MD THESIS

METAL IONS AND THE AETIOPATHOGENESIS OF PARKINSON'S DISEASE IN NIGERIANS

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TO WHOM IT MAY CONCERN

Re: Dr Sunday BWALA

Dr Sunday Bwala discussed his MD thesis with me at its inception and asked me if I would be prepared to act as an external supervisor, which I was delighted to do.

The work, however, within this thesis is entirely Dr Bwala's own and was carried out in collaboration with Mr David Dexter in the University Department, King's College, London.

Signed: .....
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THE DEAN
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30.1.1991

Dear Sir,

RE:MD THESIS ON "THE AETIOPATHOGENESIS OF PARKINSON'S DISEASE IN NIGERIANS" BY DR.S.A.BWALA.

This is to certify that Dr.S.A.Bwala who is a consultant physician in this hospital conceived the above thesis and carried out the clinical and investigative procedures. The laboratory analysis was performed in collaboration with Kings College Hospital,London and the Biosciencia laboratories in Mainz,Germany.

Yours Faithfully,

Professor Idris Mohammed MD,FRCP,FAS.
(Internal Supervisor and Chief Medical Director.).

DEDICATIONS.

To my late father,Angaya Mshelbwala,a registerd nurse of the Royal College,who inspired me to learn the art of medicine at the early age of 11.He took me along to watch him anaesthetise patients for surgery and subsequently observe the surgeons perform procedures that ranged from hernial repairs to gastrectomies.Rest In Peace Daddy.You sacrificed your time ,energy and personal comfort to see to my education.Your efforts came to fruition and I pray to live such an examplary and selfless life as yours.Again,Rest In Peace Daddy.

To my mother,Ya Gana Nkwarbwala for her longsuffering and cheerfulness during my childhood and education.To you Iam grateful mother.

And to my wife Mary and our children Grace,Joy,Charles and David for their patience,understanding and support.

Sunday A.Bwala
Saudi Arabia 1990.

'Dust as we are, the immortal spirit grows
Like harmony in music; there is a dark
Inscrutable workmanship that reconciles
Discordant elements'.

Wordsworth.

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CHAPTER ONE

HISTORICAL PERSPECTIVES OF PARKINSON'S DISEASE.

(A) Clinical Aspects.

When James Parkinson (1755-1824) wrote his essay on "the shaking Palsy" in 1817, little did he know that his colleagues will do him the well deserved honour of naming the constellation of symptoms he described after him as a disease entity.[1,2] Thus Idiopathic Parkinson's disease (IPD) has come to be recognised in clinical neurology and is best described by James Parkinson himself when he wrote in the famous, celebrated essay,"..... The first symptoms percieved are, a slight sense of weakness with a pronesess to trembling ..... most commonly in one of the hands and arms.... As the disease proceeds, ...difficulties increase: writing can now be hardly at all accomplished,... the propensity to lean forwards becomes invicible, and the patient is forced to step on the toes and fore part of the feet,..... irresistably impelled to take much quicker and shorter steps, and therby adopt unwillingly a running pace." As the disease progressed James Parkinson went on to write "his words are now scarcely intelligible.... saliva is continually draining from mouth...." He was careful to distinguish "the shaking" palsy from similar disorders and wrote what one might call a differential diagnosis. "It is sufficient, in general, to point out the characteristic differences which are observable between disease/s in some respects resembling consequent to indulgence in the drinking of spiritous liqours; that which proceeds from the immoderate employment of tea and coffee; that which appears to be dependant on advanced age; and all those trembling which proceed from the various circumstances which

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induce a dimunition of power in the nervous system." He took time to point out that the tremour of "the shaking palsy" was present at rest and tended to disappear with action. Such masterly observation and description of a disease, which is the bed-rock of clinical medicine in general and neurology in particular, certainly deserves the recognition given to James Parkinson.

Clinical states akin to some features of "the shaking palsy" are speculated to have been described as far back as 2,500 BC.[3] This stretched from Ayurvedic medicine through Egyptian papyrus records to the Old Testament, Galen, Leonardo da Vinci and to Gabius in 1758. Expectedly, the signs observed were tremors and gait disturbances which are likely to attract the attention of people. Brady-kinesia and perhaps sialorroea were also noticed. After James Parkinson's essay, physicians became more aware of the disease and took interest in making more observations. Descriptions of "dementia-like" symptoms and rigidity emerged and 1888, Gowers made an astute observation of the epidemiologic characteristics of the disease. He noted it was common in the later years of life with a male preponderance and an apparent hereditary tendency in a minority of the patients.[4]

To summarise, Parkinsonian features seem to have been present in patients centuries before James Parkinson made his observations. After the essay on "the shaking palsy", progressively increasing descriptions of the disease came to light over the decades. Today, a clinical diagnosis of Parkinson's disease is generally accepted when two or more of the following are present:-rhytmic rest tremor of 4-6/second, bradykinesia, rigidity and postural disturbances.[5]

(B) Pathology.

James Parkinson did not have the privilage of autopsy cases to study.

Nevertheless, he speculated the anatomical site of the lesion to be at the medulla as he wrote"... in that part of which is contained in the canal, formed by the superior cervical vertebrae, and extending, as the disease proceeds to the medulla oblongata". After the "essay, an increasing number of autopsy descriptions of the disease came to light and in 1925, Foix and Nicolesco re ported on the basic pathology.[6] Since then clearer definitions of the pathology of Parkinson's disease has become available. It is established that currently, 66-85% of the cases of Idiopathic Parkinson's disease have characteristic focal neuromelanin cell loss in the central and caudal parts of the zona compacta of the substancia nigra.[7] Similar variable changes are also seen in the subcortical nuclei such as the noradrenergic Locus Coelereus and Cholinergic Nucleus Basalis of Meynert. In additon, Lewy bodies (intracytoplasmic inclusions) are found in 82-100% of the cases.

(C) Aetiology.

Down the ages, tremor similar to the parkinsonian type had been associated with old age.[3] Added with the observations of Gowers that IPD was a disease of later life, the notion that it has to do with the ageing process becomes hard to ignore. To crown it all, present epidemiologic data confirms Gowers observations, and progressive dimunition of striatal dopamine with age has been well documented.[8] It seems therefore inescapable that the ageing process is part of the underlying factors in the genesis of IPD. Since not everybody develops the disease on getting old, an intrisic genetic or environmentaly acquired factor must predis poseto the disease. Gowers noted only a 15% tendency to inheritance and recent studies of monozygotic twins show a

very low concordance rate for IPD.[9,10] Although this does not totally exclude a genetic predisposition to the disease, the environmental factor becomes more important. James Parkinson himself did ponder on trauma to "the medulla spinalis" as a cause but was of course limited in his observation by the state of knowledge in pathology and opportunities of his time. In a curious way, "demetia pugilistica" is post traumatic but is of course a syndrome of multiple neurologic deficits with parkinsonism as one of its features.[11] The pandemic of encephalitis lethargica (von Economo's disease) between 1917-1926 resulted in parkinsonism but clinically different from IPD and cases have since died out.[12,13] Nevertheless, this did raise the possibility of a slow virus as a cause of IPD. Recent resurgence of the viral hypothesis is that of influenza virus infection in intrauterine life but has been promtly disputed.[14,15] Thus far, infectious agents have been contemplated as possible causes of IPD and as long as no other definite factor is found the matter will not be put to rest.

With the ever present background of a disease like Wilson's disease, in which a metal ion (copper) is known to be deposited in the basal ganglia causing movement disorders, the search for an environmentally acquired metal ion intoxication only seemed logical.[16] The documentation of manganese induced parkinsonism kindled the search further.[17] Curiously, some studies found negative associations with factors such as cigarette smoking but the exact reason why it is not clear.[18] Consumption of pesticide contaminated underground (well) water in a rural community has been postulated to cause a local increased incidence of Parkinson's disease.[19,20] Another case in point is

the Amyotrophic Lateral Sclerosis/Parkinson Demetia Complex (ALS/PD) of the Chamaro people of Guam in the Pacific.[21] Metal ions hypothesised to be involved are clacium and magnesium. The picture is however complicated by the presence of other potential neurotoxins in a plant diet, the Cycad.[22]

The climax in the search for an environmental neurotoxin was when parkinsonism was stumbled upon in a young drug addict.[23] This was subsequently shown to have been induced by 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP). This was present as a by product in a synthetic analogue of pethidine. MPTP is a known compound first synthesised in 1947 as a therapeutic agent then but caused rigidity in primates. The compound produces a selective damage to the zona compacta of the substancia nigra simulating parkinsonian clinical features and has been used to produce experimental models of parkinson's disease in animals.[24,25] So, it seemed an answer to the problem was at hand. However, the toxin has not yet been shown to occur naturally in the environment. The search for MPTP-like analogues has been promted as a result.[26] This is a worthwhile effort because the pathophysiologic mechanism of neuronal damage by MPTP has therapeutic implications as it can be inhibited by drugs like selegiline, a monoamine-B inhibitor (MOA-B).[27,28] MOA-B is necessary for the bio-transformation of MPTP to the neurotoxic radical MPP^+ which is thought to affect mitochondrial function perhaps at the level of Complex 1 or by the generation of free-radicals.[29,30] Free-radicals cause neuronal death.[31,32]

In summary, the pieces of the jigsaw puzzle of the aetiopathogenesis of Idiopathic Parkinson's disease include the ageing process, some undefined predisposition, and environmental neurotoxic agents such as metal ions or

MPTP-like analogues. As to which piece will complete the picture, is still open. This thesis endeavors to contribute in this search by looking at metal ions in Nigerian Parkinsonian patients.

CHAPTER TWO

DIFFERENTIAL DIAGNOSIS

Clinically, James Parkinson himself distinguished "the shaking palsy" from other neuro-degerative syndromes that had tremor.[1] Today, the clinical diagnostic criteria of Martilla is widely used for Idiopathic Parkinson's disease (IPD).[5] Over the years however, an array of neurodegerative syndromes with parkinsonian symptoms as one of their many deficits have emerged. To distinguishe them from IPD, several classifications have been employed. Martilla reserved (a) Primary Parkinsonism for IPD and (b) Secondary parkinsonism for infectious (post-encephalitic), Toxic (manganese, carbon monoxide), drugs (phenothiazines, butyrophenones, reserpine) vascular (arterio-sclerotic) trauma, tumours and miscellaneus causes. Presently, a widely used system places them under a broad umbrella of The Akinetic-Rigid syndromes.[33] This is subdivided into:-

(a) Pure Parkinsonism (60-75%)

(b) Parkinson-plus syndromes (15%)

(c) Diffuse with parkinsonism

(d) Childhood syndromes with parkinsonism

Idiopathic Parkinson's disease forms the bulk of the first group distinguished by the classical features of rhytmic, rest tremor (4-6/second) rigidity; bradykinesia and postural disturbances any two of which is accepted for a clinical diagnosis. Diagnostic pitfalls include hemi-parkinsonian symptoms (rigidity and bradykinesia particulay) misinterpreted as "stroke". Drug induced parkinsonism can mimic IPD and a careful history of ingestion of the phenothiazines, butyrophenones, and reserpine should be looked out for. Sporadic toxin-induced parkinsonism is included in this group such as as manganese, carbon mono-

xide and of recent the celebrated MPTP-induced parkinsonism in drug addicts.[23] A history of exposure is usually present and there may be a local increased incidence depending upon the mode of exposure. The post-encephalitic parkinsonism of Von Economo[12] has distinctive clinical features, prominent among which are oculo-gyric crises, pupullary abnormalities, dyskinesia and an earlier age of onset. The incidence of the disease has declined since the pandemic of 1951 eventhough the inceidence of IPD has remained relatively stable.[34]

The parkinson-plus syndromes include:- Progressive Supranuclear Palsy (Steel-Richardson-Olszewsky syndrome)[35], Multiple System Atrophy (olivo-ponto-cerevellar degeneration, striato-nigral degeneration, Progressive autonomic failure),[36] and parkinsonism with basal ganglia calcification. Progressive Supranuclear Palsy is distinguished clinically by the presence of supranuclear gaze palsy (down gaze), dysarthria and axial rigidity. The tendon reflexes are brisk and the plantar response may be extensor. Multiple System Atrophy (MSA) is a welcome simple term for the hitherto, bewildering array of neurodegenerative syndromes that involve pyramidal, extrapyramidal, cerebellar and autonic pathways. This was earlier termed the Shy-Drager syndrome [37] which has been dropped in favour of MSA which is less confusing. The syndrome presents at an earlier age than IPD and the response to DA replacement is poor. Progressive Autonomic Failure (PAF) which is a component of MSA presents with disturbances such as postural hypotension and impotence from the degeneration of cells of the intermediolateral column of the spinal cord and brainstem equivalents. Isolated pure PAF without other system involvement does occur and was formerly called Idiophatic Orthostatic Hypotension (IOH).

The group of diffuse brain disease with parkinsonism as one of their symptomatology are many and include:- Alzheimer's Dementia (AD), Creutzfeldt-

Jacob disease, Multi-infarct dementia (MID) and Dementia Pugilistica ('Punch-drunk' syndrome).[11] Apart from akinetic-rigid features, this group has demetia as its hallmark of presentation. The problem of course is that some patients with Idiopathic parkinson's disease present with dementia and 14.6-62.5% of those that come to autopsy have AD type of plaques, neurofibrillary tangles and cortical neuronal loss.[7,38] To compound the problem, nigral Lewy bodies which are the pathological hallmark of IPD are found in 18% of AD brains.[39] Because of the overlap in the clinical and pathologic features of IPD and AD, some have wondered whether IPD is a form of AD or vice versa.[40,41] Another view is that the co-existence of these two diseases in some patients might be purely co-incidental because of the high prevalence of these pathological lesions in the population of that age group anyway.[42,43] Then there is the neuropathological entity, Diffuse Lewy body disease that may present clinically as a Parkinsonian syndrome with subsequent dementia or vice versa. Lewy bodies are present diffusely in the brainstem and cerebral cortex.[44] Nevertheless, the consensus at the moment for a diagnosis of IPD follows the clinical criteria of Martilla, 60% depletion of neurones in the substancia nigra and the presence of Lewy bodies in almost 99% of cases that come to autopsy.[5,7,] Where there is doupt, IPD is excluded if Lewy bodies are absent in two nigral sections, 330 pigmented nigral nerve cells, or 150 pimented nerve cells in the locus coelureus.[45] Of course these pathologic criteria still leaves the clinitian in a dilemma at the bedside or clinic. A way out of this might come with recent developments in Positron Emission Tomography Scanning (PET). The Akinetic-rigid syndromes are said to have distinctive features in terms of

regional cerebral blood flow (rCBF), regional cerebral oxydative metabolism ($rCMRO_2$), regional cerebral metabolic rate for isoptic glucose (rCMRGlu) and pre-and pos-synaptic dopaminergic systems.[46] Similar PET studies in the dementias reveal certain characteristics of rCBF, $rCMRO_2$, and rCMRGLu that differentiates them from parkinson's disease.[47] Though still a research tool, PET studies are improving and hold great promise in offering the clinician a bedside assesment by activation studies while the patient performs mental and physical activities.

Of the various childhood akinetic-rigid syndromes, Wilson,s disease and Hallervorden-Spatz disease, are well known, and curiously, they are associated with metal ion deposition and damage to the basala ganglia.[48,49]

In Hallervorden-Spatz disease, an iron-containing pigment is deposited in the substancia nigra but a metabolic defect of cysteine accumulation has been proposed.[50] 8% of Hutington's disease presents in childhood, half of whom have the Westphal variant with akinetic-rigid symptomatology.[33] There are several parkinsonian syndromes presenting before the age of 20 years and tend to run in families.[51] However the nosology is yet to be settled as pathological studies have not been made in most of them. For practical purpose, the term Idiopathic Parkinson's disease is restricted to adults in this study.

CHAPTER THREE

GEOGRAPHICAL LOCATION OF THE STUDY.

The study was carried out in Maiduguri, the capital of Borno State of Northern Nigeria. (Fig. 1) Borno State is at the north eastern corner of Nigeria lying between longtitude 10^{O}E to 15^{O}E and latitude 10-14^{O} north of the equator with the Lake Chad forming its north eastern border. It has an area of about 11,658,900 M^2 and a population of about 5 million with occupations centered around commerce and civil service in the urban areas and farming in the rural areas. The University Teaching Hospital, where the study was done, is located in Maiduguri ($11^{0}51'$ North,$13^{0}15'$ East) with a population of 800,000 consisting of six to ten diferrent tribes. The University hospital has a bed capacity of 350 and serves as a tertiary referal centre for the state and neighboring Gongola and Bauchi States.

The climate of Borno State consists of a long dry season (October to May) and a short wet season (June to September).[52] The vegetation is that of a Savannah with the Sahel, Sudan and Guinea varieties from the northern to the southern part of the state in that order. The Sahel is made up of thornbush mainly and has a mean annual rainfall of 250-500 mm. It is found in the extreme northern part of the state and is sparsely populated. There are worrying evidences of encroachment of the Sahara desert in this part of the state with consequent migration of the local populace to the southern regions. The Sudan savannah is located in the middle part of the state with more rainfall (mean annual rainfall 500-800 mm.) and has a shrubby vegetation with tall trees. It is densely populated and the main Staple food is millet, soghum and groundnuts, supplemented with rice in economically well-to-do or

cassava in the poorer people. The Guinea savannah covers a small portion of the southernmost part of the state with a mean annual rainfall of 800-1000 mm. and has a vegetaion of more grass and trees.

Most of the published work of Parkinson's disease in Nigeria has been done in the southern part of the country. A hospital based study in Ibadan documented a frequency of 5/10,000 (1966 to 1976).[53] A door-to-door community survey in 1986 in Ibadan Nigeria [54] showed a prevelance ratio of 59/100,000. This is considered to be genuinely lower than in the developed countries. No such documented study has been done in Borno State before. In a four year practice in the University of Maiduguri Teaching Hospital (1985-1988), which is a tertiary referal hospital and where the only practicing neurologist (author) in the state saw virtually all cases of Parkinson's disease, an impression of a low prevelance of the disease was made. Doctors in the peripheral hospitals were personally requested by the author to refer all neurologic cases in general and Parkinsonism in particular. This hospital-based study is the first of its kind in the Borno State by documenting the clinical features of Parkinson's disease in this part of Nigeria. In addtion, the study of the role of metal ions in the aetiopathogenesis of the disease is the first ot its kind not only in Borno State but in Nigeria as a whole.

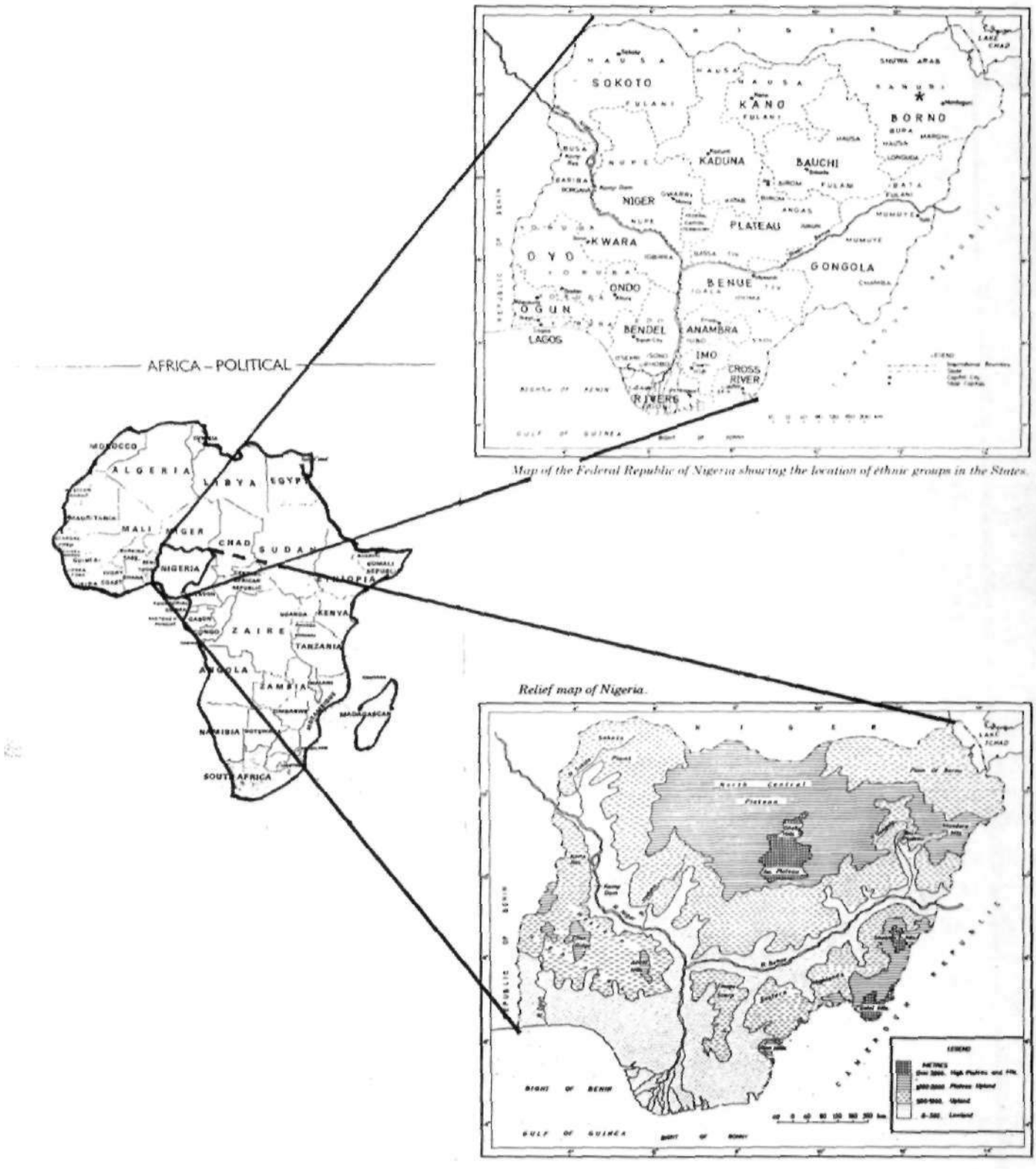


FIGURE I. Maiduguri is indicated with asterick *

CHAPTER - FOUR

PREFACE

THE THESIS:

Large, long-lived cells like the neurons may be susceptible to the cumulative effect of trace elements over a long period of time.[55] Some of these have been implicated to cause parkinsonism such as copper, iron, manganese and magnesium.[17,56] The diet and water supply of people in the third world, 80% of whom are peasant agriculturists, are not monitored for such trace elements contaminants. Food and water are scarce and whatever is available is consumed in order to survive without due regard to contamination. Quite often, one sees, sinks and water containers heavily stained red possibly from iron content of the drinking water. Such societies eat a single crop as staple food. If this is contaminated with a toxin, cumulative effects are likely as has been shown in lathyrism and the Nigerian Ataxic Tropical neuropathy.[57,58] Therefore the thesis is that:

(a) Analysis of drinking water and staple food where Parkinsonian Patients reside should reveal trace element contamination.

(b) Trace element estimation in the serum and cerebrospinal fluid (CSF) of Parkinsonian patients is likely to reflect such abnormalities.

(c) Since only a small proportion of the population living in the same environment and consuming the same food and water develop Parkinson's disease, there should be a group susceptible to the cumulative effect of trace elements.

CHAPTER - FIVE

REVIEW OF THE AETIOPATHOGENESIS OF PARKINSON'S DISEASE.

Useful aetiological clues can be obtained from the epidemiological characteristics of a disease and in the case of Idiopathic Parkinson's disease (IPD), exciting possibilities have been encountered over the years.[20,23] The age specific incidence rate of IPD in well studied, stable communities has been shown to be steady.[34] This implies that new neurotoxins are unlikely to have caused the disease over the period of study spanning about 35 years. Rather, the casuative agent is likely to have affected the population at risk over a long period of time in contrast to the sporadic cases of parkinsonism due to identified exposure to neurotoxins like manganese and MPTP.[17,23] Thus the pointer is towards a neurotoxin that has been present in the environment for a long time, perhaps since the disease was described decades ago by James Parkinson; an agent in constant contact with the population at risk; may be consumed along with food or drink. The metal ions make good candidates for such an agent because they have ever been present with man in his environment as part of his biological constituents.[59] The degree of exposure to such an agent is likely to vary from one environment to the other because the incidence rate of IPD differs from country to country.[60] This will not be surprising because the metal ion content of soils and water vary according to the local geologic make ups. A natural experiment has been going on in this context, because door-to-door surveys have shown that blacks in a rural West African Community (Nigeria) have a very much lower prevalence rate for IPD than blacks in rural

Mississippi, United States of America (USA).[54] At the same time, the blacks born and living in the USA seem to have acquired the same higher prevalence rate of the whites / 341/100,000. This further indicates that environmental factors have a greater role in the aetiology of IPD rather than genetic factors solely.[61] The age-specific incidence curve for IPD rises steadily from 35-40 years, speaking at about 75 and then declining. This lends support for an exposure to an agent at a point in time, then 'incubating' over a certain period as a 'latent neurotoxin' before the disease manifests clinically. On the other hand, the exposure might be chronic and continous over time but this will like give a progressive increase in the age specific incidence rate after the age of 75. This might be the case if diagnosis of IPD is not made after that age and can arise if people beyond the age of 75 attribute their symptoms to old age and hence do not declare it even in door to door surveys. This is particularly so in the early stage of the disease when the symptomatology may be subtle. Either way, it is established that there is progressive dimunition of striatal dopamine with increasing age at about 5-8% per decade until 80% threshold when the disease manifests clinically.[8] Until that stage, hyperactivity of remaining DA Neurons and post synaptic receptor supersensitivity maintains DA transmission.[62,63] In addition, recent evidence has shown that patients dying of IPD have 'on-going' toxic neuronal damage, indicating a possible chronic exposure to a neurotoxin.[64] This is in keeping with the current frustration in the therapy of disease which progressively fail to respond to dopamine replacement. 'On-going' neuronal damage also has important implications to the present enthusiam in brain transplant surgery for IPD

in the sense that, unless the possible 'toxic' agent is identified and eliminated, the efforts of the neurosurgeons may not come to fruitfull results because the transplanted brain tissue may be 'attacked' by whatever the 'toxic' process may be.

The neurotoxin hypothesis can be sustained by studying migrant populations. If the migrant population retain the same risk of developing IPD as their country of origin, then a toxic environmental substance might have affected them in some way before migration. This may be before a certain critical age as in multiple sclerosis where this has been observed eventhough the responsible agent (? a virus) has not been indentified.[65] A similar observation has been made in the case of ALS/PD complex of the Island of Guam.[66] People have developed the disease years after migrating from the Island. The suspected agents include metal ions and eventhough it is not been conclusively proven, it indicates that such a mechanism is possible in a disease like IPD in view of the epidemiological pointers of it being strongly influenced by environmental causes.

From the foregoing, a close look at metal ions will indicate that they are likely candidates in the aetiopathogenesis of IPD because of the following properties:-

i. They are ubiquitous in the environment and therefore likely to affect the population at risk evenly.

ii. They form part of the constituents of food and drink and are therefore likely to be consumed for a long time, albeit, in different quantities and at different time.

iii. The regional distribution of metal ions in the brain of normal

people show high concentration of certain metal ions. In addition, the brains of patients with Wilson's disease show copper deposition in the basal ganglia which is thought to cause the movement disorder in the disease. Recent evidence also shows high Iron deposition in the substancia nigra of Parkinsonian brains.

iv. Metal ions are part and parcel of normal physiologic metallo-enzyme systems closely linked to neurotransmitter mechanisms.

iii. Regional Distribution of Metal Ions in the Brain.

Using various tecniques such as neutron activation analysis, the distribution of brain trace metals in animals has been shown to be heterogenous and vary with the region of the brain, the type of trace metal and is age-dependant.[67] As an example, Iron levels in the midbrain and striatum of mice initially increase between the ages of day five to day ten and declining thereafter to adult levels. At the same time, cerebral cortical levels behave in the reverse direction. Midbrain copper levels increase tremendously between day ten and weaning, only to decrease sharply to adult levels. Striatal copper levels also decrease to adult levels after weaning. Striatal and midbrain zinc levels are high initially but decrease temporarily before weaning, then rising to the adult level. Similarly, manganese, selenium and aluminum levels fluctuates in the various regions of the brain at different times before attaining their respective adult levels.

Thus the developing brain is vulnerable over the period of critical development after birth to either a defficiency or toxicity of trace metals. This especially so when the development of the Brain Blood Barrier (BBB) is taken into account. The BBB is not fully developed at the time when these fluctuations of brain metal ions take place.[68] As such, the developing brain is likely to be influenced by any change in the internal environment which is in turn affected by the metal ion intake from the external environment, In fact there are documented brain teratogenic effects of trace elements defficiency in animals. Defficiency of trace metals have also been associated with alterations in neurotransmitter levels and metallo-enzyme systems. Copper defficient animals had low noradrenalin and dopamine levels and it is known that the key enzyme in dopamine synthesis is a cuproenzyme, dopamine-beta-hydroxylase.[69] As regards the toxic effects of metal ions on the brain , this has been studied mainly on synaptosomal and neural cell culture models in vitro and will be dicussed later.

Similar post-natal, age dependant regional brain distribution of metal ions in humans is not available possibly for ethical and methodological reasons. Technically, this should be feasible by studying the brains of aborted fetuses of different gestational periods and then the brains of children who die at various age up to adolecense. Such an information will throw more light on the normal pattern of distribution of metal ions at different ages and clarify the role or metal ion damage to the brain. Presently, differing regional concentrations of metal ions have emerged due to technical and methodologic problems.[70,71] In many

studies the normal brain has been found to have high iron and copper concentration in the basal ganglia and substancia nigra.[72]

Using inductively coupled plasma spectroscopy, a substancial increase of iron in parkinsonian substancia nigra by 35% in contrast to controls has been documented.[64] Zinc levels were also increased in the substancia nigra and caudateputamen but the copper contents were reduced. Manganese and lead levels were unaltered. The cause-effect relationship of metal ions in these regions of parkinsonian brains has been the subject of debate. However, it is emerging that similar neurodegenerative syndromes like MSA and Progressive Supranuclear palsy might have increased metal ions but in a different inactive form than in IPD. In Wilson's disease, it has been long established that copper is heavily deposited in the basal ganglia due to a deficiency of the plasma carrier protein, caeruloplasmin. This leads to neuronal damage and subsequent extrapyramidal disease although the exact mechanism is not yet clear.[73]

iv. Metallo-enzyme Systems and Neuro-transmitters.

Metal ions are involved in neurotransmitter synthesis as in case of dopamine-beta-hydroxylase which is a cuproenzyme, playing a key role in the synthesis of dopamine.[69] Tyrosine hydroxylase requires Iron and glutamate dehydrogenase is a zinc metalloprotein.[74,75] Neurotransmitter storage is a metal-ATP dependant process.[76] and the release of neurotransmitter is partially calcium dependant.[77] Manganese and magnesium influence neurotransmitter-coupled adenyl cyclases.[78] Even the catabolism of neurotransmitters like catechol-O-methyl-transferase (COMT) require magnesium.[79] These examples illustrates the close link between metal ions

and neurotransmitter functions. Therefore it will not be surprising that metal ions in the same vein are potential neurotoxins.

In vitro synaptosomal studies indicate that metal ions are neurotoxic in high tissue concentrations by interfering with synaptic transmission. This either affects neurotransmitter release or uptake mechanisms: the final common pathway being Sodium-Potassium-ATPase inhibition.[80] Cadmium at a concentration of 1-100 uM slightly decreases spontenous dopamine release. Copper, zinc and selenium are potential inhibitors of dopamine uptake while neurotransmitter metabolising enzymes like Monoamine oxydase (MOA) are inhibited by cadmium and aluminum in toxic levels.[81] MOA-B has a role in MPTP toxicity and is currently under study by employing its inhibitors like segeline. Neural cells cultures show that toxic levels of manganese and magnesium stimulate glial cell amine catabolism and lesions Gamma amino butyric acid (GABA-Ergic) and cholinergic neurones.[82]

v. Free radical damage.

Another way by which metal ions can produce neuronal dysfunction is through the formation of free radicals that lead to the lipid peroxidation. They form many complexes and have more than one stable oxydation state and can therefore take part in oxydation-reductions reactions.[83] This subject will discussed in detail in chapter nine.

In summary, metal ions are good candidates in the search for an aetiopathogenic cause of IPD by virtue of being ubiquitous in the environment and are potential neurotoxins directly or via the generation of free radicals.

Since metal ions are consumed over a long period of time and some have been demonstrated to be in high concentration in parkinsonian substancia nigra, it is likely that they contribute to the jigsaw puzzle of the aetiology of this disease. However the answer is not complete because there is still the enigma as to why only some people in the population at risk in the same environment develop the disease in the face of lack of evidence for genetic predisposition. In addition there is the question of the normal aging process and its relation to Parkinson's disease.

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CHAPTER SIX.

SUMMARY.

An impression of excessive amounts of metal ions in the drinking water of Maiduguri city by inspection,led to the speculation of possible roles of metal ions in the aetiology of parkinson's disease.A thesis was thus put forward that these excessive amounts of metal ions would affect a predisposed group of the population and subsequently lead to free radical neuronal damage in the substancia nigra and eventualy present as parkinson's disease.Twelve consecutive patients with parkinson's disease were clinically evaluated and their serum and cerebrospinal fluid metal ions were estimated along with controls.The drinking water metal ion levels were estmated in comparison to central London drinking water.The finding of high iron content of the drinking water along with mildy high levels in parkinsonian cerebrospinal fluid was not surprising and indicates that exposure of the brain to high iron levels before maturation of the blood brain barrier might predispose to the development of parkinsons's disease.A surprising finding was that of low cerebrospinal fluid manganese levels in the parkinsonian patients in face of high levels of the metal in drinking water.This could be due to concurrent high intake of calcium and phosphates in the parkinsonian patients.Low levels of manganese in the brain may lead to reduced mitochondrial superoxide dismutase,a known physiologic antioxidant.Another surprise was the absence of copper and zinc in the drinking water of

Maiduguri.Low levels of these metals in the diet of babies could lead to reduced brain levels of copper and zinc dependant superoxide dismutase which are important protective anti oxidants.Thus,the conclusion is that metal ions might have a role in the development of of parkinson's diease if present in excessive or defficient amounts before maturation of the blood brain barrier.

CHAPTER SEVEN

MATERIALS AND METHODS. Seventeen consecutive patients with parkinsonism were admitted to the University of Maiduguri Teaching Hospital after an informed consent to the study between August 1987 to September 1988. The patients came from Maiduguri metropolis and surrounding villages directly or by referal from the Maiduguri General Hospital. As the only neurologist in Maiduguri, the author saw all neurological cases in general and parkinsonism in particular having infomred colleagues in the city earlier on. As an incentive to the patients, admission was free in contrast to other patients who had to pay normally. In addition, the author obtained free samples of Sinemet from Pharmaceutical companies and gave the drug to the patients freely at subsequent outpatient follow-ups.

A diagnosis of Parkinson's disease was made based on the clinical criteria of Martilla[5] i.e. when there were two or more of the following:- rhythmic rest tremor (4-6/second), bradykinesia, rigidity and disturbance of postural reflexes. Based on this criteria, three patients were excluded from the study. One had Multiple System Atrophy (MSA) of the Olivopontocerebellar variety clinically in that he had cerebellar ataxia of all limbs, brisk knee and ankle jerks in addition to moderate bradykinesia enough to prevent him from dressing up or walk as briskly. He had moderate mask-like facies and rigidity at the wrists. The second patient had MSA of the Striatonigral atrophy variety clinically as he presented with severe bradykinesia, tremor and cogwheel rigidity and was bed ridden. Response to progressively large doses of levodopa and anti-cholonergics was minimal and

he had an unrelenting course and died with pneumonia three months after diagnosis. Postmortem was not granted unfortunately due to cultural and religious beliefs prevailing in the community studied. This mode of presentation and clinical course (unreponsiveness to L-DOPA) of parkinsonism is thought to be that of striatonigral atrophy variety of MSA.[84]

A third patient presented with bradykinesia, tremor and rigidity six months after a stroke secondary to longstanding hypertension that was treated with reserpine. He was excluded from the study for these two reasons which may be contributory or responsible for his parkinsonism.

A fourth patient fulfilled the clinical diagnostic criteria of Parkinson's disease but had primary liver cell carcinoma as well. He had six centimetre enlarged knobly tender liver which was firm and had a bruit. Serum afoetoprotein was markedly elevated which was taken as diagnostic of hepatoma as this has been fond to be consistent with the histology in 80% of cases of the tumour which is common in Nigeria.[85] Unfortunately a postmortem was not granted as in the second patient for similar reasons.

The clinical features of thirteen patients that fulfilled the clinical criteria for Parkinson's disease is as illustrated in table 1. Video recording facility was not available. The disease severity was assessed by the North Western University Dissability Rating Scale (NUDS).[86] (See appendix for details). This scale was choosen for the following reasons:-

1. Its simplicity.
2. It is a clinical assesment not requiring elaborate equipments which are not available in developing countries.

3. It is relevant to the mode of presentation of Parkinson's disease in the community studied because it relates to the performance of daily essential activities of livelyhood. Patients in the community studied generaly put up with their symptoms of disease process and only report to hospital when disabilities interferes with their livelyhood. This is because access to hospitals is difficult due to long distances and costly transport fares. In addition, hospitals were not well stocked with drugs due to economic depression and patients were less inclined to attend. This was one of the reasons of providing free hospital admissions and drugs to attract patients to the study.

The NUDS rating scale is based on the practical fact that Parkinson's disease affects the patient in useful activities such as Walking, personal hygiene, Dressing, Eating/Feeding and Speech. These are basic functions needed by a person in any community in general but particularly in developing countries where manual labour of some form is the means of obtaining food, shelter and clothing. It is a well known fact that tremor of Parkinson's disease is more alarming to the onlookers than the patient who is more concerned with the rigidity and bradykinesia that impairs his mobility.

Thus the NUDS scale has FIVE rating functional activities (walking, personal hygiene, dressing, eating/feeding and speech) and each is given twenty points. This enables the expression of the degree of disability in percentages. The points enable relative changes over time to be assesed

in a patient. On admission, patients were clarked in presence of relatives who provided supplementary information. Physical examination was done several times during admission. The NUDS score was recorded accordingly. The diference of the total score and 100 gave the degree of disability. Routine haematologic and biochemical tests and chest X-Rays were performed. Computed Tomographic Scan was not available. 10 milliitres of clotted blood was extracted from Parkinsonian Patients and from age and sex matched controls. The latter were patients with various neurologic disorders that required lumbar puncture. (See table 111). The sera obtained were stored in plastic containers at-70^0 Centigrade. These were thawed in a batch at the end of the study (September 1989) on the day of transportation to London. They were packed in an insulated container after two days in the freezer at-70^0C. The following morning, the container was flown to Lagos (one and a half hour flight time) and stored over night in a deep freezer at -20^0C. The following day, the container was flown to London(6 hour flight time) where it was placed in a freezer at -20^0C overnight afterwhich it was stored at-70^0C until the time of analysis. The container was never opened by the customs during transportation. Specimens were subsequently analysed in Germany.

Lumbar puncture was performed after an informed consent at the fourth/fifth lumbar intervertebral space in the left lateral decubitus position using a 22 gauge stainless steel needle. All lumbar punctures were done by the author under usual aseptic conditions first thing in the morning between 8am and 9am. The first 5 ml of cerebrospinal fluid (CSF) was analysed for cell count, sugar, and protein by routine methods at the laboratory of the

Maiduguri University Teaching Hospital. There was one CSF that had more than 10 red blood cells and a second lumbar puncture was done three weeks later. After the first 5 ml of CSF, six aliquotes of 1.5ml were collected in six consecutive plastic tubes and were immediately st^{0}red at -70^{0}C for subsequent metal ion estimation. Similar collection of CSF was performed in age and sex matched controls who had various neurologic disorders requiring lumbar puncture. (See table 111). All cerebrospinal fluids were packed and transported along with the sera to London in the manner described above and then to Germany.

Anti-Parkinsonian therapy was witheld in most of the patients until after the above procedures but in few this was not possible as the refering physicians had initiated therapy.

Geological data of the Maiduguri area and the rest of the state was collected from the department of geology of the University of Maiduguri to give an idea of the metal ions present in the environment. Drinking water samples were collected from the three main bore holes of the Maiduguri Water Board for metal ion estimation. The three bore holes were from different geologic strata that contain water, called aquifers. (See figure 11). The first bore hole was the Borno Industrial Development bore hole which is from the upper aquifer. The water was crystal clear and had a temperature of 35°C. The second bore hole was the Government House bore hole which was fromm the middle aquifer. The water had a temperature of 38°C. The third bore hole was the Dalori bore hole from the lower (3rd) aquifer with water at a temperature of 46°C. 75 ml was collected from each bore hole tap

where citizens collected their drinking water. Collection was in 25 ml metal free plastic tubes i.e. three tubes for each bore hole. They were tightly sealed and packed in a plastic bowl and transported at ambient temperature by air through couriers service (DHL) to London, then Germany.

The physico chemical varie ties of Borno State Soils were obtained from the department of Soil Science of the University. This was necessary to have an idea of metal ions present in the immediate environment which has a bearing on the metal ion content of food crops. Data was also collected from the department of agriculture on the metal ion content and intake of staple foods (Millet and sorghum) .

ANALYTICAL METHODS.

a).Cerebrospinal Fluid.Due to prohibitive costs of analysis in Germany(see appendix six and seven),only total iron and manganese levels were performed.These two metals were chosen because of their high levels in the Maiduguri drinking water and their speculated roles in the aetiopathogenesis of parkinsonism by previous workers. The analysis was done by the Bioscientia Laboratories,Mainz,West Germany using electrothermal atomic absorbtion spectrophotometry(see appendix eight for details of methodology).Essentially,atomic absorbtion spectrophotometry means the vapourisation of the medium containing the metal ions e.g.CSF into small aerosol particles with consequent dissociation of the solvent molecules and metal atoms.This vapourisation is achieved either through air-acetylene flame or graphite furnace(electrothermal atomosation).The latter method is more sensitive and was employed in this study because of the range of CSF metal ion levels.After the vapourisation,the resultant free metal atoms are subjected to a beam of discrete wavelength from a light source(hollow cathode lamp or electrodeless discharge lamp) .The majority of the atoms in question are in the ground state and when exposed to the radiant energy,they absorb it and become excited to a higher energy state.Subsequent emission of this energy produces a spectral line(i.e.the line arising from a transition from the ground state to the lowest exited state.) .This forms the basis of atomic absorbtion spectrophotometry.To keep out contaminating noises,the detection electronics are appropraitely tuned.Other background correction methods

are also available and for details,see appendix eight.

b).Serum.Only five sera from parkinsonian patients and two from controls were analysed for total iron because of accidental loss of the other sera during transportation.The atomic absorbtion spectrophotometry described above was used in the same laboratory.Control toal serum iron was obtained from the University of Maiduguri Teaching Hospital.These were healthy first-time blood donors.

c).Drinking water.These were analysed by courtesy of Mr.David Dexter using atomic absorbtion spectrophotometry(flame) at the Chalsea College,London.London drinking water was used for comparison.

d).Statistical methods.The non-parametric method of Wilcoxon Rank Sum Test for unpaired data was used.(see appendix nine for details).This method is appropriate for data that do not fall into the normal Gaussian distribution curve.However,it is less sensitive than the student t test in detecting a change.

CHAPTER EIGHT

RESULTS

I. CLINICAL FEATURES:

The clinical features of the Parkinsonian patients is as shown in table (1) based on the four clinical diagnostic criteria of Martilla.[5] The disabilities as determined by the North Western Disability Scale (NUDS) is shown in table (11). The age range for Parkinsonian patients was 31-85 years with a mean of 55 years. The mean age for the controls was 60 years. The male: female ratio for Parkinsonian patients was 1:4.5. Tremor was the commonest presenting symptom followed by bradykinesia and rigidity. The mean duration of symptoms was 24.7 months before presentation. The average disability NUDS score was 16.8%. Hospital frequency of Parkinson's disease over the period of study was 6/10,000.

ILLUSTRATIVE CASES:

A] Evolution from early mild disease to late severe form.

A 56 year old Fulani Arabic teacher first presented to his doctor at the age of 51 with a one year history of generalised body aches and pains that were partially relieved by analgesics. "Weakness" of the limbs set insidiously shortly. He thought his left arm was tremulous occassionally. The only physical sign then was very mild facial immobility. The patient continued on analgesics. Two months later the patient noticed increasing tremor of his left hand that became embarrassing. He overcame this by leaving the left hand in his pocket most of the time. Physical examination this time by a different doctor revealed obvious rhythmic tremor of the left hand, mask-like facies but there was no rigidity.

Treatment was witheld because he was able to function well and concealed his tremor in his pocket! However, six months later he developed more bradykinesia and rigidity impairing ordinary house activities. This time he was started on L-DOPA 125 mg twice daily and benzehezol 1 mg twice daily, which relieved his tremor but not the bradykinesia. The L-DOPA was gradually increased to 500 mg four times daily but his bradykinesia continued unabated. Four years later he developed on-and-off attacks. He fluctuated from extreme akinesia to dyskinesia all to the distress of his relatives, some of whom became exasperated and thought the poor fellow was deliberately being difficult. He was readmitted and drugs were withdrawn (drug holiday) for three weeks and sinemet 110 was slowly introduced up to four tablets daily. This seemed to have helped but to date he is still bradykinetic but can just manage to perform his daily activities. Other modalities of treatment are being sought.

B] Unilateral Parkinsonian Symptoms initially diagnosed as "Stroke".

A 56 year old retired policeman presented to his doctor with a four year history of sensation of "weakness" of the right arm and leg. Occassionally he had transient tremors of the right hand at rest. These were attributed to stroke. His gait was not affected. A year later, he presented to an eye clinic with cataract of the right eye where he was found to have non-insulin dependent diabetes as well. He was subsequently referred to the author with his 'stroke'. On examination, he had a mask-like facies, pill-rolling movement of the right hand and a positive glabellar tap. There was cog-wheel rigidity of

right wrist only. Bradykinesia was generalised but more so on the right. When walking the right arm did not swing. These features markedly abated with benzehexol and laevodopa therapy. His diabetes was controlled with diet and chlorpropamide 250 mg once daily. Two years later, he had continued to benefit from antiparkinsonian drugs.

C]. Parkinson's disease diagnosed as thyrotoxicosis.

A 50 year old lady presented to her doctor in a peripherial hospital with a three months history of generalised body aches, pains and weakness. She also has had a goitre for ten years (common in their village). The doctor thought the patient had a fine tremor and tachycardia and made a diagnosis of thyrotoxicosis at which point he referred her for confirmation. Physical examination revealed an obvious 'mask-like' facies, pill-rolling tremor of hands and cog-wheel rigidity at the wrists. Her gait was also slow. The signs and symptoms dramatically improved with benzehexol and laevodopa. The thyroid swelling was subsequently shown to be non-toxic. She returned to her doctor for further management.

D] Classical form of the disease.

A 68 year old farmer was brought by his children with a 2 year history of tremor, slowness and drooling of saliva. He had progressively became unable to walk without falling and later was unable to get out of bed. There was a galore of physical signs. He was so tremulous that it even involved his lips and eye lids. He was profoundly bradykinetic and was ben ridden on admission. When he improved and was able to walk, he had

TABLE I (CLINICAL FEATURES)

PATIENTS.	AGE(YEARS)	SEX.	DURATION OF SYMP.	TREMOR.	RIGIDITY.	BRADYKINESIA.	POSTURE DIST.
1.	50	FEMALE	6 MONTHS	++	++	+	+
2.	50	MALE	2 YEARS	++	++	+	+
3.	56	MALE	4 YEARS	-	+	+	-
4.	35	MALE	3 YEARS	+++	+++	+++	++
5.	56	MALE	7 YEARS	+	+	++	+
6.	85	MALE	3 MONTHS	++	-	-	+
7.	45	MALE	2 YEARS	+	-	+	+
8.	45	MALE	5 MONTHS	+	+++	++	+
9.	70	MALE	2 YEARS	++	+	++	+
10.	50	FEMALE	1 YEAR	+++	+	++	+
11.	68	MALE	2 YEARS	++	+	+	+
12.	71	FEMALE	2 YEARS	++	+	-	-
13.	31	MALE	7 MONTHS	+	-	+	-

KEY.

\+ =MILD
++ =MODERATE
+++ =SEVERE
\- = NONE

SYMP.=SYMPTOMS
DIST.=DISTURBANCE

TABLE II.(DISABILITY RATING.)

PATIENTS.	NUDS SCALE. WALKING.	DRESSING.	HYGIENE.	EATING/FEEDING.	SPEECH.	TOTAL (NUDS) SCORE.	DEGREE OF DISABILITY (%)
1.	17	17	18	19	16	87	13
2.	18	19	17	16	18	88	12
3.	17	18	19	18	14	86	14
4.	13	15	14	10	14	66	34
5.	16	17	18	17	16	84	16
6.	19	19	18	17	18	91	9
7.	18	19	18	17	18	90	10
8.	11	14	13	16	19	73	27
9.	16	15	13	17	16	77	23
10.	18	17	18	16	15	84	16
11.	18	17	18	19	17	89	11
12.	16	16	18	19	17	86	14
13.	17	16	17	17	18	85	15

KEY.

NUDS=NORTH WESTERN DISABILITY SCALE.

TABLE III CONTROL GROUP

PATIENT.	AGE.	SEX.	DIAGNOSIS.
1.	55	MALE	PERIPHERAL NEUROPATHY
2.	68	MALE	CARCINOMATOUS NEUROPATHY
3.	40	MALE	RADICULOPATHY
4.	20	MALE	RENAL FAILURE/NEUROPATHY
5.	72	MALE	HYPERTENTION/NEUROPAHTY
6.	50	MALE	MELANOMA/NEUROPAHTY
7.	57	MALE	GUILIAN BARRE SYNDROME
8.	70	MALE	GUILIAN BARRE SYNDROME
9.	60	MALE	PERIPHERAL NEUROPATHY
10.	42	MALE	PERIPHERAL NEUROPATHY
11.	49	MALE	PERIPHERAL NEUROPATHY
12.	68	MALE	PERIPHERAL NEUROPATHY
13.	39	MALE	PERIPHERAL NEUROPATHY
14.	26	FEMALE	PERIPHERAL NEUROPATHY

the classic posture and gait with propultion and retroputon. He had continued to benefit from L-DOPA and benzehexol a year after diagnosis. His children brought him for follow-up 300 kilometers away regularly as they valued the benefit of treatment.

11. ENVIRONMENTAL SOURCES OF METAL IONS IN BORNO STATE.

A. Geologic Features. Borno state is situated mainly in the Chad Basin (Fig. 1). This is an area of sediment filled depression around the lake over the ages.[87] Beneath are layers of a sand and clay sediments with alluvial deposits and are collectively called The Chad Formation. Also found in this Formation are mudstones and sand stones (the Kerri Kerri formation). The basement complex comes next and consists of migamite and granites. These geologic strata have differing metal ion content and influence the ionic content of the soils for cultivation (and therefore crop metal ion content) and drinking water.

B. Soils of Borno States. Factors that determine soil properties are mainly the geologic parent materials described above, climate and the hydrologic influences. Based on these, the soils of Borno are the following in order of surface area covered:-[88]

i. Alfisols. (3,047,170 ha) The parent materials of this type of soil consists of alluvium, basalt, shales, sandstones,

mudstones, Chad sediments with sands. The proportion of these vary in different parts of the state.

ii. Inceptisols (1,262,990 ha). These are mainly of sandy clay with loam. There is present exchangeable calcium.

iii. Entisols (5,772,720 ha) These consists of parent materials from alluvium, sands, and drift materials, colluvium.

iv. Vertisols. (845,100 ha). The parent materials are clay shales, alluvial deposits, lacustrine deposits and basalt-colluvium.

v. Mollisols. (112,200 ha). Parent material, basalt and sandy clay with iron concretions, and found mainly in the southern part of the state.

C. Crops. From the foregoing, in general the soils of Borno state have a low iron content in the Sahelian savannah of the northern part while the soils of the guinea savannah in the south have a high iron content.[89] Crops like spinach reflect these as their iron content is determined closely by the soil content. However, this crop is not consumed on a large scale. On the other hand, grains like millet and sorghum which are the staple foods have a narrow limit of variation in their element content, as such they are not greatly affected by the soil metal ion content.[90] Rather, it is the contamination of these grains by soil, cooking utensils and processing by machinery that determines the final amount of metal ione consumed.

This will vary from place to place and from season to season. In addition, these staple foods are transported from one part of the state to the other and hence the metal ions consumed will tend to average out. In the area of study, the crops are harvested in a traditional method on the ground with consequent contamination by the soil. Eventhough as much of this is removed as possible, the grains taken for grinding by machinery contains soil contaminants. The grinding machines have a metal grinding surfaces which add to the metal contamination. Cooking is done in baked clay pots in the rural areas and aluminum pots in the urban areas.

The food and Agriculture Organization (FAO) of the United Nations in collaboration with the United States Department of Health, Education and Welfare has compiled a Food Composition Table for use in Africa.[91] This is still widely used but does not have most of the metal ions. Obviuously this needs to be done and only such large organizations have the resources. Using these data for iron, a study done in Maidiguri township showed a low mean iron intake from the staple food millet and sorghum.[92] On average, male adults had a daily intake of 66% of the 5-9mg recommended by FAO/WHO. Women took less than 55% of the 28 mg recommended intake. Haem iron intake was negligible in both sexes. Iron from water intake was not determined. However, the mean daily intake of water was estimated to be 1514ml for males and 1111 for females.

D. Hydrology. From the geologic sequence of lacustine, fluviatile clays and sand in the Chad formation of Borno State, three distinct zones of water bearing sandy sediments called aquefers can be distingushed. These are separated by the layers of clays. They are refered to by by the Borno State Water Board as Upper, Middle and Lower aquefers.[93] (see Figure 11). The upper aquefer is exposed to the surface in some places. The middle aquefer has a wider lateral extent throughout the state with concentric dips and rises. About 60% shows free flowing artesian pressure with a general direction of ground water flow towards the lake Chad. Because most of the State is in the semi-arid sahel, these layers of ground water are virtually the sole sources of water supply. Water is obtained from the upper aquefer by hand-dug wells as it is very near the surface in several places. With increasing demand for water, the government has made many bore holes to the second and third aquefers. The metal ion content of drinking water from the three aquefers is shown as in table. These metal ions are present after prolonged contact with the the sands amd rocks of the aquefers through geologic time. The percholation of rain water through the atmosphere and various geologic strata of the Chad Basin before reaching the aquefer layers also do contribute to the metal ion content of the drinking water depending upon the metal ion content of these media. Thus the metal ion content of the drinking water from the Chad Basin bore holes is the sum total of these climatic and geologic factors.

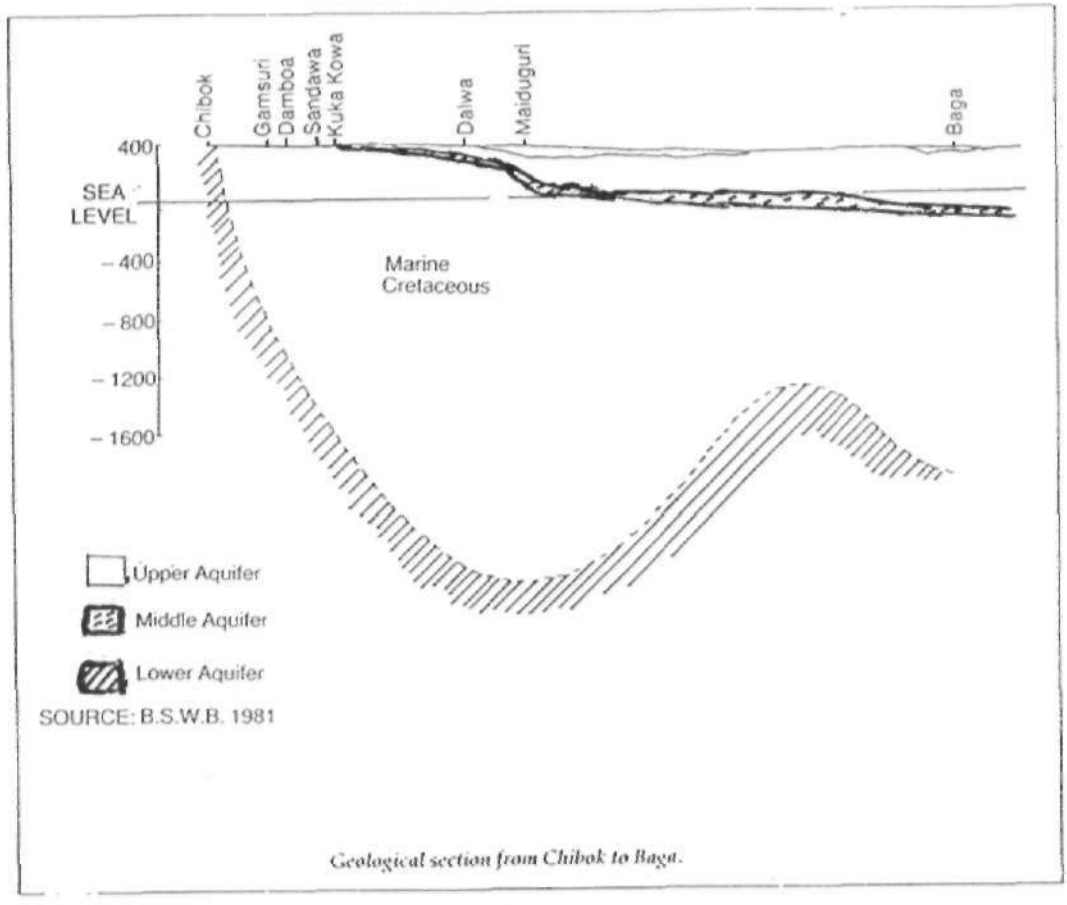


FIGURE II
Underground water sources in Borno State
BSWB = Borno State Water Board

III.CEREBROSPINAL FLUID METAL IONS. *

Tables IVa and IVb show the CSF iron and manganese levels in patients and controls.Parkinsonian patients tended to have higher CSF iron levels with a mean of 94.6mcg/L.However,this was not statistically significant(Unpaired Wilcoxon Rank-Sum Test>0.05) .For manganese however,parkinsonian patients had a lower mean level of 2.0mcg/L as against that of the control which had a mean of 4.3mcg/L.This was statistically significant(Wilcoxon Unpaired Rank-Sum Test<0.05).This is displayed in figure III.

IV.SERUM TOTAL IRON.

The sera available from the five parkinsonian patients had a mean level of 16.8mcmol/L(see appendix 7 for raw data).The two controls had a mean of 12.8mcmol/L.

V.DRINKING WATER SAMPLES.

Compared to London drinking water,the Maiduguri samples reveal high levels of iron and manganese(see table V.).In contrast,copper and zinc levels were undetectable.Unfortunately,the limitation of funds prevented the analysis of these two metals in the CSF and serum.Compared to World Health (WHO) recommended safe water levels,the Maiduguri iron and manganese levels are high(see appendix 5).

* One parkinsonian CSF was lost during transportation.

Number of cases	Iron levels in micrograms/L	
	Parkinsonian patients	Control
1	45	31
2	288	33
3	37	55
4	61	179
5	27	20
6	55	146
7	383	40
8	75	271
9	20	78
10	81	18
11	32	41
12	31	31
13	-	18
14	-	31
TOTAL	1135 mcg/L	992 mcg/L
MEAN	94.6 mcg/L	70.9 mcg/L

TABLE IVa. Cerebrospinal Fluid (CSF) Total Iron levels of Controls and Patients. Unpaired Wilcoxon Rank Sum Test > 0.05

Number of cases	Manganese levels in micrograms/L	
	Parkinsonian Patients	Controls
1	2.6	1.3
2	1.5	1.3
3	2.3	1.9
4	1.9	1.9
5	1.0	4.3
6	2.3	4.4
7	2.1	7.0
8	3.7	3.9
9	0.9	17.0
10	2.4	4.2
11	3.2	1.4
12	1.3	1.9
13	-	5.6
14	-	0.9
TOTAL	24.1 mcg/L	56.1 mcg/L
MEAN	2.0 mcg/L	4.3 mcg/L

TABLE IVb. Cerebrospinal Fluid (CSF) Man ganese Levels of Controls and Patients. Unpaired Wilcoxon Rank Sum Test < 0.05.

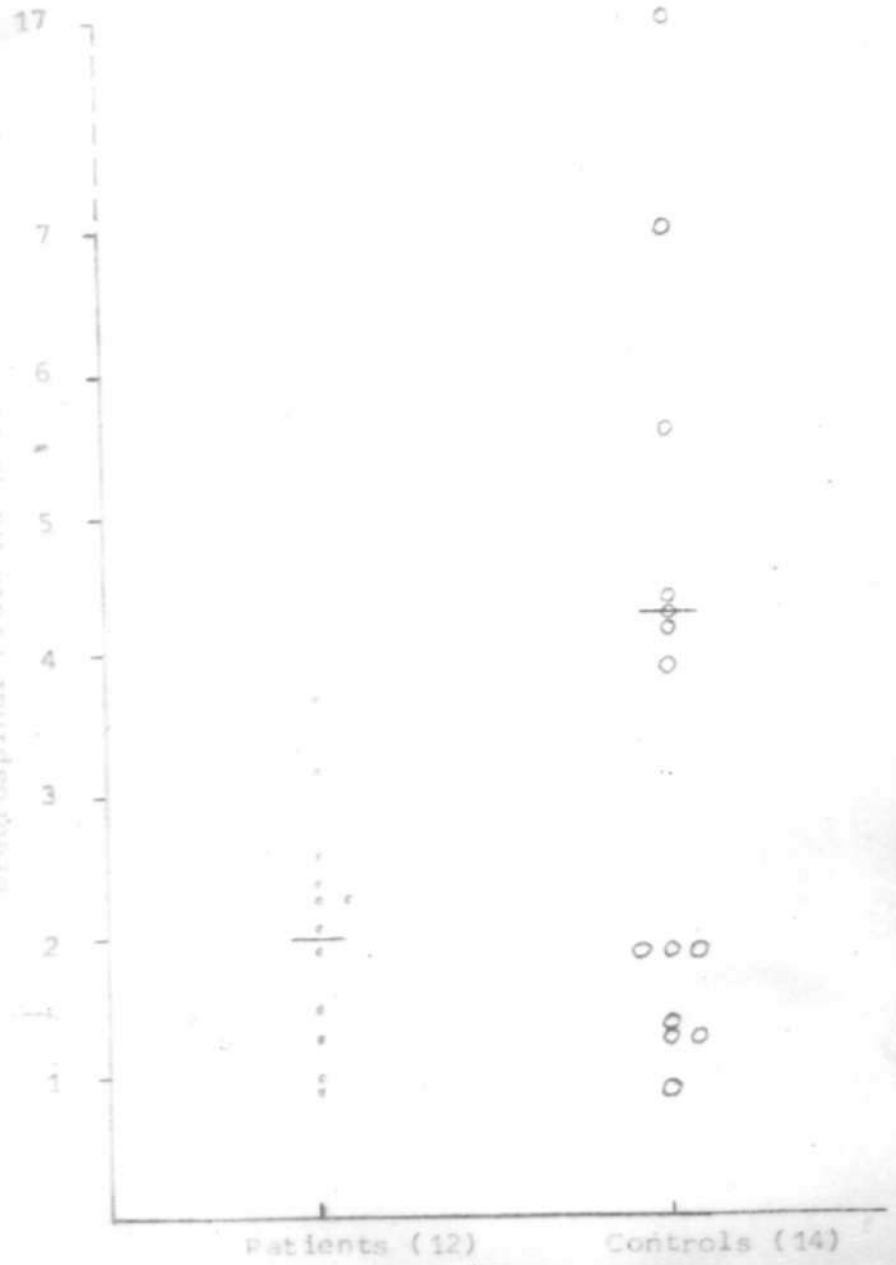


Figure II: Cerebrospinal Fluid (CSF) Manganese Levels of Patients (mean 2.0 mcg/L) and Controls (mean 4.3 mcg/L). Unpaired Wilcoxon Rank Sum Test < 0.05

WATER SOURCES.		METAL ION CONCENTRATION IN nmol/L.					
AQUIFER	BORE HOLE	IRON	COPPER	ZINC	MAGNESIUM	MANGANESE	ALUMINIUM
UPPER	I.D.B.	2770	ND	ND	56780	23.4	9858
MIDDLE	GOVT.H.	26760	ND	ND	128060	115340	14784
LOWER	DALORI	51000	12.4	149.2	90720	2706	9934
LONDON DRINKING WATER		ND	1362.4	540.4	178740	6.2	207400

TABLE V. Metal ion content of drinking water from Maiduguri bore holes and London tap water. Concentration in nmol/L. I.D.B.=Industrial Development Borehole. GOVT.H=Government house. ND=Not Detected.

CHAPTER NINE

DISCUSSION

1. CLINICAL FEATURES:

Clinically, Parkinson's disease starts with vague, non specific symptoms before the cardinal features become obvious. The diagnosis can thus be missed in the early phase. The early symptoms include fatigue, which is described as "weakness" by the patients aches and pains, restlessness and internal tremulousness. Sensations of pins and needless and muscle cramps can occur. Case A illustrates these subtle early symptoms easily dismissed as due to aging then progressing to the full clinical form. When faced with such diagnostic problems, it is helpful to remember the clinical diagnostic criteria of Martilla[5] of which any two are acceptable for a diagnosis.[94] Most doctors have a mental image of the Parkinsonian patient as a "tremulous old man". However, as pointed out there need not be tremor and hence the diagnosis is easily missed. The prompt response to anticholonergics or L-DOPA helps in clinching the diagnosis. Case A also demonstrate the long term side effects of L-DOPA therapy i.e. on and off attacks.[95] The pathophysiology of these complications is not known, but may be related to peripheral and drug-induced alterations of central dopaminergic receptor sensitivity.[96] Some of these side effects are dose-related. However, careful fractional reduction in the dose is needed to avoid increasing the severity of Parkinsonian symptoms. The introduction of bromocriptine has not solved the problem, but may be useful in ameliorating the on-off phenomena.[97] Transient drug withdrawal (Drug holidays) indicates that some patients might benefit

but the pharmacologic basis of the effectiveness of drug withdrawal is not clear.[98] Altered dopamine receptor sensitivity with chronic administration may be a factor. Most patients prefer dystonia to bradykinesias which are more disabling. The dystonic mevements are often more worrying and alarming to friends and relatives as happened in this patient. New developments of continous infusion of dopaminergic drugs and slow release Sinemet hold some promise in reducing the motor fluctuations in therapy.[99,100] However, these are beyond the reach of third world patients who are unable to afford even levodopa alone. Case B illustrates a common mistake of misdiagnosing unilateral Parkinsonian symptoms as stroke. Cursory observation led the doctor to mistake the rigid right arm and leg as due to stroke. The episodic tremor might have been absent on examination and could have been elicited from careful history taking. Case C is interesting. The presence of a long standing goitre with tremor that was not properly observred were interpreted as thyrotoxicosis. The definite physical signs with prompt response to anti-parkinsonian drug clinched the diagnosis. The goitre was subsequently found to be non toxic. Case D demonstrates the classical florid form of the disease which can be considered to be at one extreme end of the spectrum of linical presentation.

The strategy of management is to treat the patient according to the dissability present and not to treat all.[101] This is because drugs such as anticholinergics do cause unpleasant side effects in old people and

can precipitate glaucoma, urinary retention and confusion. More important however, is the wearing-off effect of the antiparkinsonian drug after four to five years of continous treatment.[102] The reason for the progressive dimunition of therapeutic effect is not clear. Progression of the disease process or alteration of receptor sites has been postulated.[103] In addition, there are some who hold the view of "L-DOPA -autoxidation" in vivo, because dopamine oxidation increases free radicals and neuromelanin as byproducts that in turn may lead to neuronal damage.[104] The oxidation of dopamine can be blocked by the inhibition of monoamine oxidase-B using selegiline. The selegiline trials should throw more light on this which was the initial rationale of introducing selegiline in the mid-1970's until when MPTP-induced parkinsonism was discovered. This has opened a new horizon in the management of IPD as selegiline might slow down the disease process by inhibition of the biotransformation of MPTP to the toxic metabolite MPP^+.[27,28] Thus, Parkinson's disease presents with a variety of signs and symptoms that form a spectrum ranging from the early subtle form of the disease easily ignored as old age to the extreme end of classic form with florid well recognised signs enabling spot diagnosis even on the street. In between are various less obvious presentation masquerading as "stroke", drooling of saliva attributed to loose dentures, thyroid disease etc. These diagnostic pitfalls should be kept in mind for early diagnosis and therefore the relief of dissability. However, in general the clinical features in Nigerians are similiar to that in Caucasians except that it is rarer.[53,54] In the group studied, the disease tended to present late due to lack of easy access to hospitals, the average time of presentation after onset of disease being 24.7 months. The low hospital frequency of 6/10,000 is similar to that of the Ibadan study.[53]

Although Levodopa therapy has improved the quality of life and expectancy of many patients, a lot is yet to be learned about the progressive nature of the disease if it is to be halted.[105,106].

II. METAL IONS AND BODY HANDLING:

More than 60 metal elements have been discovered in living things of which a few are termed 'trace' elements.[107] These are: aluminum, antimony, arsenic, barium, boron, bromine, cadmium, chromium, cobalt, copper, gallium, lead, lithium, manganese, mercury, molybdenum, nickel, rubidium, selenium, silver, strontium, tin, titanium, vanadium and zinc (see periodic table in appendix [1]). They are termed 'trace' elements because of their very low biologic concentrations and the earlier uncertainty of their exact metabolic roles. In man a few of these 'trace' metals are referred to as "essential trace elements" because in their absence, deficiency states do occur which are reversed by the appropriate dietery supplements.[108] These essential trace elements include:Iron, Iodine, chromium, copper, cobalt, manganese, molybdenum, selenium, zinc and flourine. Heavy metals like mercury, lead and cadmium serve no apparent biologic function and are toxic to man even at very low concentrations.[109] Other metal ions have less well-defined metabolic roles. These are categorized as probable essential trace elements: - silicon, vanadium, nickle, tin, arsenic, aluminum and boron. They function as cofactors for cell enzymes and as structural components in special tissues.

The soil-plant-man relationship of metal ions have already been highlighted earlier on in chapter eight in terms of the environment.

Whatever, the source of metal ion (food or drink), the intake and subsequent body handling will determine the effects on man.

(a) Intake:

From the data gathered from the University of Maiduguri, department of agriculture, the average daily intake of metals (iron in this case) from food was found to be low.[92] Since the average volume of water intake was known, the intake of metal ions from drinking water is illustrated in Table V. The intake of metal ions from food is the sum total of iron absorbed by food crops from the soil, ions contaminating the food in the process of preparation, and from the drinking water. This is the sum-total of geologic and meteorologic phenomena determining man's consumption of metal ions. In the case of iron, the low dietery intake is made up for by the high iron contents of drinking water.

(b) Absorption:

The mechanisms and regulating factors for the absorption of metal ions vary with each element. Factors such as solubility, oxidative state, chelaton, PH and carrier proteins all combine to determine the net amount of metal ion that enters the circulation.[110] Iron is a case in point. It is ingested from food mainly in the ferric (Fe^{3+}) state and is solubilized and ionized to the ferrous (Fe^{2+}) state by gastric juice. Consumption of ascorbic acid (fresh fruits like oranges and guavas) and amino acis tend to promote its absorption by forming chelates. In the community studied, dietery intake of vitaminic C and proteins were below FAO/WHO recommended levels.[92] Thus the very

high intake of iron from drinking water is partially reduced by these factors i.e. a deficiency of dietery factors protecting against the excessive absorption of iron from drinking water.

Other food factors are, phytates and high fibre diet that reduce the absorption of metal ions by forming complexes.[111] Calcium bind phytates thereby enhancing absorption of iron.

In the case of iron, there are systemic regulatory mechanisms that influence absorption. This is attributed to 'mucosal block' of iron absorption when the body is iron-loaded.[112] Normally, the ferrous iron binds with apoferritin to form ferritin. When the apoferritin is exhausted, excess iron ingested is rejected and excreted with faeces. By this mechanism of muscosal block, only 10-30% of ingested iron is absorbed. In deficiency states, little iron is incorporated from transferrin (the carrier protein macroglobulin) in the mucosal cells there by enhancing absorption.

(c) Transportation:

After absorption, metal ions are transported to various parts of the body by carrier proteins.[107,113] Examples include tranferrin (9 beta 1-globulin of molecular weight 86,000 binding to two atoms of ferric aron); cearulopasmin (an alpha-2 globulin of molecular weight 150,000 containing 8 gm. atoms of copper per mole); an alpha 2

macroglubin for zinc and transmanganin (beta 1-globulin) for manganese. Some small amounts of metal ions are transported by albumin. In the mucosal cells of the deudenum and proximal jejunum, iron is bound to 20-35% of the plasma transferrin for transportto the liver and bone marrow mainly.

(d) Metabolism:

The metabolic role of metal ions as metalloenzymes have been discussed in chapter five. The toxic effects on the brain could be direct or secondary through lipid peroxidation and the latter will be fully discussed later.

(e) Excretion:

The body has limited capacity to excrete excess metals via the renal gastrointestinal tract and biliary system and is therefore at the mercy of the intake and rate of absorption.[114]

III. The Blood Brain Barrier (BBB) and blood cerebrospinal fluid barrier (B-CSFB).

Anatomically, the blood brain barrier is considered to be the sum total of the tight junctions between capillary endothelial cells of the brain parenchyma (BBB) and those of the modified ependymal cells lining the surface of the choroid plexus in the lateral, third and fourth ventricles (the blood CSF barrier).[115] Physiologically, it is thought that the tight junctions permit the selective permeability of fluids and solutes with water soluble molecules crossing poorly, while lipid-soluble ones cross easily. This results in the formation of

cerebrospinal fluid (CSF) continously from the choroid plexuses mainly, at the rate of 500 ml daily and to some extent from the ventricular ependymal cells and vessels of the arachnoid membrane over the spinal cord.[116] The CSF has a volume of 150 ml and a turn-over of 3 times per day. It is not of the same composition as extracellular fluid and has 7% greater concentration of sodium, 30% less glucose and 40% less potassium. The exact mechanism by which this differential and protective concentration of solutes is attained is conjectural.[117] One explanation is that of active secretion of sodium ions by the choroid plexus into CSF which secondarily attracts chloride ions with consequent increase in osmotic pressure greater than that of plasma. This generates a gradient with diffusion of fluids and solutes towards the chronoid plexus and capillary tight-junctions that selectively permit the passage of solutes. Substances like glucose have carrier-mediated energy dependant transport (facilitated diffusion). Most metal ions are able to reach the brain parenchyma before establishment of the BBB i.e. in utero and in the first few years of life.[16] It is during this interval of time in an individuals life that the levels of metals are acquired for brain hyperplasia and maturation.[59] Once the BBB is established, the metal ion environment is resistant to change.

The BBB keeps the central nervous system (CNS) interstitial space under precise control with constant levels of solutes (metal ions included). The cerebrospinal fluid is in dynamic exchange of fluids and solutes wiht the CNS interstitial fluid, the chemical content of which are specific products of neurones and supportive glial cells. Thus,

by sampling the CSF, the biochemical state of the CNS can be obtained, although certain variations should be kept in mind. They include the existence of rostralcaudal concentration gadients for many substances in the CSF, circadian rhythms, state of hydration, diet, age and stress. These effects can be minimized by using other neurological disorders as controls as was the case in this study (table 111).

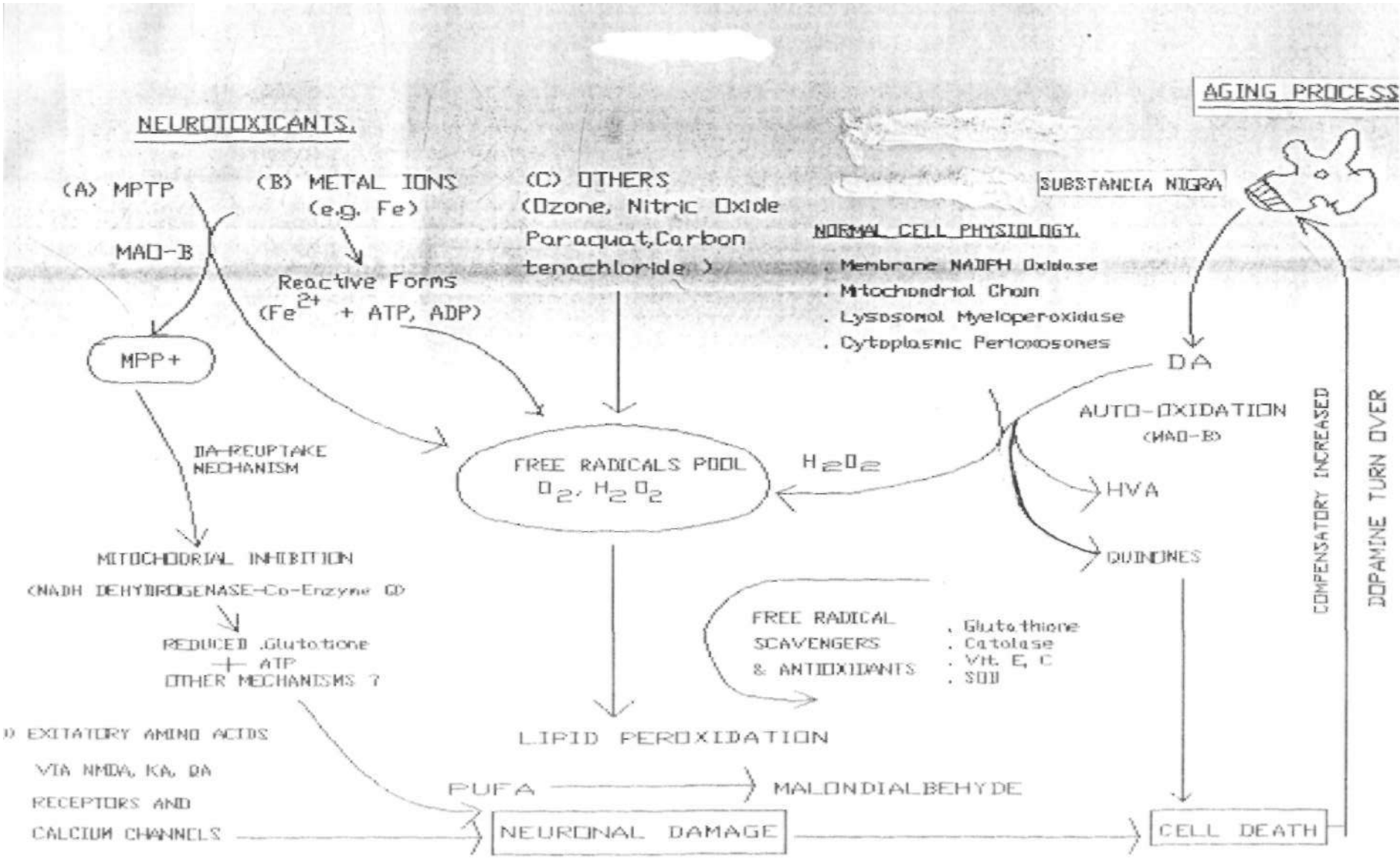
It is proposed that, it is the flux of metal ions between the brain and body fluids (and therefore the environment) before maturation of the blood brain barrier in conjunction with age-dependant auto-oxidation of dopaminergic neurones that determines the development of Parkinson's disease.

Metal Ions and Lipid Peroxidation. Metal ions are capable of producing neuronal damage through the formation of free-radicals that subsequently leads to lipid peroxidation (Fig. IV).[31.32] Cell membranes are formed of a bilayer of phospholipid molecules that contain polyunsaturated fatty acids (PUFA) such as arachidonic acid.[118] The unsaturated double bonds of PUFA are susceptible to attack by oxidation. Once oxidised (A process called peroxidation because of the attachement of oxygen atoms as peroxide to the double bonds), the cell membrane becomes rigid and lyses.[119] A similar process leads to lysis of subcellular organelles such as perioxosomes, lysosomes, and mitochondria which in turn release their enzymes. This results in the denaturation of proteins and subsequent neuronal death.

A free-radical is an atom or a chemical group with a single electron in its outer shell and is highly reactive. It therefore attempts to

acquire another electron by attacking other atoms like the PUFA at their double bond sites. Free-radicals are normally generated at membrane NADPH-oxidase, mitochondria, lysosomes and perioxoxsomes for physiologic functions. Metal ions form many complexes and have more than one stable oxidation state e.g. Iron (Ferric and Ferrous forms) and can also provide free radicals for oxidation-reduction reactions. However, these are kept in check by physiologic anti-oxidants many of which are metal ion-enzyme systems. These include selenium-dependant glutathione peroxidase (GSH-Se) in the cystosol, and manganese dependant mitochondrial Superoxide dismutase, copper and zinc-dependant superoxide dismutase (CuZn-SOD).[120,121] Some diatary nutrients like vitamins C and E are also essential anti oxidants.[122] Thus brain metal ions have a dual role in free-radical damage as they are involved in the production of lipid peroxidation (by providing free-radicals) as well as protecting against lipid peroxidation (by being closely associated with anti-oxidants). The proper balance between these two opposing functions of metal ions is essential for homeostatic integrity of the neurone structurally and bio-chemically. When this dual role of metal ions in neurodegeneration is superimposed on the natural age-dependant auto-oxidation of the substancia nigra cells, it can be seen that the proxidant metals like iron will tend to hasten the degenerative process. In the same vein, acceleration of the degenerative process process via lipid peroxidation will be enhanced by a deficiency of anti-oxidant helpers such as copper, zinc and selenium.

Eventhough consistent low levels of these metals have not been demonstrated in parkinsonian substancia nigra, with continuing search, this may prove to be the case or some biochemical block in their relation with anti-oxidant enzymes.



THE AGING-NEUROTOXIC DAMAGE HYPOTHESIS.

KEY. MPTP= 1-methyl-4-phenyl-1,2,3,6-tetrohydropyridine; MPP^+= 1-methyl-4-phenyl-pyridinium
MAO-B= Monoamine Oxidase type B; Fe= Iron; ATP= Adenosine Triphosphate; ADP= Adenosine Diphosphate
O_2= Oxygen; H_2O_2= Hydrogen Peroxide; HVA= Homovanillic Acid; Vit= Vitamin; PUFA= Polyunsaturated Fatty Acid
NADH= Nicotinamide Adenine Dinucleotide; NMDA= N-methyl-D-asportate; KA= Kainate; QA= Quisqolate
SOD= Superoxide dismutase

FIGURE IV

Heavy metals and Essential Trace Metals.

Another factor in the metal ion role in parkinson's disease is the interaction of the heavy metals with the essential metals. The heavy metals, cadmium, lead and mercury are toxic to man by interacting with the functions of essential metals.[109,123] Eventhough they do occur naturally in the earth's crust, exposure to them reflects industrial contamination.[124] Assuming that the essential metal ions such as copper, zinc and iron have a leading role in the pathogenesis of IPD, then heavy metals can indirectly influence the development of parkinson's disease. If this is so, it will help explain why parkinson's disease is more prevalent in industrialised communities or communities exposed to pesticides, fertilizers and contaminated well water.[125,126]

Cadmium:

Cadmium is a prototype toxic heavy metal that is absent from human tissue at birth. It slowly accumulates in the body by ingestion and inhalation. Its effects are counteracted by zinc (a group 11 metal in the periodic table). In-vitro studies have shown that cadmium does reduce the activity of zinc dependant enzymes.[127] Although no such data of in-vivo studies in man are available, it is conceivable that cadmium might reduce the activity of CuZn-SOD and subsequently enhance lipid peroxidation and therefore neuronal degeneration of the substancia nigra. Cadmium does effect iron by reducing its absorption from the intestines and has affinity to ferittin. This has a tendency to lower serum iron and in fact iron defficiency anemia results from this in animals.[128] The importance of this in man has not been shown, but theoretically, if exposure to cadmium occurs

before maturation of the BBB, low brain levels of iron stores could result. This may cause reduced D_2-dopamine receptor binding sites[129] and subsequently predispose to parkinsonism in later life. In addition, iron-dependant catalase (anti-oxidant) could be reduced. It should however be remembered that low brain iron levels will tend to reduce the free-radical pool which will in turn tend to reduce lipid peroxidation and thereby protect against neuronal damage. The net effect of cadmium in the context of iron metabolism will thus depend upon the predominant effects of other anti-oxidants present .

Apart from being an antioxidant-associated metal, selenium also protects against cadmium toxicity. It achieves this by shifting cadmium from one protein site to the other.[130] Consumption of selenium in this role could possibly reduce its availability for gluthathione peroxidase and thereby predispose to lipid peroxidation.

Cadmium and copper have a mutual antagonism and cadmium toxicity in sheep has been shown to cause copper deficiency along with demyelination. Cadmium has also been shown reduce placental transfer of copper with subsequent deficiency in the new born.[131] When viewed in conext of CuZn-SOD this will enhance lipid peroxidation with predisposition parkinson's disease in later life. In addition, the copper deficiency could affect dopamine neurotransmitter homeostasis by reducing the copper-dependant Dopamine-B-hydroxylase.[69]

Data on the effect of cadmium on manganese metabolism in man is limited but cadmium has been shown to increase renal and hepatic manganese in animals.[132] If this does occur in the human brain during the pre-BBB period, parkinosnism can happen in later life as has been shown in adults previously.[17]

This will have to exeed the anti-oxidant effect of mitochondrial MnSOD.

Lead:

Lead is a highly toxic heavy metal in man with no essential biologic function. Non-industrialised, remote societies have only a neglible amount in the blood.[133] Sources of contact include paints, plaster, air(gasoline additives) and newsprint. Placental transfer does occur and after birth, the body tissue acquire lead by ingestion and inhalation.[134] Concentrations increase with age because exretory measures (faeces) do not keep up with ingestion. The most disturbing fact is that subclinical neurologic damage, behavioural and neuropsychologic deficits can occur at blood and tissue levels near those considered as 'normal'.[135] Certainly the issue needs reappraisal by society. The exact mechanism of neurologic damage is not elucidated but might have to do with the disturbance of dopamine release [136]and therefore concievably predispose to parkinsonism in later life. Marked reduction of zinc, copper and magnesium have been seen in rats treated with lead but data in man are not available.[137] If lead does cause such as effect in man, then it could reduce the brain CuZn-SOD and catalase-Fe activities thereby enhancing lipid peroxidation in later life.

Mercury:

Mercury is a highly toxic heavy metal that occurs naturally as well as from agricultural processes (herbicides) and industrial products. It is ingested and absorbed from the intestine and substantial amount diffuses into the nervous system, placenta and the foetus. The organic form of mercury (methyl mercury) is neurotoxic probably through interaction with sulphydryl groups in enzymes and membranes. Human disasters have happened from mercury

poisoning as in Minamita disease in Japan.[138] Multiple neurologic deficits occured in those cases although movement disorders such as parkinsonism were not documented. Perhaps slow, low-dose poisoning might affect the brain especially before establishment of the BBB. Secondly mercury could lead to deplation of selenium which is known to protect against the toxic effects of mercury.[139] If this does happen, then reduced function of GSH-Se could predispose to lipid peroxidation.

The Case for Metal Ions:

Metal ions are part and parcell of normal physiologic metallo-enzymes systems and neurotransmitter mechanisms. In addition, they partake in the enhancement and reduction of lipid peroxidative tendencies. In this regard, they are potentially capable of causing neurodegenarative changes at those site where they have a high concentration i.e. the basal ganglia and substancia nigra. In fact reduced levels of free-radicals scavengers like glutathione peroxidase and catalase which are metal dependant, have been demostrated in parkinsonian substancia nigra.[140,141,142] High levels of products of lipid peroxidation e.g. malondiadehyde have been demonstrated in parkinsonian substancia nigra along with reduced substrates of lipid peroxidation such as polyunsaturated fatty acids.[143,144]

Generally,the Nigerian new born baby feeds on breast milk up to the age of one to two years supplemented by the adult food in most of the population who are rural dwellers.The working class which forms a minority(about 20%),wean their babies earlier (3 to 6 months) and threreafter,feed the baby with on artificial feeds' based on cow milk.The population studied in Maiduguri originate from the rural areas mainly and have settled in the township in search of business or government employment.The metal ion content of human breast milk at term is poorer than cow milk except for iron and selenium,the levels of which are higher in the former. There is need for a study of the metal ion content of the Nigerian breast milk to help identify any metal ion excess or deficiency that could affect the post natal brain before maturation of the blood brain barrier.

As the Nigerian infant grows,its drinking water is generally the same as that of the adult and is therefore exposed to the excesses and deficiencies of the various metal ions of the Maiduguri drinking water(see table V).It can thus be seen that absorbs too much of the high amount of iron present in the water will tend to have excess free radical that can cause neuronal damage in later life.The absorbtion might occur in significant quantities perhaps ony in those that have concurrent increased intake of di etary factors such as vitamin C that are known to enhance the absorbtion of iron.Thus,a predisposed group to excess iron absorbtion results.In contrast,those that have

concurrent presence of phytates in the diet which inhibit iron absorbtion,will be protected from excess of the metal. From the foregoing,it is therefore not surprising that parkinsonian patients tended to have higher CSF and serum levels of iron than the controls.As regards manganese,it is known that the presence of calcium and phosphates in the diet,inhibits its absorbtion.[111] It is therefore conceivable that,the parkinsonian patients were unable to absorb enough of the metal despite of excessive levels in the drinking water due to concurrent presnce of excess calcium and/or phosphates.Inadequate manganese in the post natal brain could lead to reduced mitochondrial Superoxide Dismutase(SOD),a well known physiologic anti oxidant that protects against auto-oxidation and subsequent parkinsonism.[120] This will especially be so if the suceptible group lack other dietary anti-oxidants such as vitamin E which could not be ascertained in this study to to limited funds.It is true that manganism has been shown to lead to parkinsonism albeit not classic parkinson's disease. The very fact that no case of manganism was encountered in this study that evaluated most cases of parkinsonism occuring in the area,implies that there must be dietary protective factors such as phosphates in the general population.

The undetectable levels of copper and zinc in the Maiduguri drinking water implies that if an infant is unable to obtain these metal ions from other dietary sources,its brain levels of copper and zinc-dependant Superoxide Dismutase will tend to be

low.Thus it will lack this important protective anti-oxidant and be predisposed to parkinson's disease in later life.Limited funds prevented the estimation of these two metals in the CSF and serum which might have been low.I would also have been intresting to know the level of selenium in the drinking water and CSF for the afore mentioned reasons because glutathione peroxidase,an important free radical scavenger,is selenium-dependant.

The hevy metals(lead,mercury and cadmium) were not studied because of limited funds but also because they are unlikely to be present in significant amounts in such relatively unindudtrialised societies as Maiduguri compared to Europe.[125,126] On the other hand,surprises are ever present and when funds do become available,it would be worthwhile ascertaining the heavy metal status of such rapidly changing Nigerian environments.

SYNTHESIS: What does this picture of metal ion function imply? Firstly, access of the metal ions to the brain is limited to the pre-blood brain barrier maturation period during which a complement of metal ions are acquired which dictates the evolution of parkinson's disease. It is the view of this thesis that if migrant populations are studied, a critical age before migration is likely to be found after which the same risk of IPD as the country of origin will be retained. This is similar to the case of multiple sclerosis where this has been observed eventhough the responsible agent (virus) has not been identified.

A similar observation has been made in the case of ALS/PD complex of the Island of Guam.[21] People have developed the disease years after migrating from the Island. The suspected agents include metal ions and excito toxic amino acids. Eventhough this has not been conclusively proven, it indicates that such a mechanism is possible in a disease like IPD in view of the epidemiological pointers of it being strongly influenced by environmental causes. An interesting study was that of the comparison of a rural West African community and a rural United States born black communities as a natural experiment because the American blacks were ancestorily from West Africa.[54] It was demonstrated that the American born rural blacks had a much higher prevalance of IPD. This implies that the migrant blacks had acquired their new environments risk for IPD. It will be interesting to study generations of blacks from West Africa that migrated at various ages from birth and had remained in the high risk environments of Europe and America. If the thesis of the Pre-BBB metal ion loading is correct,

a critical age before migration is likely to be found. This will be an age around i.e. after the BBB has matured. A reverse study of course should confirm the hypothesis. However, because of the long time it takes for the 80% threshold of dopamine depletion before the disease declare's itself clinically, prospective studies will take generations to reach an end-point. Retrospective studies in this case will yield earlier results if feasible.

The higher prevalence of IPD in industrialised nations might be accounted for by the presence of heavy metal pollutants in such communities because they have an indirect effect on essential metal ions as earlier pointed out. That the disease has existed before industrialisation is in no doupt.[143] This can be explained by the fact that the essential metals have been with man from time immemorial and as earlier pointed out, are potential neurodegenerative agents. These points imply that there are at least two-sub-populations of IPD as far metal ions are concerned. The first will be that caused by an imbalance of the esential metal ion loading of the brain in the pre-BBB period and the second is that due to super-inposed heavy metal ion interaction in the pre-BBB period. The former group will predominnate in non-industrialised societies while the latter will predominate in industrialised communities. In addition, non-industrialised communities tend to have diets containing greater amounts of anti-oxidants which might contribute to the lower prevalence of IPD.[144] The essential metals likely to be found responsible for the first sub-population of PD are excess iron, relative defficiency of copper, zinc and selenium, and perhaps molebdenum.

Excess pre-BBB iron loading will accelerate lipid peroxidation by providing free-radicals. Deficient pre-BBB loading with copper, zinc and molebdenum will reduce the amount of CuZn-SOD, while deficient loading with selenium will reduce the activity of GSH-Se. On the other hand there would be those with deficient pre-BBB iron loading who will develop the disease in presence of relative deficiencies of Cu,Zn and Mo in addition to reduced iron-dependant catalase activity. A search for heavy metal ion influence on the pre-BBB loading period should be looked for in the developed communities as this may be occuring at presently acceptible levels. An insight into the roles of these metals could be gained by metal ion studies of normal brains of children of various ages post mortem. Maternal, cord blood and post natal blood levels of metal ions will also be helpful as in the case of lead.

The MPTP factor.

The well publicised MPTP-induced parkinsonism cannot be ignored in face of the remarkable resemblance to IPD. However, the effect of this toxin could be linked to free-radical generation and therefore lipid peroxidation (fig.1). The free-radical generation occurs in the initial step of the convertion of MPTP by monoamine oxidase type B (MOA-B) to the metabolically active metabolite $MPP.^{+}$[27,28] Secondly, by the effect of MPP^{+} on mitochondrial system, reduced activity of gluthione (a senium-dependant free-radical scavenger) could enhance lipid peroxidation. The DATATOP study using MAO-B inhibitor selegiline, has demonstrated slowing of IPD and this could be due to the inhibition of a MPTP-like toxin.

However it should also be remembered that the same drug also inhibits the MOA-B dependant auto-oxidation of dopamine that normally occurs with ageing, and indirectly it also reduces lipid peroxidation as the auto-oxidative process generates free radicals. In short, MPTP could be a second factor in the development of parkinson's disease either through mechanisms unrelated to lipid peroxidation or by enhancing lipid peroxidation.

CONCLUSION.

The exact causative agent of parkinson's disease remains an enigma.Various metal ions,amongst other causes have been implicated directly or indirectly.This study of Nigerian Parkinsonian patients in Maiduguri indicates that the young neonatal brain is exposed to excessive and deficient metal ion concentrations before maturation of the blood brain barrier.A small susceptible group of the population in Maiduguri consequently grow up with either an increased tendency to metal ion-induced free radical neuronal damage(iron) or a decreased level of protective metal ion-dependant anti-oxidants(copper,zinc and manganese).As to why only very few patients do develop the disease in comparison to industrialised soscieties,might be due to a variety of reasons.Those range from the presence of anti-oxidants in the diets of the former to the absence of heavy metals and to factors such as absence of MPTP-like agents.

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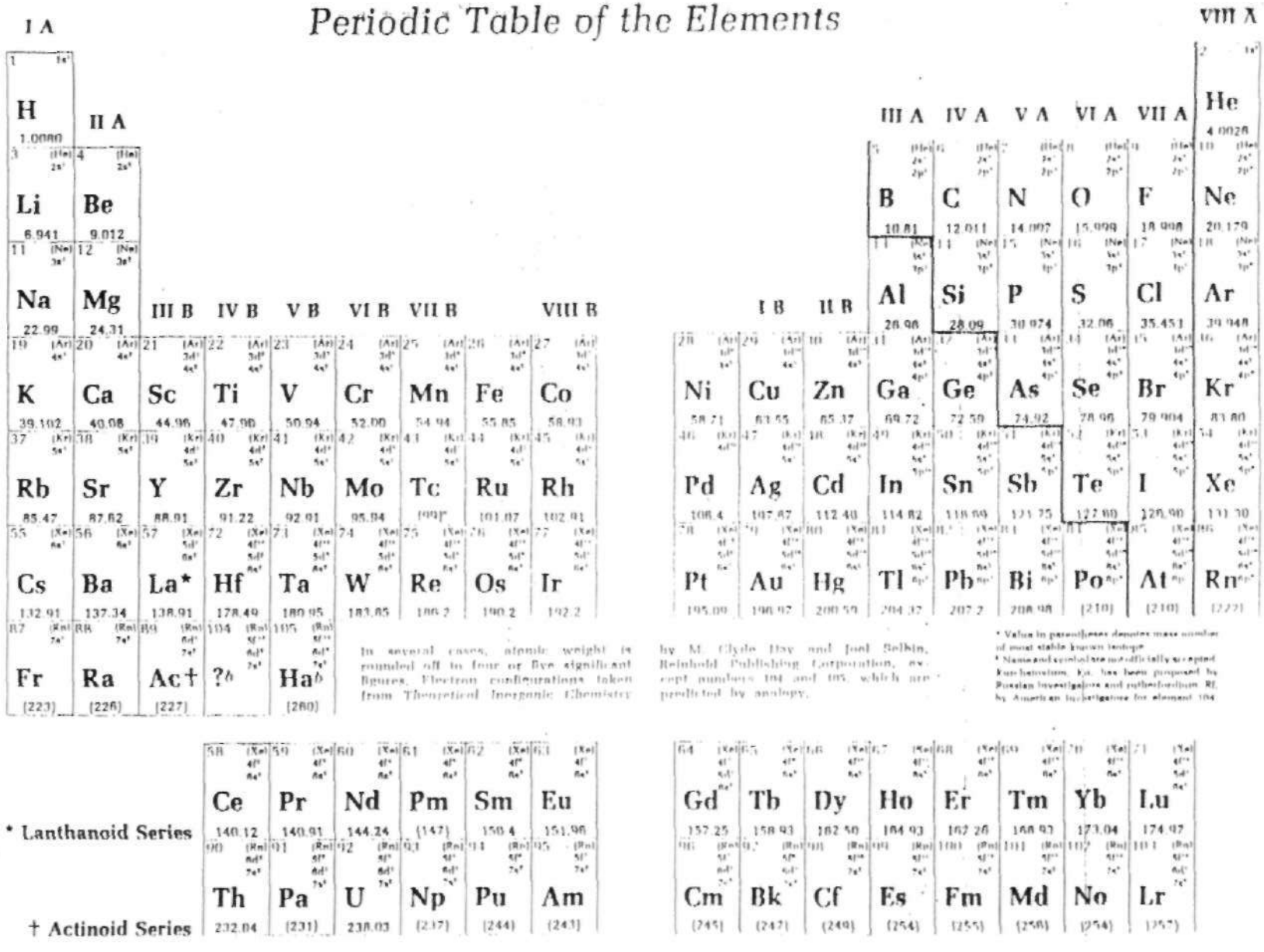
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Periodic Table of the Elements



In several cases, atomic weight is rounded off to four or five significant figures. Electron configurations taken from *Theoretical Inorganic Chemistry* by M. Clyde Day and Joel Selbin, Reinhold Publishing Corporation, except numbers 104 and 105, which are predicted by analogy.

* Value in parentheses denotes mass number of most stable known isotope.
* Names and symbols are not officially accepted for hahnium, Ha, has been proposed by Russian investigators and rutherfordium, Rf, by American investigators for element 104.

Appendix 2a

PARKINSON'S DISEASE STUDY

CLINICAL INFORMATION

A. Patient's Personal Characteristics

Name Hospital Number

Age Address

Sex

Tribe Occupation

B. History of Illness and Duration of Symptoms

(a) Tremor

(b) Bradykinesia

(c) Postural Disturbances

(d) Miscellaneous; e.g. muscle cramps, fatigue, etc.

C. Past Medical History

(a) Other diseases

(b) Medications (with dates)

D. Family History of Parkinson's Disease

E. Physical Signs

(a) Tremor (part of body affected, stress relation)

(b) Bradykinesia (rate of blinking/minute, facial expression, speed of pronation-supination of hands and foot tapping, arm-swing on walking and time of maintenance of positions).

(c) Rigidity (limbs affected. 'Activated rigidity test).

(d) Posture (gait features, etc.)

(e) Miscellaneous (Sialorrhoea, seborrhoea, dementia speech).

F. Disability Rating (Northwestern University Disability Scales (NUDS) For details see appendix 1

Scale	Score
1. Walking	
2. Dressing	
3. Hygiéne	
4. Eating	
5. Speech	
T O T A L	

Appendix 2c

Investigations

(a) Routine - Stool, urine, full blood count, urea, electrolytes, fasting blood sugar, chest X-ray.

(b) Special

1. Serum for proteins and trace elements (stored at $-70^{\circ}C$)

2. Cerebrospinal Fluid (CSF)

   * Sugar)
   * Protein) done on the day of lumbar puncture
   * Cell count)

   * Trace Elements - 1.25 ml x 6 tubes at $-70^{\circ}C$

(c) Sample of Drinking water stored at $-70^{\circ}C$

(d) Sample of staple food

Appendix 3

Northwestern University Disability Scales

Scale A: Walking

Never Walks Alone

0 Cannot walk at all, even with maximum assistance.
1 Needs considerable help even for short distances; cannot walk outdoors with help.
2 Requires moderate help indoors; walks outdoors with considerable help.
3 Requires potential help indoors and active help outdoors.

Sometimes Walks Alone

4 Walks from room to room without assistance, but moves slowly and uses external support; never walks alone outdoors.
5 Walks from room to room with only moderate difficulty; may occasionally walk outdoors without assistance.
6 Walks short distances with ease; walking outdoors is difficult but often accomplished without help; rarely walks longer distances alone.

Always Walks Alone

7 Gait is extremely abnormal; very slow and shuffling; posture grossly affected; there may be propulsion.
8 Quality of gait is poor and rate is slow; posture moderately affected; there may be a tendency toward mild propulsion; turning is difficult.
9 Gait only slightly deviant from normal in quality and speed; turning is the most difficult task; posture essentially normal.
10 Normal.

Scale B: Dressing

Requires Complete Assistance

0 Patient is a hindrance rather than a help to assistant.
1 Movements of patient neither help nor hinder assistant.
2 Can give some help through bodily movements.
3 Gives considerable help through bodily movements.

Requires Partial Assistance

4 Performs only gross dressing activities alone (hat, coat).
5 Performs about half of dressing activities independently.
6 Performs more than half of dressing activities alone, with considerable effort and slowness.
7 Handles all dressing alone with the exception of fine activities (tie, buttons).

Complete Self-Help

8 Dresses self completely with slowness and great effort.
9 Dresses self completely with only slightly more time and effort than normal.
10 Normal.

Scale C: Hygiene

Requires Complete Assistance

0 Unable to maintain proper hygiene even with maximum help.
1 Reasonably good hygiene with assistance, but does not provide assistant with significant help.
2 Hygiene maintained well; gives aid to assistant.

Requires Partial Assistance

3 Performs a few tasks alone with assistant nearby.
4 Requires assistance for half of toilet needs.
5 Requires assistance for some tasks not difficult in terms of co-ordination.
6 Manages most of personal needs alone; has substituted methods for accomplishing difficult tasks (electric razor).

Complete Self-Help

7 Hygiene maintained independently, but with effort and slowness; accidents are not infrequent; may employ substitute methods.
8 Hygiene activities are moderately time-consuming; no substitute methods; few accidents.
9 Hygiene maintained normally, with exception of slight slowness.
10 Normal.

Scale D: Eating and Feeding*

Eating

0 Eatings is so impaired that a hospital setting is required to get adequate nutrition.
1 Eats only liquids and soft food; these are consumed very slowly.
2 Liquids and soft foods handled with ease; hard foods occasionally eaten, but require great effort and much time.
3 Eats some hard foods routinely, but these require time and effort.
4 Follows a normal diet, but chewing and swallowing are labored.
5 Normal.

Feeding

0 Requires complete assistance.
1 Performs only a few feeding tasks independently.
2 Performs most feeding activities alone, slowly and with effort; requires help with specific tasks (cutting meat, filling cup).
3 Handles all feeding alone with moderate slowness; still may get assistance in specific situation (cutting meat in restaurant); accidents not infrequent.
4 Fully feeds self with rare accidents; slower than normal.
5 Normal.

Scale E: Speech

0 Does not vocalize at all.
1 Vocalizes but rarely for communicative purposes.
2 Vocalizes to call attention to self.
3 Attempts to use speech for communication, but has difficulty in initiating vocalization; may stop speaking in middle of phrase and be unable to continue.
4 Uses speech for most communication, but articulation is highly unintelligible; may have occasional difficulty in initiating speech; usually speaks in single words or short phrases.
5 Speech always employed for communication, but articulation is still very poor; usually uses complete sentences.
6 Speech can always be understood if listener pays close attention; both articulation and voice may be defective.
7 Communication accomplished with ease, although speech impairment detracts from content.
8 Speech easily understood, but voice or speech rhythm may be disturbed.
9 Speech entirely adequate; minor voice disturbances present.
10 Normal.

* Eating and feeding are figured separately and the two scores added.

Appendix 4

King's College London

UNIVERSITY OF LONDON

BIOMEDICAL SCIENCES DIVISION

MANRESA ROAD, LONDON SW3 6LX TELEPHONE: 01-351 2488 FAX: 01-352 7376

Head of Division, Professor S. L. Howell

Pharmacology Group

HEAD OF PHARMACOLOGY
Professor P. G. Jenner

FAX TO:- DR. S. A. BWALA DEPARTMENT OF MEDICINE
KING KHALID MILITARY CITY HOSPITAL
HAFR AL-BATIN 31991.
SAUDI ARABIA.

FAX N° 00966 03 7871777 ~~6913 4648~~
FAX FROM: DR. DAVID DEXTER PHARMACOLOGY GROUP.
FAX N° 3768150

DEAR DR. BWALA;

HERE ARE THE METAL ION RESULTS FROM THE WATER SAMPLES IN nMOLES METAL ION/LITRE.

	Fe	Cu	Zn	Mg	Mn	Al
LONDON WATER	N.D	1362.4	540.4	178740.0	6.2	207400.0
GOUT HORZE BORE HOLE	26760.0	N.D	N.D	128060.0	115340.0	14784.0
DALORI BORE HOLE	51000.0	12.4	149.2	90720.0	2706.0	9934.0
A.I.D BORE HOLE.	27700	N.D	N.D	56780	23.4	9858.0

N.D = NOT DETECTED.

AS YOU CAN SEE THERE IS VARIATION IN THE WATER FROM EACH BORE HOLE AND FROM LONDON WATER. GENERALLY THERE IS MORE Fe, AND Mn IN THE BORE HOLE WATER BUT LESS Al, Mg AND Cu. COMARED TO LONDON WATER. DO YOU WISH ME TO RETURN THE REMAINING WATER FROM THE SAMPLES?

YOURS SINCERELY,
David Dexter

TABLE 7.1. Standards for Potable Water Supplies

(1)	(2)	(3)	(4)	(5)
Substance or property	High class water	WHO Highest desirable concentration	Generally attainable standard	WHO Maximum permissible concentration
Total dissolved solids, mg/l	300	500	1000	1500
Colour*	3	5	20	50
Turbidity, JTUs	0.2	5	3	25
Taste (TON)**	0	<2	<2	<3
Odour	0	<2	<2	<3
Iron, mg/l	0.1	0.3	0.3	1
Arsenic, mg/l	0.05	0.05	0.05	0.05
Manganese, mg/l	0.02	0.1	0.2	0.5
Cyanide, mg/l	0.1	0.2	0.2	0.2
Copper, mg/l	0.2	1	1.3	1.5
Zinc, mg/l	1	5	10	15
Lead, mg/l	0.05	0.05	0.05	0.05
Calcium, mg/l	50	75	150	200
Magnesium, mg/l	40	50	100	150
Sulphate, mg/l	100	200	300	400
Chloride, mg/l	100	200	450	600
pH	7.0—8.3	7.0—8.5	7.0—9.0	6.5—9.2
Magnesium and sodium sulphate, mg/l	200	500	750	1000
Phenol, mg/l	0	0.001	0.001	0.002
Carbon chloroform extract (CCE) organic pollutants, mg/l	0.05	0.2	0.2†	0.5
Alkyl Benzyl sulphonate (ABS) surfactants, mg/l	0	0.5	1.0	1.0
Hardness ($CaCO_3$), mg/l	80	200	300	300

* Platinum Cobalt Scale

**TON = threshold odour number

†Concentrations in excess of 0.2 mg/l require further investigation to ascertain cause

Appendix 6

BIOSCIENTIA TEST LIST

Heavy metal group 1 — 88 Dm each

Arsenic
Bismuth
Cadmium ✓
Chromium
Cobalt
Manganese ✓
Nickel
Selenium ✓
Thallium
Molybdenum ✓

Heavy metal group 2 — 58 Dm each

Aluminium ✓
Bromide
Copper ✓
Fluoride
Gold
lead ✓
Zinc ✓

Confirmatory Drug Assay — 29 Dm each

Codeine
Tylenol/Paracetamol
Morphine

IRON DM 40 ✓

MERCURY DM 180 ✓

Drug Assays — 80 Dm each

Amitryptaline
Acetominophen
Aetochlomalone
Chlomipramine
Chloramphenicol
Clonazepam
Dentracin
Desipramine
Dibenzapine
Doxepin
Erganyi
Imipramine
Isoniaziade
Melatracin
Methadone
Methylmalonic
Mexatil
Mogadon
Mylepsinum
Nitrazapen
Norpramine
Opipramol
Pethidine
Petinutin
Pirenzipin
Quinidine
Rivotril
Rohypnol
Theophilline
Tobramycin
Valium

Appendix 7(thirty three sheets).

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Report No.
Client No. 0112440
Sample Entry 24.09.90

Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
AUDU RABI ADAMU	03[illegible]			24.09.90/675006

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	31	mcg/l	
Manganese	1.3	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
I USMAN	035819	[illegible]		24.09.90/674992

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF			
Total Iron	31	mcg/l	
Manganese	4.3	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
A GASI	037131	[illegible]		24.09.90/674982

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	33	mcg/l	
Manganese	1,3	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
KADIRI KADRA	036862	???		24.09.90/674981

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	45	mcg/l	
Manganese	2,6	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
AMSA MODU	038483	[illegible]		24.09.90/674984

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/			
Total Iron	37	mcg/l	
Manganese	2,3	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
USMAN ABDULLAHI	015321	[illegible]		24.09.90/674983

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	288	mcg/l	
Manganese	1,5	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
USMAN MALLINKE	009023	[illegible]		24.09.90/674985

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1938			
Total Iron	61	mcg/l	
Manganese	1,9	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
JAURO HASSAN M	035709	???		24.09.90/674987

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	55	mcg/l	
Manganese	1,9	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
A YASALE	039155	[illegible]		24.09.90/674986

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1987			
Total Iron	27	mcg/l	
Manganese	1,0	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
L BAKARI	035882	???		24.09.90/675003

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	31	mcg/l	
Manganese	5.6	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
ISA MATAYA MAMMAN	020575	???		24.09.90/675004

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	32	mcg/l	
Manganese	3,2	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
TIJJANI AHMED IBRAHIM	035059	???		24.09.90/675005

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	18	mcg/l	
Manganese	0,9	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
BUKAR ALASURU	013965	22/		24.09.90/674988

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	55	mcg/l	
Manganese	2,3	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
YERIMA ATINWA	033814	???		24.09.90/674989

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	383	mcg/l	
Manganese	2,1	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
AUDU MOHAMMED		[illegible]		24.09.90/674991

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	179	mcg/l	
Manganese	1,9	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
K BAPPA	037520	???		24.09.90/674994

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	20	mcg/l	
Manganese	0,9	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
UDOH MACK	037533	???		24.09.90/674993

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	75	mcg/l	
Manganese	3.7	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
A ABUBAKAR	017274	???		24.09.90/674980

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	31	mcg/l	
Manganese	1,3	mcg/l	

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Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
MOHAMMED BUHARI	035995	???		24.09.90/674999

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	81	mcg/l	
Manganese	2,4	mcg/l	

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Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
ZARADE MALA	022359	???		24.09.90/674998

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF			
Total Iron	271	mcg/l	
Manganese	17,0	mcg/l	

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
J MDAHARA	NIL	???		24.09.90/674997

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	40	mcg/l	
Manganese	3.9	mcg/l	

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Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
G BUAHLEN	NIL	???		24.09.90/674996

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	146	mcg/l	
Manganese	7,0	mcg/l	

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Reporting date 23.11.90
Report No.
Client No. 0112440
Sample Entry 24.09.90

Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
YAGANA ALHAJI	024702	???		24.09.90/674995

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1983			
Total Iron	20	mcg/l	
Manganese	4,4	mcg/l	

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Client No. 0112440
Sample Entry 24.09.90

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
BAWA ALKALI	020943	???		24.09.90/675000

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	78	mcg/l	
Manganese	4.2	mcg/l	

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Sample Entry 24.09.90

Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
L DANEYEL	037647	???		24.09.90/675002

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF /1988			
Total Iron	41	mcg/l	
Manganese	1,9	mcg/l	

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Reporting date 23.11.90
Report No.
Client No. 0112440
Sample Entry 24.09.90

Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
ETZLA NKEDU	027869	???		24.09.90/675001

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
CSF/1988			
Total Iron	18	mcg/l	
Manganese	1,4	mcg/l	

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Reporting date 02.10.90
Report No. 1273344 Page
Client No. 0112440
Sample Entry 01.10.90

Report

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Patient	Identification No.	Date of Birth	Reporting Date	Request Code
ABSULLAHI USMAN	035321	--.--.--		01.10.90/75952 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	17.2	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

035321

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Reporting date 02.10.90
Report No. 1273343 Page
Client No. 0112440
Sample Entry 01.10.90

Report

***

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
MODU AMSA	038483	--.--.--		01.10.90/75951 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	14.9	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

038483

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Reporting date 02.10.90
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Client No. 0112440
Sample Entry 01.10.90

Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
IBRAHIM-TIJJANI AHMED	035059	--.--.--		01.10.90/75957 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	7.16	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

035059

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Reporting date 02.10.90
Report No. 1273342 Page
Client No. 0112440
Sample Entry 01.10.90

Report

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
MALLINKE USMAN	009023	--.--.--		01.10.90/75950 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	25.6	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

009023

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Reporting date 02.10.90
Report No. 1273345 Page
Client No. 0112440
Sample Entry 01.10.90

Report

***

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
KADARA KADIRI	036862	--.--.--		01.10.90/75954 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	18.3	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

036862

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Reporting date 02.10.90
Report No. 1273347 Page
Client No. 0112440
Sample Entry 01.10.90

Report ***

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
BUHARI MOHAMMED	035995	--.--.--		01.10.90/75961 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	7.88	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

035995

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Reporting date 02.10.90
Report No. 1273348 Page
Client No. 0112440
Sample Entry 01.10.90

Report

***1

Patient	Identification No.	Date of Birth	Reporting Date	Request Code
NKEDU EZTLA	027869	--.--.--		01.10.90/75966 S

Sample Material/ Sampling Date/Analysis	Result	Units	Reference Range
Serum			
Iron, total	18.6	mcmol/l	Male: 9.5-29.9 Female: 8.8-27.0

027869

CLIN. CHEM. 30/8, 1358–1360 (1984)

Appendix 8.

Concentrations of Ag, Al, Au, Bi, Cd, Cu, Pb, Sb, and Se in Cerebrospinal Fluid of Patients with Cerebral Neoplasms

Adnan El-Yazigi,[1] Iman Al-Saleh,[1] and Ossama Al-Mefty[2]

We measured the concentrations of nine trace elements in cerebrospinal fluid of 11 patients with malignant brain tumors, 11 with benign brain tumors, and 10 control patients, using flameless atomic absorption spectrophotometry. The mean and standard deviation for these concentrations (μg/L) in the control group were 5.1 ±2.9 (silver), 326.6 ±171.2 (aluminum), 38.5 ±32.2 (gold), 36.6 ±23.7 (bismuth), 1.5 ±1.3 (cadmium), 39.8 ±24.7 (copper), 15.7 ±11.5 (lead), 20.9 ±3.8 (antimony), and 19.1 ±13.3 (selenium). Concentrations of silver and lead were markedly increased in patients with malignant cerebral neoplasms. The malignant-tumor/control patient concentration ratios were 2.31 for silver and 2.11 for lead. We observed no significant differences between the results for the benign tumor patients and the control group.

Additional Keyphrases: *atomic absorption spectrophotometry · cancer · trace elements · brain tumors*

Trace elements in blood and urine have been amply studied, but reports dealing with these elements in cerebrospinal fluid (CSF) are few. We found none on the concentrations of trace elements in the CSF of patients with cerebral neoplasms. Many neurological and other diseases are associated with changes in the chemical composition of the CSF. Trace elements play an important role in the metabolic, biosynthetic, and biochemical activities in the living cells. Malignancy-induced imbalances in these activities often produce abnormal values for these elements in the tumor cells or the surrounding biological fluids. Indeed, the concentrations of copper (*1–4*), zinc (*5*, *6*), and selenium (*7*) in serum have been related to different types of malignancies. The iron, antimony, chromium, cobalt, and scandium content of DNA in sarcoma [illegible] also vary with the disease progresses (*8*). Schr[illegible] et al. (*9*) found [illegible] and selenium in benign cerebral tu[illegible] normal brain tissues; other elements, rubidium, and zinc) differed slightly from normal. The concentrations of copper, iron, zinc, manganese, calcium, magnesium, cobalt, and nickel in bone of patients with osteogenic sarcoma differ significantly from those in controls (*10*).

We undertook to measure the concentrations of silver, aluminum, gold, bismuth, cadmium, lead, antimony, copper, and selenium in the CSF of patients with malignant or benign brain tumors, and, for comparison, in a control (reference) group.

Materials and Methods

Patient Selection and Sample Collection

The 32 patients included in this study (Table 1) had fasted for 8 to 12 h before surgery. A CSF specimen (3 to 4 mL) was collected in a plastic specimen container from each patient at the time of operation through a ventricular catheter or a spinal drainage needle inserted in the lumbar sac. No sample containing blood was used in this study. The patients were divided into three groups:

Malignant brain tumor: Eleven patients with histologically diagnosed astrocytoma, medulloblastoma, pinealblastoma, or chondrosarcoma were operated upon under general anesthesia between 0930 and 1630 hours for removal of the tumors. CSF from two patients was collected through the ventricular catheter and from nine by lumbar puncture.

Benign brain tumor: Eleven patients with meningioma or craniopharyngioma were operated upon and a CSF sample was collected from each. Again, the sampling sites were two ventricular and nine lumbar.

Reference group: Ten patients with various non-neoplasmic diseases (Table 1) were included in this study as a reference group. CSF was withdrawn from each patient with hydrocephalus via a ventricular catheter as part of the treatment and an aliquot used in this study. CSF samples from other patients in the reference group were collected by lumbar puncture for general chemistry diagnostic workup.

Reagents

Standard solutions (1 g/L) of the eight elements (Fisher Scientific Co., Pittsburgh, PA) were used in preparing the standard curves. Doubly distilled water, stored in a plastic container, was used. The nitric acid was AR grade.

Methods

We used a Model 975 atomic absorption spectrophotometer equipped with a Model GTA-95 electrothermal (graphite tube) atomizer (Varian Techtron Pty., Ltd., Mulgrave, Victoria, Australia). The CSF specimens were centrifuged in a

Table 1. Disease, Sex, and Age of the Patients

Disease	No. of cases	Sex (M/F)	Mean (and SD) age, yr
Malignant brain tumor group			
Astrocytoma	6	5/1	20.9 (17.7)
Medulloblastoma	3	2/1	8.7 (7.2)
Pinealblastoma	1	0/1	55.0
Chondrosarcoma	1	1/0	15.0
Benign brain tumor group			
Meningioma	8	3/5	42.6 (8.1)
Craniopharyngioma	3	3/0	26.0 (21.2)
Reference group			
Hydrocephalus	6	2/4	4.6 (4.2)
Others[a]	4	2/2	25.5 (16.4)

[a] Arterial-venous malformation, aneurism, subarachnoid cyst, headache.

[1] Department of Cancer and Radiation Biology Research and [2] Department of Neurosurgery and Neurosciences, King Faisal Specialist Hospital and Research Centre, Riyadh 11211, Saudi Arabia.

Received January 31, 1984; accepted May 29, 1984.

Table 2. Mean (and SD) Concentrations (μg/L) of the Trace Elements in CSF

Disease	Ag	Al	Au	Bi	Cd	Cu	Pb	Sb	Se
Astrocytoma	11.1	361	23.6	41.2	4.0	56.7	29.9	22.2	23.8
	(7.7)	(164)	(3.3)	(14)	(3.1)	(37.5)	(13.2)	(8.2)	(1.6)
Medulloblastoma	12.4	420	27.8	35.8	1.9	109	49.5	45.5	85.1
	(4.8)	(168)	(13.2)	(1.1)	(2.7)	(95.4)	(27.7)	(14.5)	(13.5)
Pinealblastoma	21.3	—	50.5	34.3	4.0	147	24.5	—	154
Chondrosarcoma	4	230	16.0	31.3	1.3	24	13	28.3	157
Meningioma	5.9	315	26.1	34.4	1.3	53.2	16.3	20.8	19.1
	(4.6)	(200)	(16.6)	(34.9)	(1.3)	(36.8)	(9)	(5.2)	(5.6)
Craniopharyngioma	9.1	452	35.3	31.8	0.1	41.1	11.0	25.8	18.4
	(8)	(208)	(23.7)	(15.9)	(0.1)	(15.4)	(1.1)	(14.6)	(4.1)

Values for the reference group are listed in Table 3.

refrigerated centrifuge at 3500 rpm, and 0.5 mL of the supernate was diluted with dilute (5 mL/L) nitric acid so as to yield absorbance values within the linear range for each element analyzed. Furnace operating conditions (temperature, time, and gas flow in the drying, ashing, and atomization stages) were those recommended by the instrument manufacturer (*10*). The gas used was nitrogen, except in the case of selenium, where it was argon. The lamps were the hollow-cathode type, except for selenium, for which we used an electrodeless discharge lamp with a power supply. The samples were held in autosampler plastic tubes, and the autosampler was programmed to inject 10 or 20 μL. The standard curves were prepared by use of standard solutions diluted with the dilute nitric acid under conditions identical to those used for the unknown samples. The analyses were performed in at least quadruplicate.

Results

The data for standard curves obtained for all the elements examined in this study were subjected to linear least-square regression analysis, and the resulting equations were used in calculating the concentrations of these trace elements in the CSF samples. There was good linearity between concentration and instrument reading for all the elements investigated; the correlation coefficients were 0.999 (silver, copper, and lead), 0.996 (antimony and selenium), 0.992 (aluminum), 0.990 (bismuth), and 0.998 (gold and cadmium). Reproducibility was also good, and the CV for no sample exceeded 15%.

Table 2 gives the mean and standard deviation for the concentrations of the nine elements in the CSF samples from both groups of patients. Table 3 gives the corresponding values for the refere[illegible] roup and al[illegible] revi[illegible]ly reported concentration[illegible] for t[illegible] element[illegible]

The concentrations o[illegible] he t[illegible] ments in [illegible] fro[illegible] three groups of patients wer[illegible] ted by [illegible]nt's unpaired *t*-test. There were sign[illegible] diffe[illegible]ces i[illegible] he concentrations of silver ($p < 0.012$) and lead ($p < 0.047$) between the malignant tumor group and the reference group (Table 4). Differences in the concentration of lead ($p < 0.014$) were also significant between the malignant and benign tumor group. All other differences were not significant. Specifically, we saw *no* significant differences between the benign tumor group and the control group.

Discussion

As demonstrated in Table 3, the values we obtained for most of these elements agree with those found by other investigators (*12–22*). The values for gold in CSF were higher than that reported by Kjellin (*12*), perhaps owing to differences in the analytical techniques or to protein binding differences between the two groups of patients involved in the two studies, because high concentrations of this element were found in patients with high protein concentrations

Table 3. Concentrations (μg/L) in CSF Observed in This Study and by Others

Element	Present study[a]	Kjellin (ref. *12*)	Gooddy et al. (ref. *13*)	Others (ref.)
Ag	5.1 (2.9)	<1		
Al	326.6 (171.2)	125	210	200–700 (*14*) 31–40 (*15*)
Au	38.5 (32.2)	0.0062		
Bi	36.6 (23.7)			
Cd	1.5 (1.3)	<1, 10–20		
Cu	39.8 (24.7)	16	240	95 (*16*) 76 (*17*) 196 (*18*) 133 (*19*) 40 (*20*) 7.5 (*21*)
Pb	15.7 (11.5)	0–100	Tr	44.2 (*22*)
Sb	20.9 (3.8)	<1	Tr	
Se	19.1 (13.3)	<10		

[a] Mean (and SD) of findings in the reference group of patients.
Tr, trace.

Table 4. Significant ($p < 0.05$) or Near-Significant Differences in Mean Trace Element Concentrations in CSF Observed for the Three Groups of Patients

Element	Groups compared[a]	Significance, *p*-value
Ag	MTG/RG	0.012
	MTG/BTG	N.S. (0.078)
Cd	MTG/BTG	N.S. (0.061)
Pb	MTG/RG	0.047
	MTG/BTG	0.014
Se	MTG/RG	N.S. (0.076)
	MTG/BTG	N.S. (0.074)

[a] MTG, malignant tumor group; RG, reference group; BTG, benign tumor group.
N.S., nonsignificant.

(*12*). Our value reported for bismuth appears to be the only value yet reported.

Significantly increased values for silver and lead in CSF of patients with malignant cerebral tumors (astrocytoma, medulloblastoma, pinealblastoma, and chondrosarcoma) were observed (Table 4). The ratios for tumor/reference-patient mean CSF concentrations were 2.31 for silver and 2.11 for lead. Although age reportedly influences the chemical composition of the CSF, it does not appear to be cause for the differences we observed: the benign-tumor patients, (mean age 38.1 years) had CSF concentrations similar to

years). Also, this increase can not be attributed to the difference in the sampling site (i.e., ventricular vs lumbar). Although the sampling from both tumor groups was performed identically (i.e., nine ventricular and two lumbar in each group), differences between the two tumor groups were significant, whereas the differences between the benign-tumor and reference group (six ventricular and four lumbar samplings) were not significant.

The mechanism(s) of the observed increased concentrations of silver and lead in CSF of patients with malignant cerebral tumors is unknown. The concentrations of these trace elements in CSF in cases of brain neoplasms have not been previousy reported. Schicha et al. (9) reported marked variations (higher or lower) in the cobalt, iron, rubidium, cobalt, and zinc content of human malignant cerebral tumor tissues, as compared with that in benign cerebral tumors, but the influence of such variations on the concentrations of these elements in CSF was not addressed.

The increased concentrations of silver and lead we observed in patients with malignant brain tumors may reflect disturbed metabolic and biochemical activities in the malignant cells. They may be also related to malignancy-induced alterations in the distribution of these elements between the cerebral cells and the CSF. Alternatively, these changes may be due to a change in the integrity of the blood–brain barrier and subsequent selective leakage of these elements into the CSF.

We thank Dr. Nizar Feteih, Executive/Medical Director, Dr. K. Alvin Merendino, Director of the Research Centre, and Dr. Farouk Al-Watban, the Deputy Director of the Research Centre, for their support and encouragement of the cancer research program in this institution.

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