sample and on the personal data sheet. For the convenience of the cosmetologist, names were attached to the plastic sample bags, but no names appeared on any record of confidential information. After being transported to Texas A&M University, names of all subjects were removed from their sample containers and destroyed. Also, a new set of identifying numbers were formulated to replace the initial code numbers. The only record of participants and corresponding code numbers was retained at the institu-

tion.

Preparation of samples. First, an attempt was made to standardize the length of all hair samples. Twenty samples were trimmed to a length of seven centimeters, as measured from the proximal end of the hair shaft. However, three samples were too short and kinky to be standardized. All cuts were made with the same pair of shears used in sample collection on acrylic plastic cutting boards.

Second, each sample was cleansed to remove any trace elements external to the hair structure. The washing procedure used was a modification of the one recommended by Harrison, Yuracek, and Benson (1969), and was performed in a clean room (a lab where the levels of trace elements have been minimized) to avoid contamination. Each sample was washed in a 40 dram snapcap polyethylene vial with 100 milliliters of a 0.14% solution of metal-free non-ionic detergent (Acationox, Sherwood Medical Industries). The vials were agitated on a mechanical shaker for 30 minutes at room temperature. Upon completion of the washing cycle, the samples were rinsed with five, 100 milliliter aliquots of deionized distilled water.

After rinsing, the caps of the vials were removed and replaced by perforated Parafilm. The samples were freeze-dried for 18 to 20 hours to a constant weight.

Digestion of samples. Freeze-dried samples were prepared by digestion for atomic absorption spectrophotometric (AAS) analysis using a wet nitric acid (HNO_3) - perchloric acid ($HClO_4$) oxidation procudure (Prelsey & Boothe, 1977) in a clean room. Each sample was placed in a tared, spoutless, electrolytic pyrex beaker to determine dry weight. Fifteen milliliters of redistilled 70% HNO_3 (G.F. Smith Chemical

Company) and one milliliter of double distilled 70% $HClO_4$ (G.F. Smith Chemical Company) were added. The beaker was covered with a 75 millimeter, non-ribbed pyrex watchglass and allowed to sit overnight at room temperature. The mixture was then refluxed at low heat on a hotplate for six to ten hours. A bent glass rod was placed between the beaker lip and the watchglass and the heat increased to permit HNO_3 evaporation. At the first sign of white $HClO_4$ fumes (indicating that most of the HNO_3 had evolved), the glass rod was removed, allowing the watchglass to again rest flush on top of the beaker. The sample was refluxed until the digestion mixture cleared. If the sample did not clear or if charring occurred, an additional 1.0 milliliter of HNO_3 and 0.5 milliliter of $HClO_4$ was added and refluxing continued until clearing occurred. This measure was repeated as necessary. Finally, the watchglass was removed and the mixture was allowed to evaporate near dryness. Spike recovery experiments conducted by Presley and Boothe (1977) indicate that this digestion procedure does not cause a significant loss of any of the trace metals of concern.

Each digested sample was transferred to a tared, presoaked (in 1.0 N G.F. Smith HNO_3), 13 dram polyethylene vial by washing the beaker several times with 0.1 N HNO_3 (Baker Ultrex grade) and pouring the resultant solutions into the vial. This transfer process has been shown to be quite complete (Presley & Boothe, 1978).

Each sample was brought to approximately 18 milliliters, thereby diluting the original dry weight sample anywhere from 13 to 36 times. The volume of each sample solution was determined by reweighing the filled vial and making a small correction (1.02 g/ml at pH $\sim$0.5-1.0) for the specific gravity of the sample solution. Further dilutions from the

original solution were made on a weight/weight basis in pre-soaked 13 dram snap-cap vials (using 0.1 N G.F. Smith HNO_3).

Four procedural blanks were included in each digestion to determine the amount of each metal contributed by the digestion glassware and reagents. These blanks received the same reagents and treatment as the tissue samples. An aliquot of the 0.1 N HNO_3 used to transfer and dilute the sample was placed in a vial and analyzed with each digestion as a diluent/tube blank. Reagent blanks were analyzed for all bottles of acid, prior to their use in sample digestion. These blanks were prepared by taking 10 milliliters of acid, evaporating it to near dryness in digestion glassware and transferring the residue to a 13 dram vial in the same manner described above. For each series of dilutions made using 13 dram vials, one or more vial blanks were prepared and analyzed.

To determine whether any of the metals of interest were lost from samples during digestion, spike recovery experiments were conducted during each digestion. To determine the accuracy and precision of the total analysis procedure, replicate samples and a National Bureau of Standards (NBS) standard biological reference material (bovine liver #1577) were analyzed for each digestion.

Analysis of samples. All samples, sample replicates, standard bovine liver reference material and blanks were analyzed for seven elements - copper, cadmium, iron, lead, mercury, manganese, and zinc. Copper, iron, and zinc concentrations were assessed by flame AAS using a Jarrell-Ash Model 810 atomic absorption spectrophotometer. Analyses were carried out following the manufacturer's recommended procedure. Non-specific absorption was monitored by measuring simultaneously the absorbance of a non-resonance line and the analytical line of the element

of interest. A lean air-acetylene flame with flow rates of about 15 and 2.5 liters per minute, respectively, were used for all three elements. Aspiration rate was five to six milliliters per minute. Injection volume was 0.75 milliliters. Chemical interference was checked by use of the standard additions technique. Mixed standard metal solutions were prepared in dilute HNO_3 (Baker Ultrex grade) by diluting concentrated commercial atomic absorption standards. Samples were quantitated by peak height comparison with a standard concentration curve.

Cadmium, lead, and manganese, which occurred at low levels, were measured using flameless AAS. These analyses were made using a Perkin-Elmer Model 306 atomic absorption spectrophotometer equipped with an HGA-2100 graphite furnance. Injection volume was 25 microliters. The furnace temperature gauge was calibrated using a clamp-on (inductive) ammeter and an optical pyrometer. Dry, char, and atomization temperatures and times were optimized for each metal using selected representative samples according to the manufacturer's recommendations. External and internal furnace purge gas flow rates were verified at specified levels of 0.9 and 0.3 liters per minute respectively at 40 psi delivery pressure. Corrections for non-specific or broad band molecular absorption were made by a deuterium arc background corrector. Chemical interference was evaluated and corrected as necessary by frequent use of the method of standard additions and check dilutions. Mixed standards used were prepared as described above. Samples were quantitated by peak height comparison with a standard concentration curve and bracketing standards injected before and after the samples. Consideration was given to temporal variations in instrumental sensitivity, non-linearity between bracketing standards and gross differences in peak shape.

CHAPTER IV

RESULTS

Only the results of the copper analysis performed on each sample is available at this time. Values ranged from 8.21 to 18.4 ppm with a mean of 12.2 ppm and standard deviation of 2.50 ppm. Correlation coefficients for copper level versus age, sex, race, IQ, length of institutionalization, level of activity, income of family, family size, education of parents and occupation of parents were computed. A correlation between copper levels and sex ($r=0.39$) was significant at the 0.07 confidence level. Race and copper concentration were negatively correlated ($r=-0.46$, $p<0.03$). Copper levels in hair and level of activity were also negatively correlated ($r=-0.54$, $p<0.01$). The remainder of the correlation coefficients were insignificant.

CHAPTER V

DISCUSSION

Due to the unanticipated delay in obtaining approval from the Texas Department of Mental Health and Mental Retardation (TDMHMR), the sample size for this study was limited to 23 subjects. Due to the bureaucratic delay mentioned above, delays in receiving vital reagents, and scheduling conflicts over equipment use with other researchers who had first priority, the analyses for each element have not yet been completed. Upon completion, the concentrations of seven trace elements (Cd, Cu, Hg, Fe, Pb, Mn, and Zn) will have been determined for at least 50 subjects similar to the ones used thus far.

Any conclusions based on the scant preliminary data would be highly speculative. However, a few comments concerning the obtained copper concentrations are merited. The mean copper level for hair in the group of mentally retarded children was slightly higher than that of normal children their age - 10 to 12 ppm (Underwood, 1977). Copper levels were found to be related to sex, race, and activity level in the present study. Copper levels have been shown to be slightly age dependent (Eatough *et al.*, 1974). This effect may surface in this study when copper values on additional subjects are obtained. Rice and Goldstein (1961), among others, have reported sex differences in hair copper content and these results confirm their observations. The negative correlation between activity level and copper concentration is unique, however, To the knowledge of this author, no previous studies have attempted to investigate this relationship.

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