THE STUDY OF DRUG METABOLISM IN CHILDREN WITH PROTEIN ENERGY MALNUTRITION AND TUBERCULOSIS USING THE CAFFEINE BREATH TEST

By

Dr. PAUL REMINGTON OGOLLA NYATIGI

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SUMMARY

The purpose of this study was to determine the applicability of the caffeine breath test in assessing liver drug metabolism in children with Kwashiorkor and those on anti-TB drugs. Seventeen children aged between 3-10 years were recruited into the study, of which 8 were cases of kwashiorkor and 9 on anti-TB treatment. Both groups were studied before commencing dietary rehabilitation or anti-TB treatment.

Labelled caffeine was given orally at a dose of 3 mg kg^{-1} dissolved in distilled water. Breath samples were collected by requesting the child to blow into the mask at intervals of 15 minutes for a period of 2 hours. The collected sample were transferred into a labelled headspace analyzer vial for storage pending analysis. The procedure was repeated after 10-14 days of intervention.

Breath samples were analyzed at the Scottish Universities Research Reactor Centre, Edinburgh. Unfortunately, due to faulty seal on some of the collection vials, only a limited number of specimens had adequate amount of isotope for analysis ($\geq$ 50). In the kwashiorkor group there were 4 single and 3 duplicate samples which had adequate amount of isotope labelled carbon dioxide for analysis. In the patients with duplicate samples a rise in level of labelled carbon dioxide recovered after nutritional rehabilitation, the mean (SD) percentage was 2.86 (1.3) before and 3.6

(1.39) after rehabilitation. In TB patients there were 3 single and 4 duplicate samples which had adequate amount of isotope labelled carbon dioxide for analysis. In these TB patients with duplicate samples, two had a notable rise and the other two had marginal decline in level of cumulative labelled carbon dioxide after commencement of treatment.

The study showed that the caffeine breath test is a useful test of liver drug metabolizing enzymes in children with malnutrition. The study also demonstrates that the caffeine breath test can be used in children of three years of age or possibly younger.

Further studies are required particularly to repeat the test when the children have recovered from malnutrition and tuberculosis.

Key words; Caffeine breath test, Children, Dietary rehabilitation, Anti-TB, Kwashiorkor, Tuberculosis.

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Protein energy malnutrition (PEM) is one of the major contributors to impairment of health and growth of children in developing countries. The role of infection and malnutrition, separately or in combination is an important cause of mortality and morbidity. In developing countries, infection and malnutrition continue to be responsible for the majority of paediatric health problems [1,2].

Children with kwashiorkor have a history of prolonged illness, anorexia, frequent and prolonged episodes of diarrhoea, respiratory tract infections and often recent measles. Clinical features include, hair changes, mucosal changes and an enlarged liver. Classical skin changes of crazy pavement dermatosis is only seen in oedematous malnutrition [3].

Impaired cell proliferation, reduced surface area, and disruption of the mucosal barrier are the major contributors to intestinal deficit and impaired absorption in malnutrition. Functional changes include hypochlohydria, delayed gastric emptying increased or decreased transit time [4]. As a result of the preceding factors there is growth faltering [2].

Malnourished children are more susceptible to infections particularly respiratory tract infection and intestinal parasitosis [5]. This has led to increased use of drugs in these patients in spite of inadequate knowledge in pharmacokinetics of these xenobiotics on malnourished or those children suffering from other infections such as tuberculosis [4].

With the background knowledge that PEM imposes biological alteration on various organs of the body including the liver which handles most drug metabolism and detoxification, it is important to assess drug metabolism in PEM. It is also evident that some anti-TB drugs such as rifampicin induce liver metabolism [1]. With these in mind it is sensible to postulate that drug dosage for malnourished children may require adjustment [6,7].

One of the most important difficulties encountered, however, in carrying out a study of drug metabolism in children is the need for blood sample collection which is an invasive procedure. As such it is advantageous to develop a noninvasive method of studying drug metabolism [7].

The development of the caffeine breath test as a tool of investigating liver function is promising. It has been used in some centres in Europe and America with good results, however no work has yet been done in developing countries with a huge demand for xenobiotics and a completely different cultural and ecological background [7,8]. While studying the drug interaction amongst children suffering from cystic fibrosis who are taking

ciprofloxacin, Parker et al used the caffeine breath test. The caffeine breath test involves the use of a stable isotope of carbon (^{13} C) incorporated into N-methyl branch of caffeine and administered orally. The labelled methyl group then undergoes demethylation in the liver microsomes with subsequent oxidation to carbon dioxide which is then exhaled through the lungs. The rate limiting step in this process is, the activity of the enzyme cytochrome P450 1A2 (CYP1A2) [7].

We carried out a pilot study using the caffeine breath test on 17 Uganda children aged between 3 and 10 years with kwashiorkor and tuberculosis. Most of whom also had superimposed infections.

1.2 BACKGROUND INFORMATION OF UGANDA

1.2.1 Geography

The Republic of Uganda is located in East Africa and lies astride the Equator, more than 2,000 km from the nearest ocean (Indian Ocean) [9,10]. This landlocked country lies between Kenya and Zaire to the east and west respectively, Sudan to the north, and Tanzania and Rwanda form the southern boundary. The international boundary of Uganda encloses a total of 241,038 sq km, one sixth of which consist of lakes, rivers and marshes. Lake Victoria, the third largest lake in the world, makes up most of the open water area and it is shared by Kenya and Tanzania [10].

Uganda is predominantly an elevated basin, averaging 1000 to 1300 meters above the sea level. The high altitude contributes immensely to its favourable climate [9]. Temperatures ranges between 17°C and 26°C [10]. Annual rainfall varies considerably by region, the highest levels averaging over 2,000mm are found in the fertile crescent along Lake Victoria (central, west and southwest). The rains are received in the months of March through to May (heavy rains) and light rains between September and December. In contrast, Karamoja which lies to the north east receives the lowest amount of rain averaging 500 mm annually [9,10]. Due to the combination of climatic conditions, Uganda has tropical rain forest vegetation in the south and savanna woodland and semi-desert vegetation in the north. The regional agricultural potential and the lands population carrying capacity is greatly influenced by the climatic condition [10].

1.2.2 History

Uganda is composed of many tribal groupings of Bantus, Nilotes and Nilo-Hamites and those of Sudanese origin. Uganda was basically fragmented into kingdoms or homogenous tribal groupings before Independence. They spoke various languages and had unique cultural identities. However, Luganda is the single most widely spoken language followed by Swahili and English. English is the official language of the country [10].

Uganda gained Independence from British rule in 1962. After sovereignty it became a member of the Commonwealth, United Nations, the Organisation

of African Unity, the African Caribbean Pacific States and Preferential Trade Area.

At present Uganda is divided into 43 districts for ease of adminstration and which do not necessarily represent tribal groups [10].

1.2.3 Economy

Uganda has an agricultural economy with 90% of the population dependant on agriculture and agro-based industries. Agriculture produces 98% of Uganda's exports and the country is basically self-sufficient in food. From Independence to 1970 the country had an expanding economy with Gross Domestic Product (GPD) of 4-5% per annum, compared to a population growth rate of 2-6% per annum [9,10].

However, during the past 25 years, the country has experienced a period of civil and military unrest with resultant destruction of social infra-structure and gross upset of economy. This has left a major scar on the economy, educational and health situation of the general population. In 1986 inflation was around 260% per year. Since 1986, the Museveni government of National Resistant has created an enabling environment for economic recovery [9,10]. Table 1.1 below represents some basic socioeconomic indicators [10].

Figure 1: **Map of the Republic of Uganda showing international boundaries, position in Africa and administrative boundaries**

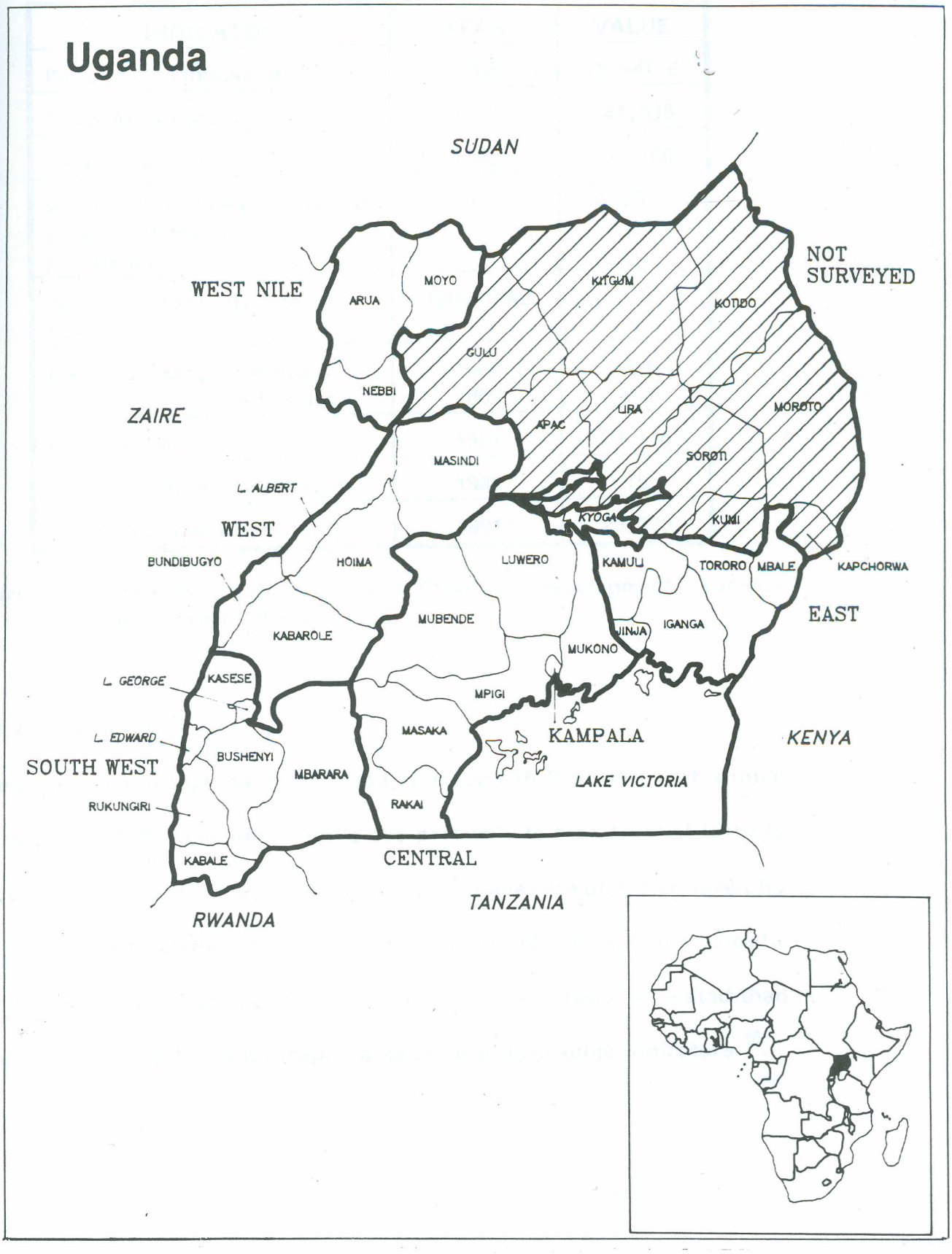

Table 1.1 Basic Socio-economic indicators in Uganda, various years

INDICATOR	YEAR	VALUE
Population (thousands)	1988	15,947.8
Total Area (sq.km.)	1988	241,038
Land Area (sq.km.)	1988	197,100
Women of childbearing age as percent of the total population	1985	23
Population growth rate (year)	1969-1980	2.8
Life expectancy - males - females	1969 1969	45.6 46.9
Hospital beds	1981	20,136
Beds per 10,000 population	1981	15
Population per physician	1981	23,000

Source: Ministry of Planning and Economic Development, various Development Plans

1.2.4 Demography

The population of Uganda is estimated at around 16.9 million, with annual increment of 2.8%. The high rate is due primarily to high level of fertility prevailing in the country, each woman has an average of 7.3 children by the end of her obstetric career. As a result of the varying climatic condition mentioned above, certain areas are more densely populated than others [9,10]. Table 1.2 below represents some demographic indicators [10].

Table 1.2 Demographic Indices, Uganda

INDEX	CENSUS YEAR			
	1948	1959	1969	1980
Population	4,917,555	6,449,558	9,456,466	12,636,179
Intercensal growth	-	2.5	3.2	2.8
Sex ratio	100.0	100.8	101.8	98.2
Crude Birth Rate	42	44	50	50
Total Fertility Rate	5.9	5.9	7.1	7.4
Crude Death Rate	25	20	19	20
Infant Mortality Rate	200	160	120	115
Percent Urban	-	4.8	7.8	8.7
Density (population/km)	25.2	33.2	48.4	64.1

Sources: Statistics Department, Ministry of Planning and Economic Development, Entebbe.

1.2.5 Health

Health services are provided by the Ministry of Health, the Ministry of Local Government and NGOs', particularly the religious groups. The government is responsible for planning and developing health policies and for providing health in all government hospitals as well as health care delivery. The NGOs' provide services both to hospitals and smaller units[10].

During the periods of civil unrest, preventive public health services such as immunization and provision of portable drinking water were disrupted leaving huge population susceptible to controllable diseases and epidemics[12]. Uganda's prospect for rebuilding the health services, begun with immunization, control of diarrhoeal diseases, nutritional surveillance and essential drugs programmes [13].

In 1990 a community based investigation (survey) was conducted in Kampala under the hospice of Uganda EPI programme. The study revealed that BCG immunization coverage was 85%, whereas measles immunization coverage was a moderate 48% [14]. Some studies in Uganda have also reported an upsurge in the number of new TB cases reported. The TB excess is a common trend associated with the AIDS epidemic. It is also a worldwide trend [11,66].

The Government of Uganda has underscored the need to gradually shift away from costly curative services to less costly and effective community based preventive services. The government also puts emphasis on the need for self-sustaining cost effectiveness, with particular stress on maternal and child health, environmental sanitation, provision of essential drugs through the essential drug programme to various outreach centres, water supply, health education and improvement of immunization services through EPI, as has already been mentioned [10,13]. The main goal is to extend health coverage to all citizens by the turn of the century [10].

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

PEM has been underlined as a major nutritional problem in almost all developing countries and coincides with many intercurrent illnesses as a result of diminished host defence against infections [1,2,17]. The lungs are the commonest site of infection and the pathogens involved are predominantly Gram negative enteric organisms [15,67]. It has also been recognised that concomitant existence of malnutrition and infection accounts for a sizeable amount of childhood mortality and morbidity [2]. Septicaemia, gastroenteritis, pneumonia and disseminated tuberculosis account for overall case fatality [15].

Mismanagement of infant feeding, abandonment of the breast for bottle feeding, the use of inappropriate, contaminated weaning foods, poor environmental sanitation and placing too much reliance on antibiotics/anti-diarrhoeals instead of oral rehydration and electrolyte replacements are some of the key problems involved in the aetiology of malnutrition [2]. As a result of malnutrition and its consequences the need of these affected children for appropriate therapies is recognised, and further to this, these therapies should be based on scientific principles rather than on empiricism [15,16,17].

Drugs are mostly lipid soluble, weak organic acids or bases of low molecular weight. These properties facilitate the process of absorption. In blood, part of the drug is bound to plasma proteins and part remains free. Bound drug is unavailable both for tissue action and biotransformation. The process of biotransformation generally converts the drugs into compounds which are inert and can easily be eliminated [18].

The metabolism of drugs by a group of enzymes with wide substrate specificity is located in the endoplasmic reticulum of the liver and other organs [4,19]. Most of these drugs undergo a series of reactions. For instance, Phase 1 reactions involves oxidation, reduction, hydrolysis and hydration. The reactions takes place principally in the endoplasmic reticulum of the liver cells. The cytochrome P450-dependent mixed function mono-oxygenase system is quantitatively the most important enzyme system in these reactions. On the other hand, Phase 2 reactions involves conjugation of drugs or their phase 1 reactants to produce polar metabolites which are usually therapeutically inactive and easily excreted[6,19,20].

2.2 PROTEIN ENERGY MALNUTRITION (PEM) AND DRUG METABOLISM

Krishnaswamy et al in his review of the literature demonstrated that nutritional deficiencies, particularly PEM result in various physiological and pathological changes that are pertinent to drug kinetics, disposition and metabolism [4]. Drug metabolism in the malnourished is likely to be

altered in children with malnutrition especially overt malnutrition such as kwashiorkor, as a result of delayed absorption, reduced protein binding, changes in volume of distribution, impaired liver function and decreased renal clearance [4,21].

Protein binding affects the plasma equilibrium between bound and the unbound drug [21,22]. Hypoproteinaemia results in high concentration of the unbound drug with increased risk of the subject suffering from the toxic effects [22].

Protein binding occurs in the plasma. Equilibrium between bound and unbound affects distribution which occurs by diffusion of free drug into the tissue. Distribution of drugs occurs within the compartment which essentially includes the blood and urine. In humans, studies have been generally limited to blood and urine. To study pharmacokinetics one has to analyze the contents of accessible fluids in order to make deductions regarding the amount of drug in non-accessible regions of the body [22]. The caffeine breath test is convenient to perform pharmacokinetic studies with, as it is a noninvasive procedure [7,8].

Studies have revealed a significant reduction in total plasma protein and albumin in malnourished children particularly the kwashiorkor group. However, dietary rehabilitation replete plasma triglycerides and plasma proteins, which is accompanied by regression of the liver size [1,2,3].

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PEM is known to produce stress in organisms affecting all systems [24]. Cortisol and growth hormone have been implicated in some of these activities [21,24]. Kwashiorkor and marasmic children showed positive correlation between cortisol and aspartate transaminase (AST), alanine aminotransferase (ALT), and calcium transport (Ca (T)) and a negative correlation between cortisol and alkaline phosphatase (ALP). However, in kwashiorkor children the correlation is to a less degree. Furthermore there is a positive correlation between growth hormone and ALP in kwashiorkor. Cortisol stimulates transaminases directly, suppresses ALP activity and indirectly increasing Ca(T) [24].

In another study, it has been demonstrated that worsening of oedematous malnutrition is associated with concomitant deterioration of liver function with subsequent increase in AST, ALT and ALP. Similarly, there is an increase in leukotrienes synthesis in children suffering from kwashiorkor, the action of which includes production of oedema [25].

The study of liver specimens obtained immediately after death of malnourished children reveals gross alteration in various organelles, diminished glycogen stores and fatty infiltration of the liver parenchyma. On the other hand biopsy from children who had recovered from malnutrition showed good cellular organisation and normal frequency of peroxisomes. This findings explains the liver's poor drug handling capability in children with kwashiorkor [26].

Cytochrome P450 represents a major metabolic frontier between the environment and the body. Inter person differences in Cytochrome P450 profiles have been noted in some studies, which explains the reason for requirement of heterogenous dosing of some individual drugs. Patients most likely to develop idiosyncratic toxicity once identified can benefit from individualisation of dosing [27]. PEM, particularly kwashiorkor, is known to result in depressed enzyme activity as well as low serum protein levels especially albumin [4,23]. These two factors may result in accumulation of various drugs with subsequent increase in their toxicity [4].

Pond et al in their study of children suffering from kwashiorkor like syndrome found that there was a significant decrease in serum albumin. They also further detected that short term, severe, protein malnutrition affected lipid, electrolytes and structural mineral metabolism and indices of liver function in absence of parasites, diarrhoea and infection [28]. A high protein energy dense diet reversed the alteration and resulted in improvement in serum albumin level [21,29].

Measurement of serum albumin has proved of great importance in assessing the response of children suffering from oedematous malnutrition to dietary rehabilitation [29]. Low albumin level also contributes significantly to increased concentration of unbound drugs in plasma with ultimate predisposition to toxic effect of these drugs [4,19,22]. As mentioned earlier albumin maybe useful in assessing the degree of liver impairment [19,29].

Ashton et al undertook a study amongst Ethiopian children suffering from malnutrition, in an attempt to elucidate clearance of chloramphenicol monosuccinate and revealed that there was decreased plasma clearance in severely malnourished children with kwashiorkor. In comparison to underweight and marasmic children, kwashiorkor subjects exhibited an increase in the AUC of chloramphenicol [31]. However, it was noted that bioavailability of chloramphenicol of both marasmic and kwashiorkor children lay between 30% and 40% [4,31]. From the study it is clearly evident that malnutrition affects absorption as well as metabolism of chloramphenicol [31].

A study carried out by Ashton (1993) et al looking into how malnourished Ethiopian children handle salicylic acid after a single oral dose, demonstrated a larger Area Under Bound (AUC) plasma concentration curve. They additionally showed that fractional excretion of salicylic was lower in children with kwashiorkor compared to control individuals, which exemplifies lower hepatocellular metabolic activity in children with oedematous malnutrition. This second study was in agreement with the previous study done by the same investigator using chloramphenicol [32] and confirms other studies [6,31].

Abdel et al in their study postulated that cytokines cause depression of cytochrome P-450 associated with drug metabolism in humans during inflammation and infection. Interleukin-1beta, interleukin-6, and tumour necrosis factor-alpha were found to be the most potent depressors of mixed

function oxidase enzymes. CYP1A2 and CYP2E1 mRNA levels and enzyme activities were depressed by interferon-gamma. This suggests that in sub-saharan Africa with many infections, infestations and concomitant existence of malnutrition, different regulatory mechanism may be involved in drug metabolism [33].

In the part of Uganda where this study was done, most young children are fed on a traditional diet based on plantain (matoke) which is low in protein and typical cases of kwashiorkor are frequent [1,21]. Other studies done elsewhere have implicated aflatoxin as a possible cause of kwashiorkor [21,34].

2.3 ANTI-TUBERCULOSIS TREATMENT AND DRUG INTERACTION

Tuberculosis is a major problem in Mulago Hospital, partly due to breakdown in health infrastructure as a result of repeated civil unrest and partly due to the HIV pandemic [11,13,14]. From 1985 to 1989 the number of tuberculosis patients attending Mulago Hospital doubled. The prevalence of TB among hospital admission also increased from 3% in 1987 to 3.7% in 1989. Most of these patients presented with pulmonary tuberculosis and were within the age group 14 to 44 years [14]. Extrapulmonary disease was most common in the childhood age group 0 to 14 years. This age group accounted for over 50% of all extrapulmonary forms of tuberculosis [11,13,14]. As a result of this, there is an increased use of anti-tuberculous drugs.

2.3.1 Rifampicin

Of the first line drugs used to treat tuberculosis, i.e. rifampicin, isoniazid and pyrazinamide, rifampicin is particularly likely to cause clinically significant drug interactions as it is a potent inducer of many drugs, in particular oral contraceptives, corticosteroids, oral anti-coagulants and cyclosporin [35,36]. The use of quinolones to treat multiple drug resistant tuberculosis, and AIDS related Mycobacterium avium-intracellular complex is not without limitation, as in contrast to rifampicin, these drugs inhibit some cytochrome isoenzymes, leading to reduced metabolism of certain drugs [7].

In humans, rifampicin is extensively metabolized and is known to induce its own metabolism [37]. Several studies have also indicated that, rifampicin specifically induces cytochrome P450IIIA subfamily and does not seem to affect the cytochrome P450IA subfamily [37,38]. Similarly, diphenylhydantion, rifampicin and barbiturates induce enzymes responsible for degradation of oestrogens. The major target is the hepatic microsomal oestrogen-2-hydroxlase (cytochrome P450IIIA4) [36].

Rifampicin induces cytochrome P450IIIA and alters blood concentration of drugs which are metabolized by this enzyme. Cyclosporin is metabolized almost completely in the liver, the rate limiting step in this process is the activity of cytochrome P450IIIA. It is necessary to detect such interaction in order to allow adjustment of drug dosage and subsequently reduce

toxicity and enhance therapeutic effects, in particular patients administered many drugs known to have pharmacokinetic interaction [39].

2.3.2 Isoniazid

Isoniazid, an anti-tuberculous hydrazine drug undergoes acetylation within the endoplasmic reticulum (ER) of the human microsomes. Individuals are phenotyped as slow acetylator homozygous for slow acetylator gene, or rapid acetylator homozygous or heterozygous for rapid acetylator gene. Acetylator status can be determined through use of caffeine, isoniazid, sulfamethazine, sulfapyridine. However, caffeine has proved useful in distinguishing between heterozygous and homozygous rapid acetylators and also identifying slow acetylators who are apparently more prone to toxic effects of drugs as a result of accumulation [40].

Like rifampicin, Isoniazid is an inducer of a specific cytochrome P450 isoenzyme [41,42,43,44]. Isoniazid induces cytochrome P450 IIE1 [45,46]. A study has shown that isoniazid induces erythromycin N-demethylase activity which is a cytochrome P450 IIE isozyme [46]. Other studies have also demonstrated isoniazid as an inducer of cytochrome P450 isoenzyme responsible for biotransformation of organohalogen anaesthetics. Thus, isoniazid enhances oxidative biotransformation of the anaesthetics [44].

Hepatotoxicity of anti-tuberculous therapy with isoniazid and rifampicin has been found to be high in malnourished children. AUC of isoniazid is

significantly high in malnourished children [18]. There is need to modify therapy appropriately to achieve therapeutic response and avoid toxicity [18,40]. The liver is involved extensively in the metabolism of endogenous and exogenous substances, and is the main organ responsible for drug metabolism. Thus, patients with liver disease may not metabolize drugs normally and are at special risk of drug side effect, especially from the above anti-tuberculosis drugs [19].

2.3.3 Pyrazinamide

Pyrazinamide (PZA) has become an essential component of the current six month regimen for therapy of tuberculosis. Susceptible strains of tuberculous bacilli converts PZA into pyrazinoic acid (POA) through pyrazinamidase (PZase), which resistant strains like mycobacterium bovis Bacilli Calmette Guerin lack [47].

Pyrazinamide in combination with rifampicin, isoniazid, and streptomycin when prescribed to newly diagnosed cavitating tuberculosis accelerates recovery by excluding the risk of hepatotoxicity. It is recommended for patients with rapid acetylation phenotype [48].

Patients with impaired hepatic function show marked reduction in pyrazinamide clearance and consequent increase in half life. The area under the curve (AUC) of pyrazinoic acid (main metabolite) is increased [49]. This has an inhibitory effect in elimination of uric acid, hence there is a

danger of toxic accumulation of uric acid. Pyrazinamide ingestion may result into hyperuriceamia [49,50].

2.4 $^{13}C/^{14}C$ TRACER/CAFFEINE AND XANTHINE DERIVATIVES

The ^{13}C isotope has been used in human nutrition studies as a tracer of choice due to its nonradioactive nature and broad applicability to all population [51,69]. Bunn et al utilised ^{13}C urea breath test to establish the incidence of Helicobactor pylori infection amongst Gambian infants. In this study ^{13}C urea in polycose was given orally and labelled CO_2 harnessed and stored in vacutainer pending analysis. The procedure is non-invasive [52].

Some studies have utilised ^{14}C as an isotopic tracer. Kruger et al and their study of significance of dioxin in paediatrics used the $^{14}CO_2$ breath test. The apparent enzyme induction of monooxygenase activity by 2, 3, 7, 8-tetrachlorodibenzo-P-dioxin (TCDD) of metabolic conversion of [3-methyl ^{14}C]-caffeine was shown to be dose dependent. The study further revealed that caffeine is a convenient substrate for studying monooxygenase activity and consequently liver function [53]. In another study Kruger et al while using ^{14}C caffeine, demonstrated the suitability of the caffeine breath test in assessing development of different monoxygenase in vivo using the animal model. The studies may well be applicable for studies in children to monitor effects of environmental pollution [54].

Theophylline a xanthine derivative has been used for prophylactic and acute treatment of asthmatic attack. When administered therapeutically, the blood level should be maintained between 10 and 20 mg litre^{-1}. However, the main disadvantage of xanthine derivatives is that the margin between therapeutic and toxic level is small and blood level over 20 mg litre^{-1} can easily tip the subject into experiencing toxicity of these drugs [2,7]. Despite the mentioned disadvantage theophylline and caffeine are used in several metabolic studies [7,8].

Fuhr et al, whilst studying the effect of grape fruit juice and naringenin and activity of the human cytochrome P450 1A2, used caffeine as probe drug or probe substitute. In the study both drugs inhibited metabolism of caffeine, however, not to a significant level [54]. Tanaka et al also made use of caffeine and trimethadione as probe drugs in their pharmacokinetic studies of drug interaction and in the investigation of the mixed function isozyme profile [57]. Theophylline is another methyl-xanthine derivative used as a probe in the study of microsomal metabolism [55].

Parker et al, while studying drug disposition in children suffering from cystic fibrosis infected or susceptible to Pseudomonas aerogenosa, utilised radio-labelled caffeine as a probe drug. The children were taking ciprofloxacillin a 4-quinolone antibiotic, which is universally known to be effective in Pseudomonas infection. Ciprofloxacin like other 4-quinolones is known to have an inhibitory effect on methyl xanthine derivatives [7].

The labelled methyl branch of ^{13}C-[N-3-methyl]-caffeine undergoes 3-N-demethylation which is dependent on cytochrome P450 (CYP 1A2). After N-demethylation the methyl group then enters one carbon pool where it is converted to formaldehyde, formate, bicarbonate and then exhaled as carbon dioxide through the lungs. There is a demonstrable significant drug interaction when caffeine is given concomitantly with ciprofloxacin [7]. Inhibitory effects of ciprofloxacin on caffeine metabolism and other quinolone derivatives has been documented [58,59]. Caffeine has proved a reliable probe drug in many studies, moreover its products of metabolism can be harnessed as gas and does not require invasive procedure, hence it is a favourable tool for study of drug metabolism in children [6,7,8].

Caffeine degradation is impaired in patients with liver cirrhosis and is greatly enhanced in patients on phenytoin therapy. Caffeine, unlike theobromine and theophylline, is degraded by the paraxanthine pathway [60]. Similarly omeprazole has been shown to induce cytochrome P4501A2 activity both in vitro and in vivo. This was evident when the caffeine breath test was performed before and after treatment with omeprazole in both poor and extensive metabolizers. The results showed a significant correlation. Omeprazole, like phenytoin induces CYP1A2 activity [61].

Measurement of the excretion of metabolites of caffeine, provides a noninvasive means of assessing enzyme induction or inhibition [6]. CYP1A2 is an inducible cytochrome P450 isozyme and has been used in some studies

to assess N-hydroxylation of numerous aromatic amines to mutagens and carcinogens CYP1A2 activity is also a useful monitor of liver damage [62].

The human cytochrome P450 1A2 isozyme mediates 3N demethylation of caffeine [7,8]. This supports the hypothesis that plasma clearance of caffeine is a specific in-vivo probe for determining human cytochrome P450 1A2 activity and ultimately liver microsomal function. Other 4-quinolone antibiotics agents such as perfloxacin are known to inhibit caffeine metabolism in-vivo and in human liver microsomes [7].

Caffeine has been utilised in many studies as a probe drug with good results, however, the caffeine breath test has been tried in few centres with successful outcome [7,8,61]. In a Zimbabwe study, children were given coca-cola, a caffeinated soft drink, and 24 hour urine was then collected in an attempt to measure the level of metabolites. The study concluded that there is a significant reduction in caffeine metabolism amongst African children. However, the experiment was rather crude and the level of caffeine was not measured accurately. But, in spite of this, the result is still valid and partly supports the idea of confounding factor which influences the outcome [62]. Another study also proved that caffeine can be used to establish acetylator status after oral adminstration of the probe followed by measurement of the level of caffeine metabolites [40].

CHAPTER 3

OBJECTIVES AND METHODS

3.1 OBJECTIVES

1. To determine the applicability of the caffeine breath test in assessing liver function in children.
2. To investigate the effect of oedematous malnutrition on drug metabolism.
3. To investigate the effect of anti-tuberculous drugs on bio-transformation of caffeine in children taking anti-tuberculous treatment.

3.2 SAMPLE SIZE DETERMINATION

The sample size (n) was estimated in reference to a study carried out by Parker et al in children with cystic fibrosis. The following formula was employed:

$$n = \frac{(u + v)^2 (s_1^2 + s_2^2)}{(x_1 - x_2)}$$

$x_1 - x_2$ - Difference between means

s_1, s_2 - Standard deviations

u. Two sided percentage point of the normal distribution corresponding to 100% - the power; e.g. if the power = 90%, **u** =1.64

v. Percentage point of normal distribution corresponding to the two sided significance level = 5%, **v** = 2.45

From the study undertaken by Parker et al;

$x_1 = 9.37$ $x_2 = 4.17$

$s_1 = 2.60$ $s_2 = 1.33$

When the above are substituted in the equation then n = 5.2

In the study 8 malnourished children and 9 children on ant-TB treatment were recruited[63] as considered adequate numbers for the study.

3.3 METHODS

3.3.1 Plan of study

The study was carried out between the months of July and August of 1995 at Mulago Teaching Hospital. Informed consent was obtained from the parents and the study was approved by the local ethics committee and Makerere Postgraduates Research Committee. Two groups of patients were studied (aged between 3-10 years) before, commencing anti-tuberculous treatment or nutritional rehabilitation. A follow up study was performed 10 to 14 days thereafter in both groups. The patients were recruited from the outpatient clinics, from various paediatric wards and Mwanamugimu Nutrition Rehabilitation Unit. TB cases were recruited from children attending either the chest clinic or TB outpatient clinic at Mulago hospital.

3.3.2 Patient selection

3.3.2.1 Kwashiokor (Oedematous malnutrition)

Malnutrition was defined according to Wellcome Classification. The other variables taken into consideration during selection of these cases are: history of prolonged illness, anorexia, prolonged recurrent diarrhoea amongst other details indicated in Tables 3.1 and 3.2. More detailed criteria is outlined in the questionnaire in Appendix 1.

Table 3.1 Wellcome Classification

	OEDEMA	
WEIGHT % OF STANDARD *	**PRESENT**	**ABSENT**
60 - 80	Kwashiorkor	Underweight
< 60	Marasmic-kwashiorkor	Marasmus

* 50th percentile weight for age

Table 3.2 Relationship between S.D, Centile, % Weight/Age and Gomez Classification

STANDARD DEVIATION	CENTILE	% WT/AGE	GOMEZ
0	50	100	Normal
	25	90	
-1	10	85	Mild
-2	3	80	
-3		75	Moderate
-4		< 60	Severe

3.3.2.1.1 Selection

3.3.2.1.1 Selection

Seven children with kwashiorkor and one child with marasmic-kwashiorkor were enroled in the study. The following details were recorded from each individual patient; age, height, weight, and detailed drug history. The children were rehabilitated on dried skimmed milk, cotton seed oil and micronutrients "***DISCO***" until oedema subsided.

DISCO 150 is a term coined after the initial letters of the main ingredients used to prepare the diet for severely malnourished children.

Recipe for the diet:

Dried skimmed milk powder	78.5g
Sugar	47.3g
Cotton seed oil	59.3g
Potassium chloride	1.0g
Magnesium hydroxide	0.5g
Total	186.6g

The quantities were weighed carefully using clean scales and clean utensils. The milk powder and sugar were mixed first with potassium chloride and magnesium hydroxide. The oil was then added gradually and the whole mixture thoroughly stirred. 186.6g of DISCO is reconstituted into one litre with boiled and cooled water. During reconstitution water is added gradually, until a mixture of smooth consistency results.

Calculating dietary requirement of a child under nutritional rehabilitation

The requirement is calculated according to weight.

For catchup growth the children are started on 120 to 200 kcal kg^{-1} day^{-1}.

Thus, a child of 7.2 kg will require;

150 x 7.2 = 1080 kcal day^{-1}

1 ml of mixture contains 1 kcal

Kwashiorkor children are given 100 kcal kg^{-1} day^{-1} initially.

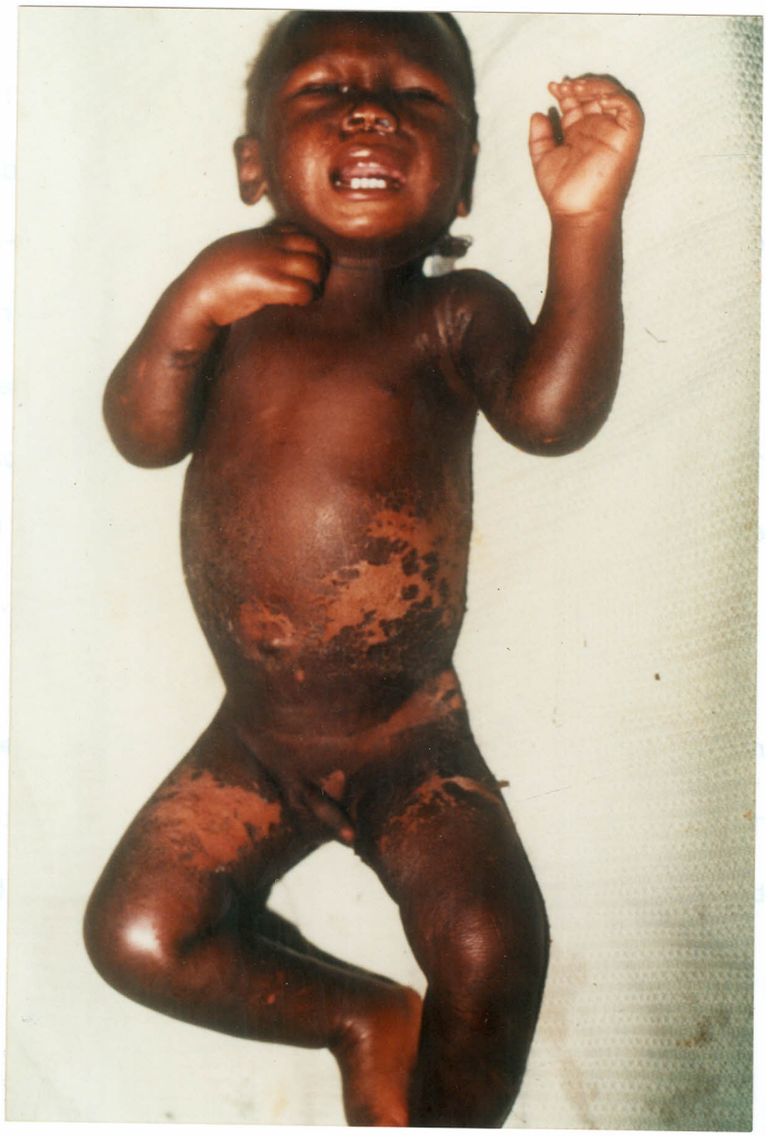
The diet is given 5 to 7 times each day.

3.3.2.1.2 Assessing improvement on nutritional rehabilitation

- The children were weighed daily before morning feeds and recorded.
- The children were also assessed clinically for disappearance of oedema.
- Improvement in skin lesion was also assessed.
- Changes in mental status was also assessed.

On attaining the required catchup growth the children were introduced to a less refined diet made from locally available foodstuffs ***"kitobero"***. This was in preparation for discharge and subsequent domiciliary care. The severity of the malnutrition was measured with the degree of the oedema, apathy, dermatoses (skin changes), and serum albumin level. Other medication besides DISCO received included, ferrous sulphate ($FeSO_4$), anti-helmenthics,

Figure 3.1: Kwashiorkor child with typical skin lesions (dyspigmentation and exfoliation) together with generalised oedema and cheilosis



folic acid, vitamin A and anti-malarials for those children with overt malaria. Severely anaemic patients were transfused carefully with packed cell in order to avoid fluid overload.

3.3.2.2 Selection of TB cases

Nine children aged between 3 - 10 years with various forms of TB (pulmonary and extra-pulmonary) were recruited for the study. All these patients were attending the chest clinic and TB clinic at Mulago Hospital. The diagnosis was based on history of contact, positive Mantoux test, history of chronic cough not amenable to conventional antibiotics or broncho-dilators and suggestive chest X-ray. The clinical details are illustrated in Table 4.3 and Figure 4.3.

The caffeine breath test was performed before commencing the patient on anti-tuberculous drugs and fourteen (14) days thereafter. Other medications received included crystapen penicillin, gentamycin and multivitamin.

3.3.3.1 The caffeine breath test procedure

The patients were trained how to blow into the bag a day prior to the test. All recruited patients abstained from caffeinated products such as tea, coffee or soft drinks containing caffeine for 24 hours and fasted for at least 4 hours before caffeine breath test. Breath tests were performed between 8am and 2pm. The patients' activities were restricted during the test.

Three breath samples were initially taken at an interval of 10 minutes before adminstration of caffeine, subsequent breath samples were taken at an interval of 15 minutes for a period of 2 hours after adminstration of caffeine, i.e; -20, -10, 0, 15, 30, 45, 60, 75, 90, 105, and 120 minutes. The labelled caffeine was given at dose of 3mg kg^{-1} dissolved in distilled water followed by a blackcurrant drink to help blunt the bitter taste. The solution was given orally and the container rinsed.

Breath samples were collected by getting the child to blow through a mouth piece and connector into a mask (Figure 3.2-5). The expired was removed from the collecting unit by means of 20 ml syringe and was transferred into a labelled pre-evacuated headspace analyzer vial.

3.3.3.2 Outline of the procedure

* The QuinTron Alveosampler a disposable Haldane-Priestley tube was used. It permits one patient use of a device to collect an alveolar sample in a standard syringe for transfer into a pre-evacuated headspace vial pending analysis. This also removes the danger of cross-infection and saves time too.
* AlveoSamplar mouthpiece was removed from its protective sealed bag.
* A stopcock was put on the syringe, the stopcock opened and syringe plunger pushed all the way in.

* The stopcock and the syringe were then attached to the AlveoSampler by inserting the male end of the stopcock firmly into the side-hole in the middle of the mouthpiece
* The patient was asked to breath normally and at the end of inspiration the mouthpiece of AlveoSampler is put into the mouth and the patient is allowed to exhale normally into the bag (not too rapidly nor too slowly).
* As the patient exhales the polyethylene bag is filled with the air, which vents through a small hole out of the bag, so exhalation can continue. The first few mls of air in normal exhalation comprises deadspace air. When the polyethylene bag was filled then it was certain that the deadspace air had been flushed out of the airways and the remaining air was coming from the alveolar.
* While the patient continues to exhale through AlveoSampler, and keeps the bag inflated, 20ml alveolar air is withdrawn into the syringe before the patient ceases to exhale. The patient must keep his mouth tightly closed around the mouthpiece until the sample is collected.
* The stopcock is turned off after filling the syringe with the sample. The whole procedure is repeated at an interval of 15 minutes and for a duration of 2 hours. The whole procedure was repeated in 10-14 days when the PEM children had improved and the tuberculous children had been on chemotheraphy for a period.

Figure 3.2: Showing pre-evacuation of the headspace vial in the caffeine breath test procedure

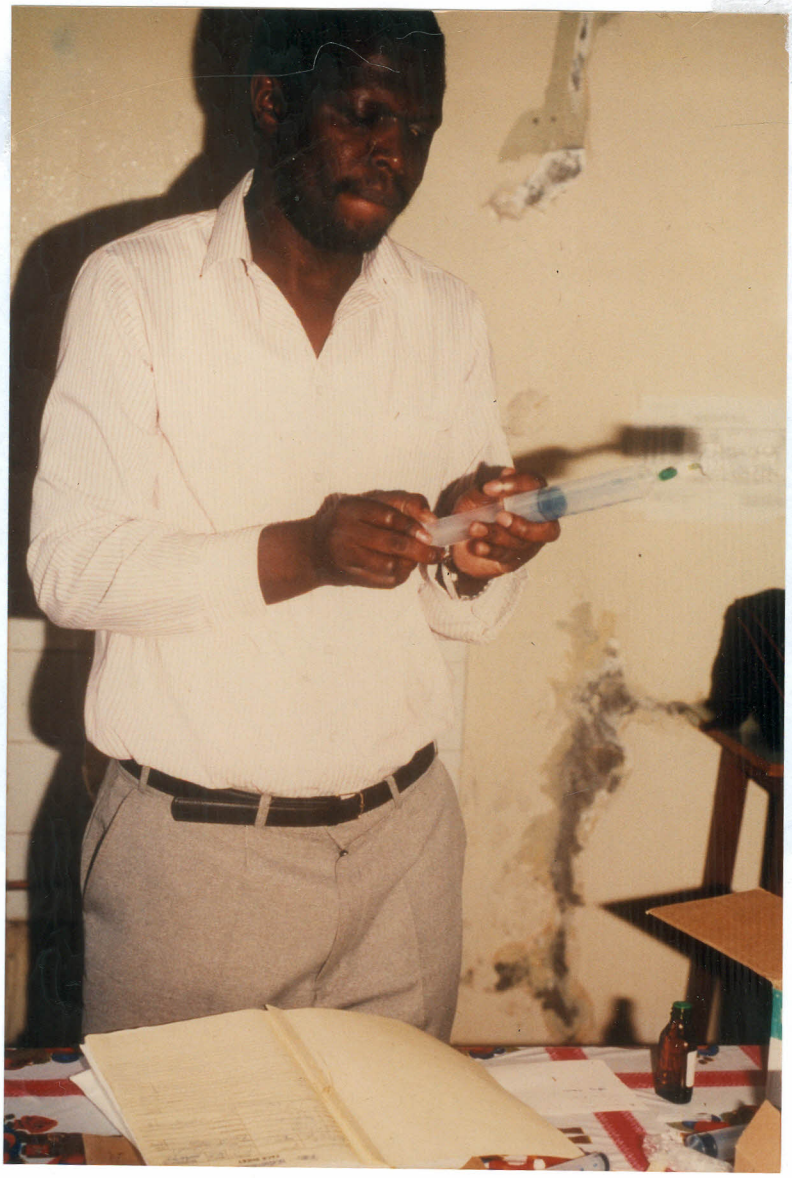


Figure 3.3: Harvesting of the labelled carbon dioxide after administration of labelled caffeine

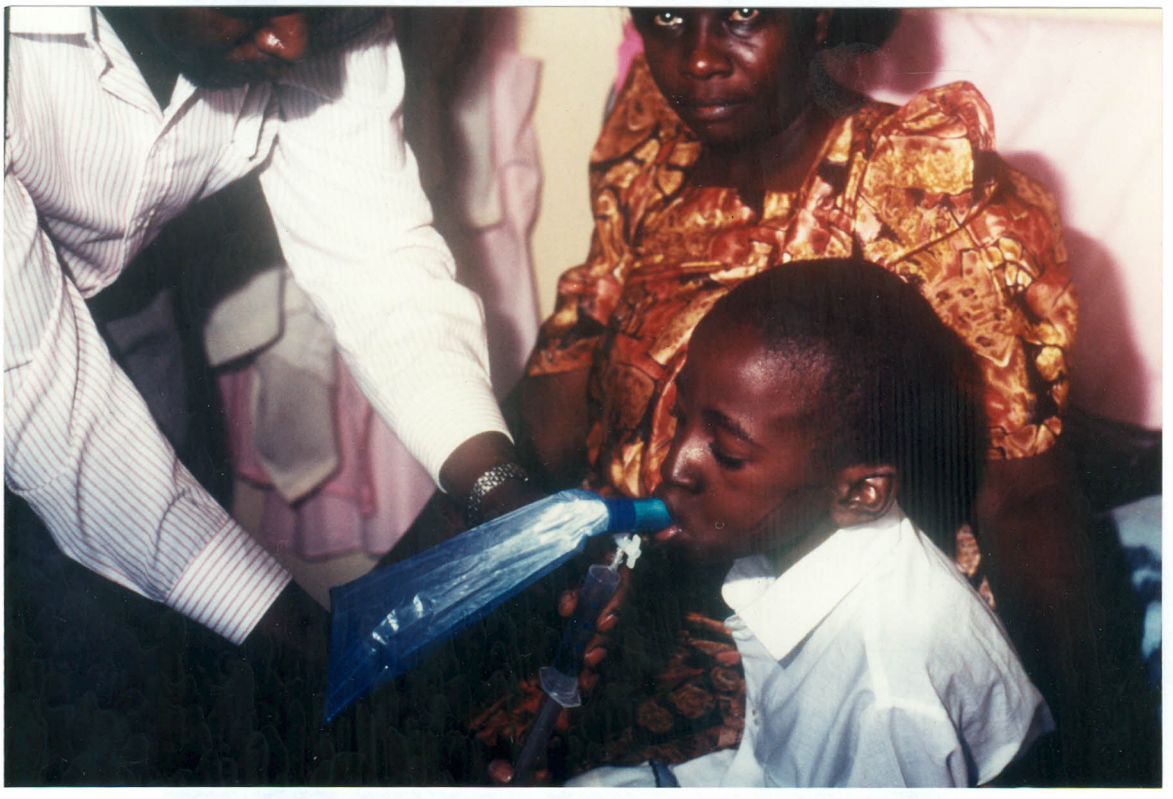


Figure 3.4: Transfering the samples into the headspace vial pending analysis

The samples were then sent to Liverpool for onward mailing to The Scottish Universities Research and Reactor for analysis. See Figure 3.2-3.5 (collecting set and picture of the procedure being performed on one of the patients).

3.3.4 Analytic methods

In human nutrition studies ^{13}C is the isotopic tracer of choice because of its nonradioactive nature and broad applicability to all populations. However, in contrast to ^{14}C, tracer ^{13}C always mixes with pre-existing pool of ^{13}C in the body, and atmosphere, thus tracer measurement involves changes in enrichment baseline. Natural abundance of ^{13}C must therefore be established [7,51].

The ^{13}C enrichment of breath samples undergoes analysis of CO_2 by gas isotopic ratio mass spectrometry (reference as above). Breath samples are injected manually into the on line gas preparation device (Roboprep; Europa Scientific Ltd, Crewe, UK) where they in turn, are dried and resolved from interfering components by gas chromatography and passed, using helium as carrier into the ion source of an isotope ratio mass spectrometer (MM602; VG Isotech, Middlewich, UK). The ion beams m/z 44 and m/z 45 will be monitored constantly and be utilized to calculate partial pressure and ^{13}C enrichment of CO_2. The reference standard must be taken into consideration all the time. Cumulative percentage exhaled labelled CO_2 throughout the 2 hours is recorded [7].

3.3.4.1 Statistical analysis

The results were compared before and after nutritional rehabilitation in kwashiorkor children and in those children suffering from TB, the results were analyzed before and after adminstration of anti-tuberculous drugs, both using student t test. A $p < 0.05$ value is considered significant.

CHAPTER 4

RESULTS

The major problem with the study was that there was a leak from the bottles due to a fault in the cap during manufacture. Altogether, around 1000 bottles were taken to Kampala. In 3 cases the amount of labelled caffeine recovered was < 50%. Samples ≥ 50% were adequate for study. In total, samples were adequate in 2 single tests and 3 duplicate tests in kwashiorkor and 3 single tests and 4 duplicate tests in tuberculosis. Results for patient 152 were not received.

TABLES OF SELECTION CRITERIA:

Table 4.1 Clinical characteristics of kwashiorkor children

STUDY No.	SEX	AGE (yr)	WEIGHT (kgs) (SD)‡	HEIGHT (cm) (centile)	DIAGNOSIS
150*	F	7	14.5 (-3)	106 (3^{rd})	Kwashiorkor
151	F	5	14.3 (-2)	108 (50^{th})	Kwashiorkor
152	M	6	12.2 (-4)	102 (3^{rd})	Marasmic-Kwashiorkor
153	F	3	10.2 (-3)	84 (3^{rd})	Kwashiorkor
154	M	4 3/12	13.1 (-2)	102 (25^{th})	Kwashiorkor
155	M	4	12.3 (-3)	98 (10^{th})	Kwashiorkor
156	M	4 2/12	11.6 (-3)	90 (3^{rd})	Kwashiorkor
157	M	4	8.7 (-4)	98 (10^{th})	Kwashiorkor

* The patient was lost to follow-up

‡ SD below the mean weight for age

KWASHIORKOR

Clinical characteristics

8 kwashiorkor patients were recruited (Table 4.1). The pre-admission weights were found to be below 3rd percentile and below 80% weight for age in accordance with the Wellcome classification. Their ages were between 3 to 7 years, with mean (s.d) of 4.7 (1.2) years. All except one child had moderate anaemia with Hb range mean (s.d) of 6.2 (1) gdl^{-1}. Only one child required blood transfusion. Of the 8 recruited patients, 3 were females and 5 were males.

CAFFEINE BREATH TEST

Table 4.2 Caffeine breath test in kwashiorkor

Patient	Caffeine dose (mg)	Interval between doses (days)	% caffeine dose recovered on admission (2 hr cumulative)	% caffeine dose recovered 10-14 days post nutritional rehabilitation (2hr cumulative)	Increase/ loss
150*	43	*	1.36	Absconded	-
151	43	13	1.36	2.05	+0.69
152	*	*	*	*	*
153	30	14	missing	0.4	-
154	39	14	3.52	4.76	+1.24
155	36	13	*	4.4	-
156	35	14	0.82	*	-
157	26	11	3.69	3.95	+0.26

Mean ± S.D (paired) 2.86 ± 1.3 3.6 ± 1.39

Mean ± S.D. (unpaired) 2.15 ± 1.35 (all initial results)

Out of the 8 patients enroled into the study, patient 150 whose condition was rather grave refused the repeat test and then absconded (Table 4.2). Patient 153 had no result for repeat test. Neither patients 155 nor 156 had any results. Three patients in this group had duplicate results with adequate amount of labelled carbondioxide. The results revealed slight improvement in liver performance following 10-14 days of dietary rehabilitation. Table 4.2 shows the labelled cumulative 2 hour CO_2 output for each of the 3 patients both before and during nutritional rehabilitation, expressed as a percentage of oral caffeine administered. 2.86% (1.3) of the caffeine dose was exhaled as CO_2 in 2 hours nutritional rehabilitation, and 3.59% (1.39) during nutritional rehabilitation. The results revealed low levels of labelled caffeine exhaled as CO_2 [7,62]. Figure 4.2 shows the mean data of the three patients with duplicate results.

The comparison of pharcokinetics between this malnourished child before and after dietary rehabilitation with the results obtained from the study of Parker et al showed a reduction in exhaled caffeine as CO_2 [7].

It was also evident, in spite of the progress made by the children on introduction to dietary rehabilitation, the 2nd test was performed too soon before adequate return to proper nutritional status. All the children studied still had palpable liver and almost all had not attained catchup growth. The other notable problem was the dwindling supply of ingredients used for reconstituting DISCO 150. There was also a problem in initiating

used for reconstituting DISCO 150. There was also a problem in initiating nutritional rehabilitation since these children were too anorexic. As a result, they were vomiting most of the feeds initially.

There was initial decline in weight of these children corresponding to loss of oedema. This decline in weight and subsequent loss of oedema was evidenced by 7^{th} to 10^{th} day during nutritional rehabilitation. Figure 4.1 shows typical initial response to nutritional rehabilitation in two patients, 154 and 155. 154 had a bout of malaria and respiratory infection during the initial stages of dietary management, whereas patient No. 155's response to dietary intervention was uneventful.

TUBERCULOSIS

Table 4.3 Clinical characteristics in children with tuberculosis

STUDYNO.	SEX	AGE (yr)	WEIGHT (kg) (centile)	HEIGHT) (cm) (Centile)	DIAGNOSIS
170*	M	10	21 (3^{rd})	105 (3^{rd})	PTB ?LIP
171	F	9	24.3 (10^{th})	113 (3^{rd})	PTB(EFFUSION)
173	M	7	21.5 (50^{th})	117 (25^{th})	PTB
174	M	10	28.3 (25^{th})	128 (10^{th})	PTB
175	M	5.5	17.4 (25^{th})	106 (10^{th})	PTB/EXT PULMONARY
176	F	6	16.5 (3^{rd})	105 (3^{rd})	PTB
177	F	3	14.6 (50^{th})	96 (50^{th})	EXT/PULMONARY
178	F	4	14.6 (10^{th})	102 (50^{th})	PTB
179	M	6	17 (10^{th})	106 (3^{rd})	PTB

The two children with extra-pulmonary tuberculosis both had TB adenitis.
* The patient got lost to follow-up - Lympocytic interstitial pneumonitis (LIP)

In this group, a total of 9 children, 4 females and 5 males, mean age ± s.d of 6.7 ± 2.5 were recruited into the study. Almost all the children had mild to moderate malnutrition as well as tuberculosis. All the children enroled into the study were commenced on anti-tuberculous regimen comprising Rifampicin (20 mg kg^{-1}), Pyrazinamide (30 mg kg^{-1}), and Isoniazid (20 mg kg^{-1}). Other medications received included under water seal drainage crystalline penicillin and gentamycin for patient No. 171 who had pleural effusion. Patient No. 176 suffered from a bout of pneumonia 4 days prior to the second test and was started on streptomycin and crystalline penicillin in addition to anti-TB chemotherapy.

Table 4.3 shows clinical characteristic on admission. All the patients had a Mantoux test >15mm, 8 patients had chest x-ray suggestive of tuberculosis, high ESR and a history of contact in some cases. A history of chronic cough not amenable to treatment, of loss in weight coupled with a high index of suspicion were used as additional factors for diagnosis (Figure 4.3). Patient No. 170 with diffuse interstitial pneumonitis, was thought to have chronic or recurrent pneumocystis carinii complicating AIDS. He was started on anti-TB, but was lost to follow up.

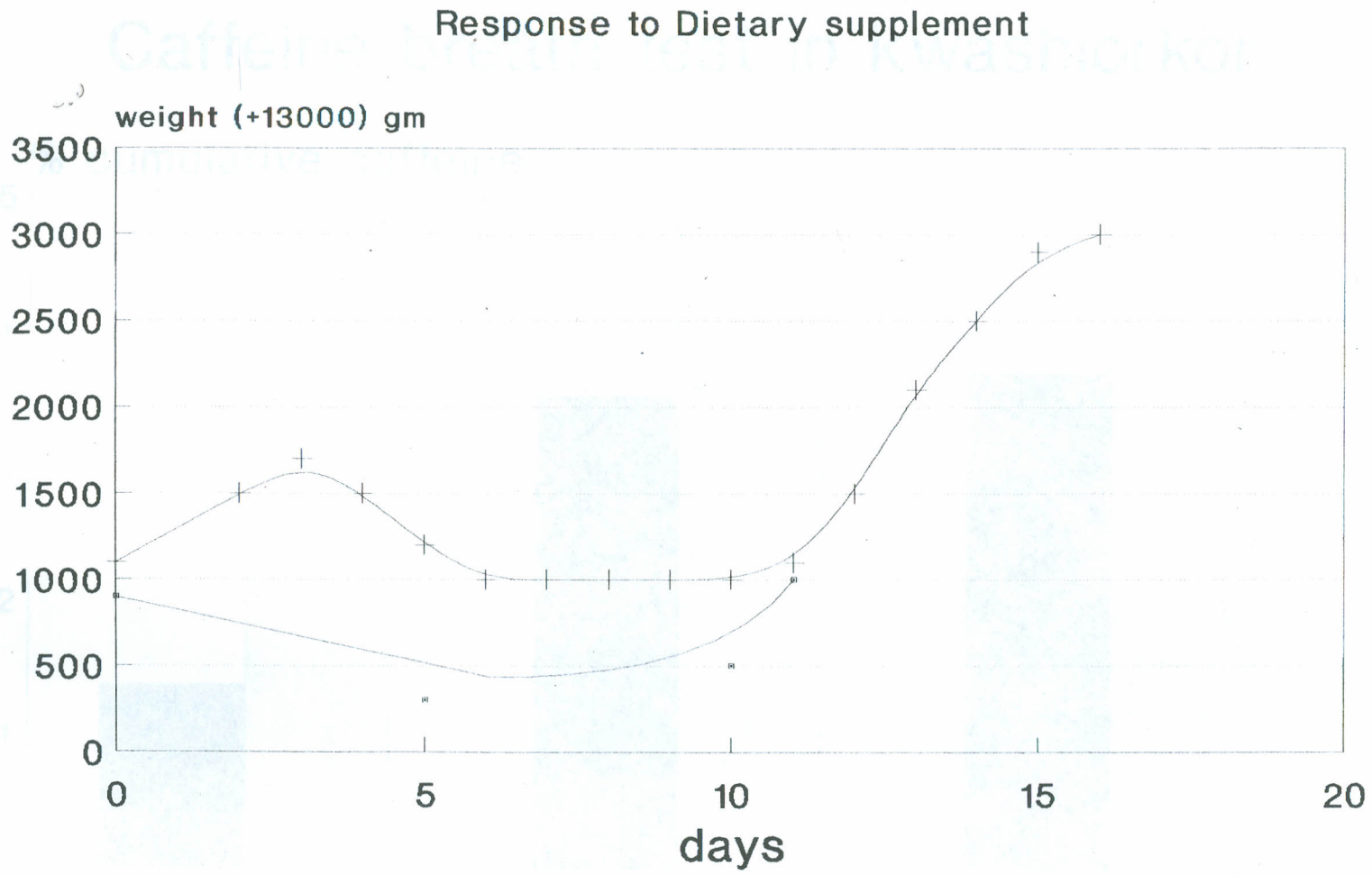

Response to Dietary supplement
weight (+13000) gm
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
days
Pts with kwashiorkor
patient No.155
patient No.154

Figure 4.1

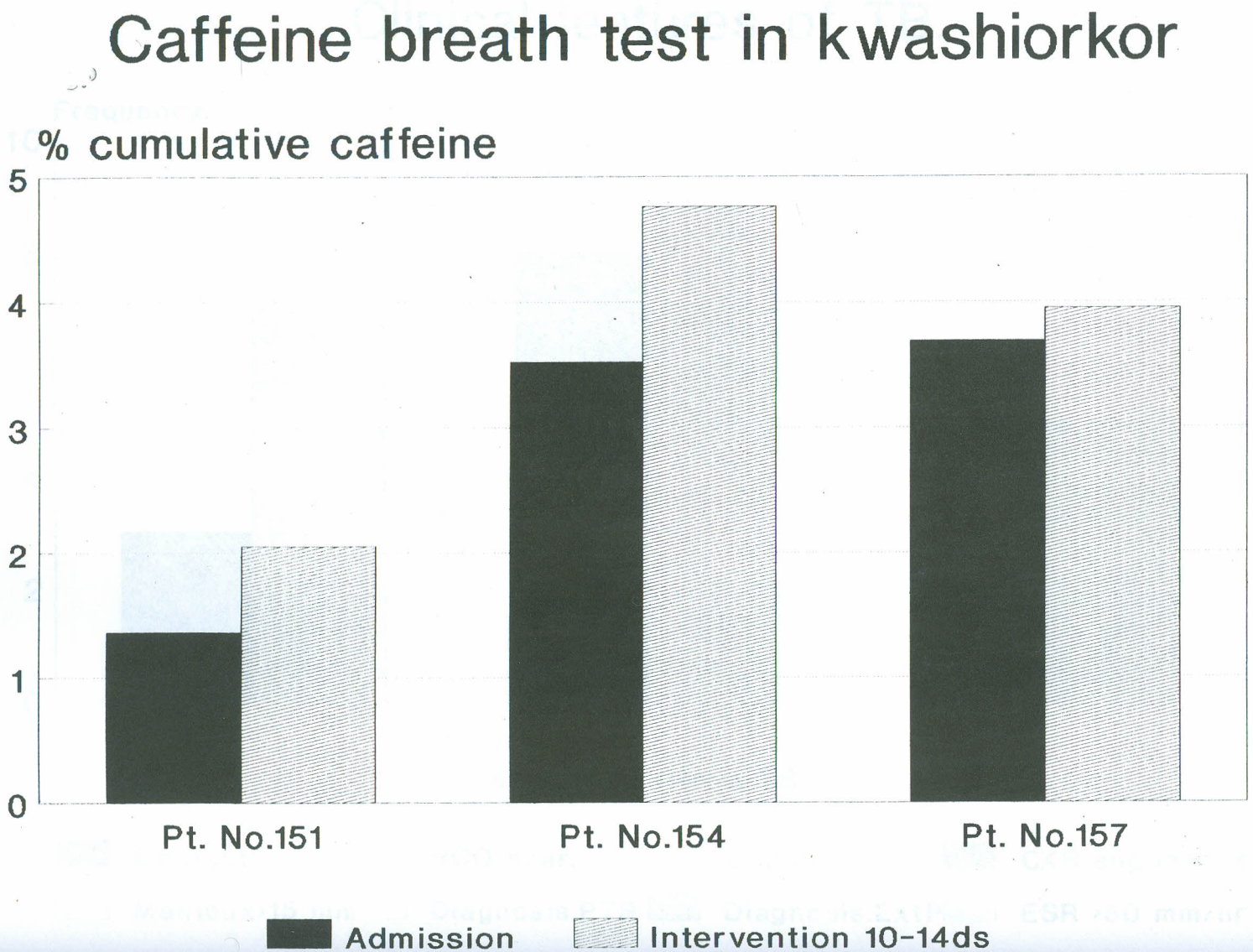

Caffeine breath test in kwashiorkor
% cumulative caffeine
5
4
3
2
1
0
Pt. No.151
Pt. No.154
Pt. No.157
Admission
Intervention 10-14ds

Figure 4.2

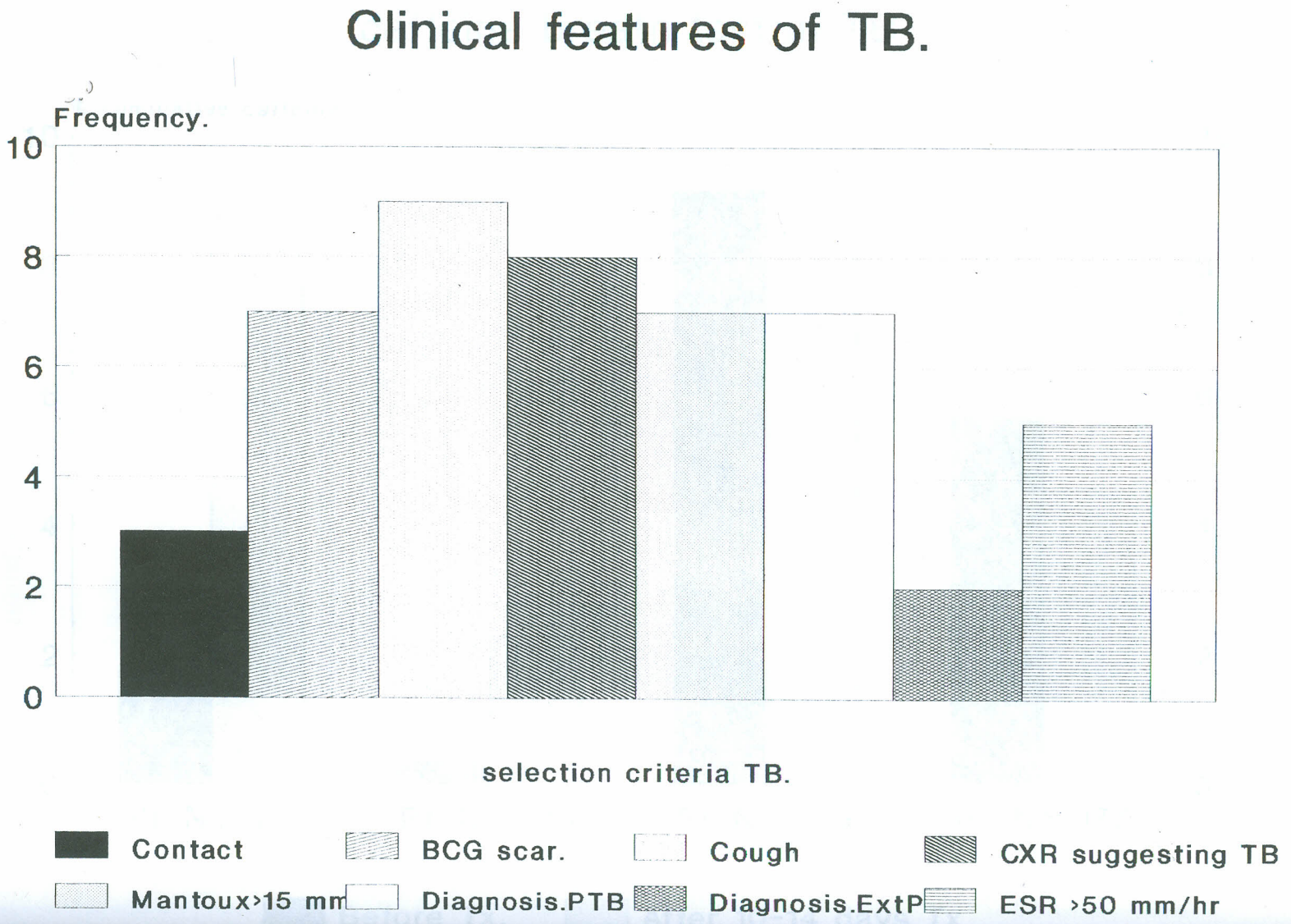

Clinical features of TB.
Frequency.
10
8
6
4
2
0
selection criteria TB.
Contact
BCG scar.
Cough
CXR suggesting TB
Mantoux>15 mm
Diagnosis.PTB
Diagnosis.ExtP
ESR >50 mm/hr

Figure 4.3:

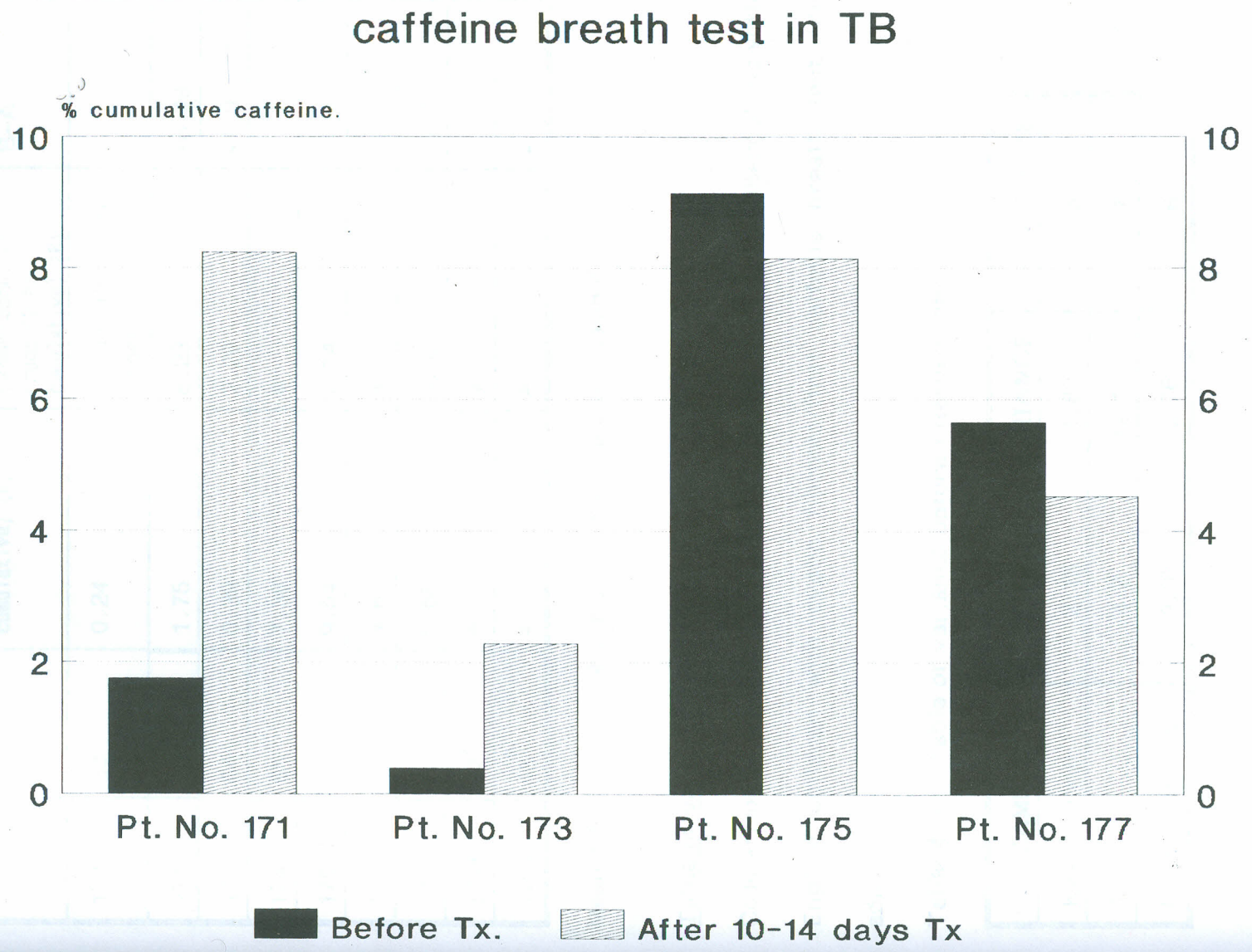

caffeine breath test in TB
% cumulative caffeine.
10
8
6
4
2
0
Pt. No. 171
Pt. No. 173
Pt. No. 175
Pt. No. 177
Before Tx.
After 10-14 days Tx

Figure 4.4

Table 4.4 Caffeine Breath Test in Tuberculosis

Patient	Caffeine dose(mg)	% caffeine dose recovered on admission (2 hr cumulative) (A)	% caffeine dose recovered 10-14 days after commencement of drugs (2 hr cumulative) (B)	Increase or loss B-A
170	63	0.24	Absconded (no repeat)	-
171	73	1.75	8.23	+6.48
173	64	0.39	2.29	+1.9
174	85	7.38	*	-
175	51	9.54	8.14	-1.4
176	48	4.61	*	-
177	43	5.65	4.53	-1.12
178*	42[‡]	*	*	-
179*	48[‡]	*	*	-

Mean ± S.D. 4.2 ± 3.5 * inadequate sample

Table of variance

Comparison of Ugandan children with kwashiorkor and tuberculosis with English children with cystic fibrosis/first caffeine breath test (on admission)

Table 4.5 Table of variance before intervention;

SAMPLE	MEAN	VARIANCE	SAMPLE SIZE
Kwashiorkor	2.15	1.82	5
TB	4.2	12.2	7
Cystic fibrosis[‡]	9.37	6.76	6

p value < 0.001 [‡]Parker et al[7]

N.B. All values are for initial test prior to any intervention such as Dietary rehabilitation, anti-TB, or adminstration of ciprofloxacin in Cystic Fibrosis.

TB patients were generally nutritionally better than their malnourished counterparts. Table 4.4 shows labelled cumulative 2 hour CO_2 output for each patient both before and during anti-tuberculous treatment expressed as a percentage of the oral caffeine administered. Out of the expected 8 duplicate and one single results, we only have 4 duplicate results and 4 single results as illustrated in Table 4.4. Of the 4 duplicate results, 4.3% (4.1) of the caffeine dose was exhaled as CO_2 in 2 hr before initiating anti-TB treatment, and 5.79% (2.8) during anti-TB treatment. The 4 duplicate results revealed some improvement in exhaled labelled cumulative 2 hr CO_2 in two patients (171 and 173) and a decrease in two patients (175 and 177), following treatment of TB. The two very sick patients especially 171 who had pleural effusion, showed remarkable improvement following commencement of ant-TB regimen (Figure 4.4).

Levels of labelled cumulative 2hr CO_2. ($P<0.001$). Table 4.5 compares the English children[7] and Uganda children.

CHAPTER 5

DISCUSSION

5.1 INTRODUCTION

Lambert et al is cited by Parker et al to have performed the first caffeine breath test in children. In there study they showed that the time to peak rate labelled carbon dioxide exhalation occurred in the first 2 hours after adminstration of caffeine [7]. It has been shown that the 2 hour cumulative exhalation reflects 3-N-demethylation and that, this is a cytochrome P450 (CYPIA2) dependent reaction [7,8].

There are many constraints involved in carrying out metabolic studies in these children. The issue of ethical consideration, availability of financial resources, development of a more acceptable procedure other than those involving venepuncture, which is basically an invasive procedure, are some of the important constraints encountered. The caffeine breath test may be an important tool in studying drug metabolism, since it is innocuous and moreover its products are easy to harness as has been demonstrated in previous studies [7,8].

5.2 KWASHIORKOR

Malnutrition is known to be potentially fatal especially in children less than 5 years. PEM and concomitant infections continues to have profound effect on children in the developing countries [1,2,20]. These children are subjected

to many drugs without necessarily knowing their drug handling capability. Hitherto, no comprehensive pharmacological study had been done to demonstrate how children can handle drugs.

The purpose of this study of Ugandan children was to determine the applicability of the caffeine breath test in assessing potential liver damage in children with oedematous malnutrition and those on anti-tuberculous drugs. PEM is known to cause biological alteration of virtually all organs in the body including the liver which handles most drug metabolism and detoxification [2,18]. Our study demonstrated a lower level of labelled caffeine exhaled as CO_2 amongst malnourished children. Of the 3 sets of complete results there was a demonstrable improvement in the value of 2 hour cumulative exhalation of labelled carbon dioxide following 14 days of nutritional rehabilitation. This reflects improvement in 3-N demethylation which is a cytochrome P450 (CYPIA2) dependent reaction (Table 4.2 and Figure 4.2). Available literature indicates that protein energy malnutrition in childhood does not lead to permanent liver damage. PEM improves on dietary treatment [1,2,4,20].

The results of the first caffeine breath test when, further compared to the study by Parker et al on English children suffering from cystic fibrosis before introduction of ciprofloxacin, shows lower levels of labelled caffeine in Ugandan children (p value < 0.001; Table 4.5) [7]. This result is in agreement with the finding of a study conducted by Masimirembwa et al on

Shona children, where they demonstrated reduction in caffeine metabolism amongst Zimbabwean children of African origin [62].

All the malnourished children under study had a palpable liver, in addition to oedema, also hair changes and skin dyspigmentation were present in some patients [1,2,65]. Studies have revealed fatty infiltration of the liver amongst children with kwashiorkor and marasmic-kwashiorkor, irrespective of the liver size [21,64]. Enlarged liver or rather fatty infiltration of the liver is associated with reduced lipoprotein production and thus inability to transport lipids from the liver. Liver microsomal enzymes, albumin and total plasma proteins are usually reduced [1,2]. With the evidence of biological alteration of liver which handles most drugs, these children are in great danger of drug intoxication as result of reduced drug metabolism[1,18].

It is well documented that children with malnutrition have poor liver function [18,34,62]. Another study has revealed decreased albumin and total protein level and associated increased level of transaminases in subjects afflicted with kwashiorkor [28]. In addition to poor liver function these children are known to have poor absorption as a result of alteration of gastric mucosa as well as change in transit time [4].

Response to dietary rehabilitation was good. However, it is important to allow these children adequate time to make a full response to dietary

treatment. There was loss of weight which coincided with loss of oedema and subsequent improvement in mental status of the patients. There was also reasonable improvement in liver function following dietary rehabilitation as shown by the caffeine breath test (Table 4.2 and Figure 4.1). This is in line with previous findings of a study done in Kampala [29]. Another study by Seifart et al on malnourished children, revealed that it was possible to see a demonstrable change in anthropometric parameters after 6 months of nutritional rehabilitation [68].

The duration of this study was too brief and was not adequate for full recovery of the liver and other organs affected by the stress of malnutrition. In future studies, children ought to be given ample time to recover before repeat test is performed in order to be able to appreciate the disparity between drug handling ability of malnourished and those children who are nutritionally rehabilitated. Three tests are suggested, on admission, 2-3 weeks after initiation of nutritional rehabilitation and on full recovery.

A dwindling supply of DISCO 150, jeopardised the recovery of these patients. In Uganda diets containing up to 200 cal kg^{-1} day^{-1} in a skimmed milk oil and sugar mixture (DISCO) are given to the children under nutritional rehabilitation. This brings about dramatic improvement in weight [1,29]. Children in India treated with 200 cal kg^{-1} have also demonstrated rapid and adequate improvement [1].

The pilot study was performed soon after the harvest and thus, there was plenty of food in store to eat. Diseases due to malnutrition tend to follow a seasonal pattern, for instance problems seem to be so rampant during the rainy periods, the times when mothers spend much of their time in the garden at the expense of breast feeding [65]. This explains why the turnover of malnourished patients was low, resulting in difficulty in recruiting suitable patients. The other major constraint we had to grapple with was patient compliance. The patients presenting to the hospital were either too sick to withstand the time-consuming and strenuous test or too young to comply with the requirements, which involved blowing into bag.

Otherwise, besides possibility of leak as a result of fault during manufacturing process of the headspace vials and also scarcity of patients as a result of harvest, the study was overall satisfactory as a pilot. In this study we were able to demonstrate that caffeine breath test is a feasible in vivo test of drug metabolism and liver function in children suffering from malnutrition. It was also possible to infer that enzyme system is not functioning at optimum rates in this children as shown in both Table 4.2 and Figure 4.2.

5.3 TUBERCULOSIS

The small sample was a limitation to our study. However, our results revealed increase in labelled caffeine in two patients. The increase could be due to improved nutrition or induction by drugs. Tests done when

patient is normal would be standard at which to assess first and second test.

Rifampicin and isoniazid are known to cause drug induction [37,41,42]. Hepatotoxicity of anti-tuberculous therapy with isoniazid and rifampicin has been found to be high in malnourished children. There is need to modify therapy appropriately to achieve therapeutic response and avoid toxicity [18,40]. Both rifampicin and isoniazid are metabolized by CYPIIIA and CYPIIE1 isoenzymes respectively [37,46] and do not have effect on CYPIA2.

Rifampicin is extensively metabolised and induces its own metabolism as well as other substrates sharing the same metabolic pathway catalysed by cytochrome P450IIIA1 [37,38,39]. However, rifampicin seems to have little influence on cytochrome P450IA2 (CYPIA2) sub-family, an enzyme which catabolizes demethylation of caffeine [36,37,38]. Our result of the paired samples demonstrated little impact if any of anti-TB drugs on the outcome of the quantity of labelled caffeine exhaled as carbon dioxide. In view of this, it will be worthwhile repeating the test after one month's treatment with anti-TB in order to allow enough time for enzyme induction to take place. It will also be necessary to perform a similar test on better nourished individuals on similar treatment, preferably English children suffering from TB.

Isoniazid, as rifampicin, is also an inducer of specific liver isoenzyme[41,42,43,44,45,46]. In some studies caffeine has been used to determine acetlylator status of people in an attempt to identify those who are prone to toxic effects of the drugs [40]. The standard triple regimen of pyrazinamide, INH and rifampicin employed in management of TB is capable of causing hepatotoxicity especially when given in high doses. Rifampicin and isoniazid are known to induce specific liver isoenzymes and, as a result, alter blood levels of drugs metabolized by these isoenzymes. It is necessary to identify such interaction in order to allow adjustment of drug dosage and subsequently reduce toxicity and therefore enhance therapeutic effect [39,40]. In our study these drugs did not appear clearly as inducers of caffeine metabolism (Table 4.4 and Figure 4.4). Further studies need to be done on these children after specified time of anti-TB therapy to be able to possibly see a discernable induction or improvement in drug metabolism [68].

Whilst performing the caffeine breath test, we were confronted with quite a number of problems, which could have most probably influenced the result. Some of the most pertinent problems encountered were on patient recruitment and patient compliance.

Diagnosis of tuberculosis in children presents a dilemma, the great variability of signs and symptoms makes it imperative that it be considered in the differential of any obscure or ill defined clinical problem [64].

Nonetheless, chest x-ray with signs of pneumonia unresponsive to standard antibiotics, enlarged peri-hilar and mediastinum were suggestive of tuberculosis [65]. History of contact though difficult to discern, positive Mantoux test > 15 mm, loss in weight and history of chronic cough arguably supported the diagnosis of tuberculosis in our study. Patients were then started on anti-tuberculous treatment, comprising pyrazinamide, isoniazid and rifampicin. However, one patient (No. 176) who went on to develop pneumonia, was additionally started on streptomycin, crystalline penicillin and gentamycin. Her response to intervention was uneventful.

Availability of drugs are some of the most difficult experiences we had to encounter. The dwindling supply of essential drugs was an issue of concern. The patients on anti-tuberculous drugs were forced to use their meagre resource to procure drugs and even when the drugs were available at the pharmacy, they were often given inadequate doses. It was a humiliating experience on the part of the patient as well as the person carrying out the procedure. The only remedial measure was to ensure that the patients got their supply of drugs during the period of study at all cost. This is one of the single most important factors which influenced patient compliance, particularly those taking anti-tuberculous treatment.

Hitherto, the caffeine breath test had not been tried in developing countries. The above mentioned problems had not been anticipated either.

However, improvement in collecting equipment will eliminate the problem of leak and improve the outcome of this very innocuous, but useful procedure. It is also important to give these children ample time to recover before a repeat caffeine breath test is done.

CONCLUSION

1. We have demonstrated that it is possible to use the caffeine breath test in young sick children as it is non-invasive and its products are easy to harness.

2. The caffeine breath test has been used successfully in young children (ages 3 years and above) acting as their own control.

3. Unfortunately, the study was limited by the failure of the seals on some bottles which invalidated some of the results and thus reduced study numbers for analysis.

4. The study shows that the function of drug metabolising enzymes in the liver in malnutrition is depressed and improves with nutritional rehabilitation.

5. The results in children with tuberculosis are more complex as the enzymes may be depressed in some patients due to malnutrition and in others they may be induced by some anti-TB drugs, both activities may present in the same patient.

6. The degree of depression of drug metabolising liver enzymes due to malnutrition could be assessed by repeating the test when the child has completely recovered.

RECOMMENDATIONS

i. Further investigations of the caffeine breath test should be undertaken in children with PEM, those on anti-TB treatment and other conditions affecting liver drug metabolising enzymes in developing countries using larger numbers.

ii. It is essential that the equipment, particularly the seals on collection vials, are adequate.

iii. Studies should be undertaken both when the child is sick and when recovered. The latter will act as standard to assess the former results.

REFERENCES

1. Stanfields-P; Brueton-M; Chan-M; Pakin-M; Waterston-T: Protein energy malnutrition: Disease of Children in the Subtropics and the Tropics 1991; 335-66.

2. Hendrickse-RG; Barr-DGD; Mathews-TS: Protein energy malnutrition: Respiratory system/Chemotherapy of Tuberculosis: Paediatrics in the Tropics 1991; Blackwell Scientific publication; 7:119-31 and 12:274-98; 22:677-85.

3. Coulter-JB et al: Protein energy malnutrition in northern Sudan: clinical studies: Eur-J-Clin-Nutrition 1988; 42: 787-96.

4. Kamala Krishnaswamy: Drug metabolism and pharmacokinetics in malnourished children: Clinical Pharmacokinet 1989; 17 (suppl 1): 68-88.

5. Lares-Asseff-I; Cravito-J; Santiago-P; Perez-Ortiz-B: A new dosing regimen for metronidazole in malnourished children: Scand-J-Inf-Dis. 1993;25:115-21.

6. Park-BK: Assessment of the drug metabolism capacity of the liver: Br-J-Clin-Pharmac 1982; 14: 631-51.

7. Parker-AC; Preston-T; Heaf-D; Kitterngham-NR; Choonara-I: Inhibition of caffeine metabolism by ciprofloxacin in children with cystic fibrosis as measured by caffeine breath test: Br-J-Clin-Pharmac 1994; 38: 573-576.

8. Lambert-GH; Schoellar-DA; Humprey-HE; Kottake-AN; Lietz-H; Campbell-M; Spielberg-SP; Budd-M: The caffeine breath test and caffeine urinary metabolite ratios in Michigan cohort exposed to polybrominated biphenyls: a preliminary study: Environment-Health-Perspect 1990; 89:175-81.

9. Emmanuel-M; Kaijuka; Kajja-E; Ann-RC; Edilberto-L: Demographic health survey Ministry of Health 1988/1989.

10. Barton-T; Wamai-G: Equity and vulnerability - situation analysis of women and children, adolescent in Uganda : Uganda-Profile 1994.

12. Dodge-CP: Health implications of war in Uganda and Sudan: Soc-Sci-Med 1990; 31: 691-8.

13. Dodge-CP: Uganda rehabilitation, or redefinition of health services?: Soc-Sci-Med 1986; 22: 755-61.

14. Okot-Nwang-M; Wabwire-Mangen-F; Kagezi-VB: Increasing prevalence of tuberculosis among Mulago Hospital admissions, Kampala; Uganda 1985-89: Tuber-Lung-Dis 1993; 74: 121-5.

15. Shimeles-D; Lulseged-S: Clinical profile and pattern of infection in Ethiopian children with severe protein-energy malnutrition: East-Afr-Med-J 1994; 71: 264-7.

16. Idiope-Tomas-A: Pharmacokinetics of antibiotics in malnutrition: Nutr-Hosp 1990; 5: 77-84.

17. Catz-CS: Malnutrition and drug metabolism in fetal and neonatal animals: Dev-Pharmacol-Ther 1990; 15: 211-4.

18. Saroja Mehta: Drug metabolism in malnourished child: The Malnourished Child 1990; 19: 329-37.

19. Finlayson-Niall-DC: Drug metabolism and the liver SO: Med-Internat-Journ 1994: 455-59.

20. Seth-V et al: Drug therapy in malnutrition: Special Article 1992; 1341-6.

21. Waterlow-JC; Protein energy malnutrition; London: Edward Arnold; 1992:40-108.

22. Notari-R; DeYoung-JL; Anderson-RC: Clinical Applications of pharmacokinetics parameters: Biopharmaceutics and pharmacokinetics an introduction 1975; 4: 101-75.

23. Ibrahim-SA et al: Correlation of some biochemical parameters with clinical features of protein energy malnutrition: East Afr Med J 1994; 71: 77-83.

24. Guler-A; Sapan-N; Salantur-E: Effects of cortisol and growth hormone on the metabolism of the liver and bone in children with malnutrition.: Turk-J-Pediatr 1992; 34: 21-9.

25. Mayatepek-E; Becker-K; Gana-L; Hoffman-GF; Leichsenring-M: Leukotrienes in the pathophysiology of kwashiorkor: Lancet 1993 16; 342 (8877): 958-60.

26. Brooke-SE; Doherty-JF; Golden-MH: Peroxisomes and the hepatic pathology of childhood malnutrition: West-Indian-Med-J 1994; 43:15-7.

27. Watkins-PB: Role of cytochromes P450 in drug metabolism and hepatotoxicity: Semin-Liver-Dis 1990; 10: 235-50.

28. Pond-WG; Ellis-KJ; Schoknecht-P: Response of blood serum constituents to production of and recovery of kwashiorkor-like syndrome in young pigs: Proc-Soc-Exp-Biol-Med 1992; 200: 555-61.

29. Department of Paediatrics and Child Health Makerere University Uganda: The Therapy of Severely of The Malnourished Child:MUN-CHAMP 1973; 1-46.

30. Brooks-SF; Golden-MH; Payne-Robinson-HM: Ultrastructure of islets of Langerhans in protein energy malnutrition: West-Indian-Med-J 1993; 47: 101-6.

31. Ashton-M; Bolme-P; Alemayehu-E; Eriksson-M; Paalzow-L: Decreased chloramphenicol clearance in malnourished Ethiopian children: Eur-J-Clin-Pharmacol 1993; 45:181-6.

32. Ashton-M; Bolme-P; Zerihun-G; Holmberg-K; Paalzow-LK: Disposition of salicylic acid in malnourished Ethiopian children after single oral dose: Clin-Pharmacokinet 1993; 25: 483-94.

33. Abdel-Razzak-Z; Loyer-P; Fautrel-A; Gauter-JC; Corcos-L; Turlin-B: Cytokines down regulates expression of major cytochrome P450 enzymes in adult human hepatocytes in primary culture: Mol-Pharmcol 1993; 44:707-15.

34. Ramjee-G; Berjak-P; Adhikari-M; Dutton-MF: Aflatoxin and Kwashiorkor in Durban, South Africa: Ann-Trop-Paediatr 1992; 12:241-7.

35. Grange-JM et al: Clinically significant drug interaction with anti-tuberculosis agents: Drug-Saf 1994; 11: 242-252.

36. Bolt-HM et al: Interaction between clinically used drugs and oral contraceptives: Environ-Health Perspect 1994; 102 suppl.9: 35-8.

37. Strolin-Benedetti-M et al: Induction and autoinduction properties of rifamycin derivatives: a review of animal and human studies: Environ-Health Perspective 1994; 102 suppl.9: 101-5.

38. Naishibe-Y et al: Induction of cytochrome P450 isoenzymes in cultured monkey hepatocytes: Int-J-Biochemistry-Cell-Biology 1995; 27: 279-85.

39. Zylber-Katz-E: Multiple drug interactions with cyclosporin in a heart transplant patient: Ann-Pharmacother 1995; 29: 127-31.

40. Weber-WW et al: Acetylation: Birth-Defects 1990; 26: 43-65.

41. Baker-MT; Vasquez-MT et al: Metabolism of 2-chloro-,1,1, difluoroethene to glycoxylic and glycolic acid in rat hepatic microsomes: Drug-Metab-Dispos 1990; 18: 753-8.

42. Ng-SF; Waxman-DJ: Biotransformation of N,N,N'-triethythylenethiophosphoramide: Cancer-Res 1990; 50; 464-71.

43. Anelov-E; Spirova-R et al: The effect of isoniazid and lipovitan on the components of microsomal electron-transport chains in rat liver: Eksp-Med-Morfol 1990; 29:24-8 (abstract).

44. Lind-RC; Gandolfi-AJ; Hall-RM: Isoniazid potentiation of a guinea pig model of halothane-associated hepatoxicity: J-Appl-Toxicol 1990; 10: 161-5.

45. Kenna-JG; Martin-JL; Satoh-H; Pohl-LR: Factors affecting the expression of trifluoroacetylated liver microsomal protein neoantigens in rats treated with halothane: Drug-Metab-Dispos 1990; 18: 788-93.

46. Hammond-AH; Fry-JR: The in vivo induction of hepatic cytochrome P450-dependent enzyme activities and their maintenance in culture: Biochem-Pharmacol 1990; 40: 637-42.

47. Salfinger-M; Crowle-AJ; Reller-LB: Pyrazinamide and pyrazinoic acid activity against tubercle bacilli in cultured human macrophages and BACTEC system: J-Infect-Dis 1990; 162: 201-7.

48. Kuznetsov-VG et al: Pyrazinamide activity of bacteria of genus yersinia: Lab-Delo 1990: 723 (abstract).

49. Lacroix-C; Tranvouez-JL; Phan-Hoang-T Duwoos; Lafront-O.: Pharmacokinetics of pyrazinamide and its metabolites in patients with hepatic cirrhotic insufficiency: Arzneimittelforschung 1990; 40: 76-79 (abstract).

50. Cohn-DL; Catlin-BJ; Peterson-KL; Judson-FN; Sbarbaro-JA: A 62-dose, 6 months therapy for pulmonary and extrapulmonary tuberculosis: Ann-Intern-Med 1990; 15; 112: 407-15.

51. Klein-P: Nutritional Application of ^{13}C: Strategic Considerations; New Techniques in Nutritional Research, Academic Press 1990; 3: 73-93.

52. Bunn-JEG; Thomas-JE; Harding-M; Coward-CA; Weaver-LT: Neonatal Helicobacter pylori infection: Proceedings of British Paediatric Association Annual meeting 1995; 67:80.

53. Kruger-N; Helge-H; Neubert-D: The significance of PCDD's/PCDF's (dioxins) in paediatrics: Monatss-Kinderheilkd 1991; 139: 434-41.

54. Kruger-N; Helge-H; Neubert-D: CO_2 breath test using ^{14}C-caffeine, ^{14}C-methacetin and ^{14}-phenacetin for assessing postnatal development of monoxygenase activities in rats and marmosets: Dev-Pharmacol-Ther 1991; 16: 164-75.

55. Kearns-GL: Hepatic drug metabolism in cystic fibrosis; recent development and future directions: Ann-Pharmacother 1993; 27: 74-9.

56. Fuhr-U; Klittich; Staib-A: Inhibitory effect of grape fruit juice and its bitter principal, naringenin on CYPIA2 dependent metabolism of caffeine in man: Br-J-Clin-Pharmacol 1993; 35: 431-6.

57. Tanaka-E; Ishikawa-A; Misawa-S: Simplified test for determination of drug-oxidizing capacity in rats with chemical-induced liver injury using caffeine and trimethadione as model drugs: Pharmacol-Toxicol 1992; 70: 177-80.

58. Davies-BT et al: Drug interaction with quinolones; Rev-Infect-Dis 1989; 11; suppl.5:S1083-90.

59. Harder-S; Fuhr-U; Staib-AH; Wolff-T: Ciprofloxacin caffeine: drug interaction established using in vivo and in vitro investigation; Am-J-Med 1989; 30; 87: 895-915.

60. Ullrich-D et al: Urinary caffeine metabolites in man. Age dependent changes and pattern in various clinical situations; Eur-J-Clin-Pharmacology 1992; 43: 167-72.

61. Rost-KL; Roots-I: Accelerated caffeine metabolism after omeprazole treatment is indicated by urinary metabolite ratios: coincidence with plasma clearance and breath test: Clin-Pharmacol-Ther 1994; 55: 402-11.

62. Masimirembwa-CM; Beke-M; Hasler-JA; Tang-BK; Kalow-W: Low CYPIA2 activity in rural Shona children in Zimbabwe: Clin Pharmacol & Therapeut 1995; 57: 25-31.

63. Kirkwood-BR: Calculation of required sample size; Essentials of Medical Statistics 1988; 26: 191-200.

64. Coovadia-HM; Loening-WEK: Tuberculosis: Paediatrics & Child Health; Oxford University Press 1992; chapter 9: 222-42.

65. Coulter-JB: Protein energy malnutrition (PEM) M Trop Paed Handout.

66. Weeks-RM; Barenzi-JF; Wayira-JR: A low cost, community based measles outbreak investigation with follow-up action: Bull-World-Health-Organ 1992; 70: 317-21.

67. Friedland-IR et al: Bacteraemia in severely malnourished children.: Ann-Trop-Paediatr 1992; 12: 433-40.

68. Seifart-HI; Donald-PR et al; Isoniazid elimination kinetics in children with protein-energy malnutrition treated for tuberculous meningitis with a four component anti-microbial regimen; Annals of Tropical Paediatrics 1995; 15; 249-254.

69. Pons-G; Rey-F; Badoual; Olive-G: Use of labelling of drugs with stable isotopes in clinical pharmacology in children: Arch-Fr-Pediatr 1989; 46: 223-8. (abstract)